Poster

Anatomy/Pathology - Poster

110 - P01-1

Increased choroidal blood flow velocity with regression of unilateral acute idiopathic maculopathy

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Purpose: The pathogenesis of unilateral acute idiopathic maculopathy (UAIM) is still unknown. The aim of this study was to quantitatively examine changes in choroidal circulation hemodynamics in patients with UAIM.
Methods: Retrospective observational case series. Five eyes of 4 UAIM patients were included. In all eyes, laser speckle flowgraphy (LSFG) was conducted to evaluate the mean blur rate (MBR), a quantitative index of relative blood flow velocity, at the UAIM lesion site. The changes in MBR at the initial visit and after 1 and 3 months were statistically analyzed. In 4 eyes, indocyanine green angiography (ICGA) was examined. In 3 eyes, enhanced depth imaging optical coherence tomography was used to measure subfoveal choroidal thickness.
Results: The mean logMAR value of best-corrected visual acuity was significantly improved (P = 0.04) with recovery of outer retinal morphology. ICGA showed macular hypofluorescence from the initial phase in all eyes examined. The average MBR was significantly increased at 1 and 3 months (+21.7% and +32.5% vs. baseline; P = 0.003, P = 0.001, respectively). The mean values of subfoveal choroidal thickness decreased with time (316.0 ± 63.5, 186.6 ± 21.0 and 167.3 ± 31.6 μm at baseline and 1 and 3 months later, respectively).
Conclusions: Choroidal blood flow velocity significantly increased at the lesion site with regression of UAIM. Our present data on LSFG and ICGA suggest that choroidal circulation impairment plays a role in the pathogenesis of UAIM.
Commercial Relationships: Yuki Hashimoto, None; Wataru Saito, None; Michiyuki Saito, None; Kiriko Hirooka, None; Shohei Mori, None; Kousuke Noda, None; Susumu Ishida, None

111 - P02-2

Diabetic Retinal and Choroidal Edema in SDT Rats

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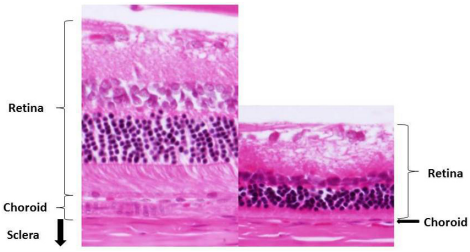
Purpose: To evaluate the relationship between diabetic retinal edema and choroidal edema in Spontaneously Diabetic Torii (SDT) rats.
Methods: We measured the retinal and choroidal thicknesses in normal Sprague-Dawley (SD) rats (n=9) and SDT rats (n=8). The SDT rats were confirmed to be diabetic based on a nonfasting blood glucose level exceeding 350 mg/dL. The SDT rats were diagnosed with diabetes by 12 to 20 weeks after birth. Both groups of rats were fed the same standard rat chow (CRF-1, Oriental Yeast, Inc., Tokyo, Japan) for more than 40 weeks. The eyes then were enucleated, sectioned to 4 μm, and stained with hematoxylin and eosin for conventional histopathologic studies. The mean retinal and choroidal thicknesses were measured at 500, 1,000, and 1,500 microns from the optic nerve disc using ImageJ software, and the mean thicknesses were calculated for each rat.
Results: The mean retinal thicknesses were 99.8 microns in the normal SD rats and 153.0 microns in the SDT rats (P=0.0015). The mean choroidal thicknesses were 9.1 microns in the normal SD rats and 17.7 microns in the SDT rats (P=0.0039). The choroidal thickness was correlated strongly with the retinal thickness in the normal SD rats and the SDT rats (r=0.72, $P < 0.05$, and r=0.81, $P < 0.05$, respectively).
Conclusions: Diabetic retinopathy and diabetic choroidopathy appeared as edema in the SDT rats. The retinal thickness was correlated strongly with the choroidal thickness in the SDT rats, which are ideal animal models of both diabetic retinopathy and choroidopathy.
Commercial Relationships: Fumihiko Toyoda, None; Akihiro Kakehashi, None; Hiroko Takano, None; Nozomi Kinoshita, None; Machiko Shimmura, None; Ayumi Ota, None; Yoshiaki Tanaka, None; Shiho Uehara, None



Comparison of the retina and the choroid (hematoxylin and eosin stain). Left; The SDT rat. Right; The SD rat.

112 - P03-3

Age related changes in the Korean pediatric human orbit on CT

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Purpose: The purpose of this study was to determine the relationships among orbital dimensions, globe diameter and age.
Methods: A retrospective analysis of 136 CT scans of subjects with no orbital or globe disease was performed and 10 lengh measurements and 3 angle measurement of various aspects of the orbit was obtained.

Results: 136 CT scans of 136 korean subjects without identifiable globe and orbital disease were included in this study. 25 subjects ≥ 17 years of age were considered mature adults and grouped together, while the remaining 100 subjects were grouped according to age. Globe diameter and all length measurements except globe protrusion increased most rapidly over the first 12 to 24 months. Globe diameter was reached to 96% of their adults value at age 8 years and highly correlated to linear orbital measurement. Central orbital axis angle and intraorbital angle in neonate group were found to be greater than their adults group and decreased rapidly over the first 12 months. Orbital protrusion continued to increase through the neonate until adulthood.
Conclusions: The growth of the korean human orbit increased most rapidly over the first 12 to 24 months and reached to 96-98% of their adults value at age 8 years. With this attempt to define normal age-related measured orbital value and orbital changes, this is helpful to treat the pediatric orbital disease and abnormalies.
Commercial Relationships: Hee-Bae Ahn, None; Woo seok Choi, None; Hyun wook Shin, None; Woo Jin Jeong, None; Yoon Hyung Kwon, None; Won Yeol Ryu, None

113 - P04-4

Correlation Between Serum Level of Vascular Endothelial Growth Factor And Subfoveal Choroidal Thickness In Patients With POEMS Syndrome

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Purpose: To determine whether there is a significant correlation between the serum level of vascular endothelial growth factor (VEGF) and the subfoveal choroidal thickness (CT) and foveal thickness (FT) in patients with polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes (POEMS) syndrome.
Methods: A cross sectional, observational case series.
We studied 31 eyes of 16 treatment-naïve patients with the POEMS syndrome with no evidence of fundus abnormalities. The subfoveal CT and FT were determined by enhanced depth imaging optical coherence tomography (EDI-OCT). The correlations between the serum level of VEGF and the subfoveal CT and FT were determined.
Results: The mean subfoveal CT was 417.9 ± 73.5 μm (right eye,416.7 ± 81.2 μm; left eye, 419.0 ± 68.1 μm), and the mean FT was 243.8 ± 35.2 μm (right eye, 248.8 ± 22.0 μm; left eye, 239.1 ± 44.6 μm). There was a significant positive correlation between the serum level of VEGF and the subfoveal CT (right eye, r=0.58, P=0.021; left eye, r=0.60, P=0.012), but the correlation between the serum levels of VEGF and the FT was not significant (right eye, r=0.007, P>0.05; left eye, r=0.25, P>0.05).
Conclusions: The significant correlation between the serum level of VEGF and the subfoveal CT in patients with POEMS syndrome suggests that serum levels of VEGF influences the choroidal structure. These results are helpful to understand not only the pathogenesis of the ocular changes in patients with the POEMS syndrome but also offer clues on the pathogenesis of other choroidal diseases.
Commercial Relationships: Hirotaka Yokouchi, None; Takayuki Baba, None; Masayasu Kitahashi, None; Toshiyuki Oshitari, None; Shuichi Yamamoto, None

114 - P05-5

Ocular Morphology and Retinal Optics Technology for the Mechanism of Accommodation Improvement in Myope with Orthokeratology

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Study Group: Ocular imaging Laboratory

Purpose: To investigate the accommodation-related changes in the ocular morphology and the visual function in young myopia with orthokeratology.
Methods: This report illustrated the state of the art of ocular morphologic technology with excellent performance including high resolution, long scan range and fast imaging speed. Moreover, this morphologic system was combined with the ocular optical measuring apparatuses for the accommodation function monitoring and the visual function supervising. With these technology systems, 12 myopic children aged from 10 to 18 years-old were enrolled and their dynamic accommodative functions were investigated before and after orthokeratology. The following-up was performed at 1 month, 3 month and 6 month.
Results: An improvement in the velocity of the accommodative response was evident from 2.40 D/s to 3.96 D/s (One-way ANOVA analysis, $p < 0.05$), which was correlated with the change in the spherical aberration at resting (Poisson's analysis, $r = 0.49$, $p < 0.05$) and the deformation of anterior segment biometry during accommodation (lens thickening, $r = 0.54$, $p < 0.01$; steepening of lens anterior surface, $r = 0.66$, $p < 0.01$).
Conclusions: The dynamic accommodative function was improved with orthokeratology in myopic children. The changes in the ocular morphology and optical property played a role in the improvement of the accommodation.
Commercial Relationships: Fan Lu, None; Meixiao Shen, None; Yilei Shao, None; Yimin Yuan, None; Weigen Zhu, None
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115 - P06-6

Evaluation of 12 myopia-associated genes in Chinese patients with high myopia

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China.

Purpose: Two recent large-scale genome-wide association studies (GWASs) identified significant associations between myopia and SNPs near the *PRSS56*, *BMP3*, *KCNQ5*, *LAMA2*, *TOX*, *TJP2*, *RDH5*, *ZIC2*, *RASGRF1*, *GJD2*, *RBFOX1*, and *SHISA6* genes. Our study is to examine whether rare high penetrant variations in these genes contribute to high myopia.
Methods: Whole exome sequencing was performed on 298 unrelated patients with early-onset high myopia (eoHM) and 195 controls. Potential variations in these genes were selected for further validation and comparison to the controls. Moreover, Sanger sequencing was used to evaluate the coding regions and the uptream 800 base pairs of *GJD2* in 395 additional subjects with late-onset moderate to high myopia (loMHM) and 403 normal controls.
Results: Exome sequencing of the 298 patients with eoHM identified 25 rare variants that were predicted to affect coding residues. The frequencies between patients and controls and the segregation analysis did not provide evidence to support their association with myopia. Sanger sequencing of *GJD2* in an additional 395 subjects with loMHM and 403 normal controls did not identify myopia-associated variants.
Conclusions: We did not find evidence to support the association of myopia with coding sequences variations in these genes. Additional studies are expected to validate these results.
Commercial Relationships: Qingjiong Zhang, None; Jiali Li, None; Dan Jiang, None; Xueshan Xiao, None; Shiqiang Li, None; Xiaoyun Jia, None; Wenmin Sun, None; Xiangming Guo, None
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116 - P07-7

Investigation of the association between LAMA2 and EGR-1 polymorphisms and high myopia in Chinese

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Purpose: Laminina2-chain (LAMA2) and EGR-1 were thought be important in human eye development. This study aimed to investigate the association of the tagging single nucleotide polymorphisms (tSNPs) in LAMA2 and EGR-1 genes with high myopia.
Methods: Four tSNPs (rs2571575, rs9321170, rs1889891 in the LAMA2 gene, rs117413810 in the EGR-1 gene) were selected, based on the HapMap database. In particular, tSNPs of rs2571575, rs9321170, rs1889891 in the LAMA2 gene were selected with accordance to the previous association reports which demonstrated possitive association with high myopia as well. All tSNPs were genotyed using ligase detection reaction (LDR) approach for 167 Han Chinese nuclear families with highly myopic offspring(<-10.0 diopters) as well as in an independent group with 485 highly myopic cases (<-10.0diopters) and 499 controls.
Family-based association analysis was performed using the Haploview package. Population-based association analysis was performed using Chi-square test.
Results: All four tSNPs tested did not show association with high myopia in our population（P>0.05）. Haplotype analysis of the three tSNPs in the LAMA2 genes did not show a significant association either（P>0.05）. Population-based association analysis of four tSNPs also showed no significant association with high myopia（P>0.05）.
Conclusions: Our family- and population-based association data in Han Chinese population both suggest that the LAMA2 and EGR-1 genes might not be associated with high myopia.
Commercial Relationships: Wei Han, None; Hui Fang, None; Fang-yu Lin, None
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117 - P08-8

Form deprivation myopia in wistar rats

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Purpose: It has been showed that experimental myopia can be induced by visual deprivation in a wide variety of animal species including chickens, common marmosets, guinea pigs, and mice. But in our knowledge, it has not been reported the myopia model of rats. The purpose of this study was to investigate whether a deprivation of form vision could induce myopia shift and axial elongation in rat eye.
Methods: We used 12 wistar rats, aged 21-23 days in this study. The Right eyes were deprived of form vision by lid suture for 4 weeks. The left eyes had a normal visual exposure as control. The refraction of eyes was measured by retinoscopy, and the axial length of eyes was measured by A-scan ultrasonography at the point of 1, 2 and 4 weeks after the application of monocular form deprivation.
Results: At the start point of this study, the refraction of the right eyes and the left eyes were 11.50 ± 0.88 D and 11.46 ± 0.94 D, and the axial lengths of these eyes were 4.97 ± 0.06mm and 4.98 ± 0.04mm, respectively. There were no significant differences between right and left eyes. After 1, 2 and 4 weeks of form deprivation, the refraction of the right eyes were +8.25 ± 0.84 D, +7.54 ± 0.92 D and +4.98 ± 0.68 D, respectively, and the refraction of left eyes were +10.75 ± 0.58 D, +9.27 ± 0.71 D and +7.81 ± 1.23 D, respectively. The axial lengths of the right eyes were 5.08 ± 0.05 mm, 5.16 ± 0.05 mm and 5.34 ± 0.06 mm, respectively, and the refraction of left eyes were 5.02 ± 0.05 mm, 5.09 ± 0.05 mm and 5.19 ± 0.03 mm, respectively. The form deprived eyes had significant myopic shift and axial elongation at all the points ($p<0.05$).
Conclusions: We demonstrated rat eyes respond to form deprivation by myopic shift and axial elongation. These biometric data are important when rat eye is used as a model to study myopia.
Commercial Relationships: Kosei Shinohara, None;

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118 - P09-9

A case of myopia progression restrained by a corneal foreign body during childhood

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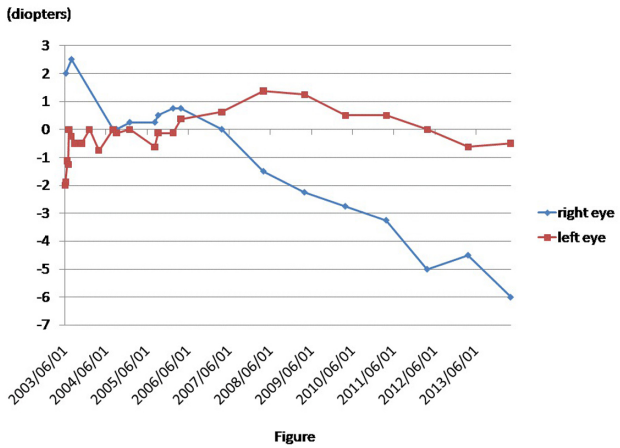
Purpose: Visual impairment due to myopia is increasing and many questions remain regarding the mechanism for myopic progression, including its prevention. Although form deprivation associated with corneal opacity during childhood is a risk factor of progression of myopia, we experienced a case where myopia progression was restrained by the reduction of off-axis aberration associated with a corneal foreign body.

Methods: A 6 year-old boy was referred to the Osaka University Hospital for the evaluation and treatment of a corneal foreign body in his left eye. A small piece of lead from a pencil was found at the mid-corneal stroma on the pupil. As there was no inflammation in the eye and corrected distance visual acuity was 20/20, the child was followed up annually, and the foreign body in the cornea remained unchanged. Following figure indicated the changes of manifest refraction (spherical equivalent) during 11 years after trauma. Although the left eye remained emmetropic, the contralateral healthy eye showed myopic progression from +2.0 to -6.0 diopters.

Results: K readings of both eyes were similar and stable during the follow-up period. Corneal higher order aberrations (HOAs, μm) during the last visit revealed that corneal spherical aberrations for 4/6 mm diameter were 0.146 / 0.465 in the left eye, and 0.039 / 0.302 in the right, respectively. Similarly, corneal total HOAs in the left eye (0.250 / 0.554) were higher than in the right eye (0.129 / 0.485).

Conclusions: In this case, form deprivation from the corneal opacity did not occur, whereas the contralateral eye had progression of myopia. Since the size of the foreign body was small, the increase of spherical aberration with minimal scattering in the cornea reduced the peripheral hyperopic defocus, which may have led to the restriction of myopic progression.

Commercial Relationships: Keisuke Kanda, None; Naoyuki Maeda, None; Takashi Fujikado, None; Kohji Nishida, None



Figure

119 - P10-10

Macular Thickness Profiles of Intra-retinal Layers in Myopia Evaluated by Ultra-High Resolution Optical Coherence Tomography

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Study Group: Ocular Imaging Laboratory

Purpose: To investigate the thickness profiles of eight intra-retinal layers and their variations in myopia using ultra-high resolution optical coherence tomography (UHR-OCT).

Methods: Eighty young people with various degrees of myopia (spherical equivalents (SEs) ranged from -1.25D to -10.25D) were recruited for this study. All the subjects had good corrected vision ability (≥ 20/20). UHR-OCT was used to obtain retinal images centered on the fovea along the horizontal and vertical meridians. The retinal images were segmented into eight intra-retinal layers with an automatic segmentation algorithm (Figure 1). The thicknesses in the central, pericentral, and peripheral regions were calculated in order to analyze the thickness variation of intra-retinal layers with myopia, and the relationships between them.

Results: The horizontal and vertical thickness profiles within the 6mm macula before and after adjusting for ocular magnification were obtained (Figure 2). Adjusting for ocular magnification, significant higher mean thickness was found only in the outer plexiform layer (OPL) in high myopia. For individual retinal layers, only the outer segment of the receptors (OS) layer thickness in the central regions was positively correlated with axial length (AL) (r=0.265, P=0.017). Myopia was not correlated with corrected mean ganglion cell and inner plexiform layers (GCL+IPL) thickness in any region, while there were regional variations in the association between myopia and other intra-retinal layer thickness.

Conclusions: Foveal thickening with increasing axial length may originate from the OS thickening. The lack of association between myopia and the corrected GCL+IPL thickness in any region suggests that macular GCL+IPL thickness might have the potential to improve diagnostic ability in the detection of glaucomatous eyes with high myopia. UHR-OCT with segmentation algorithm may be useful to detect macular changes in retinal microstructure

with respect to the progression of myopia.

Commercial Relationships: Xinting Liu, None; Yimin Yuan, None; Shenghai Huang, None; Dexi Zhu, None; Qingkai Ma, None; Xinjie Ye, None; Fan Lu, None; Meixiao Shen, None

Support: National Major Equipment Program of China (2012YQ12008004), National Natural Science Foundation of China (81400441)

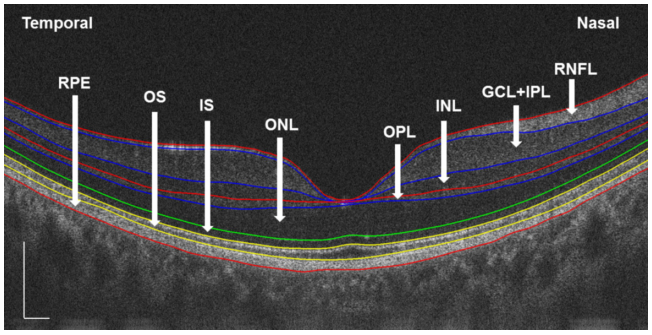

Figure 1 Segmentation of eight intra-retinal layers on a cross-sectional image from UHR-OCT along the horizontal scan.

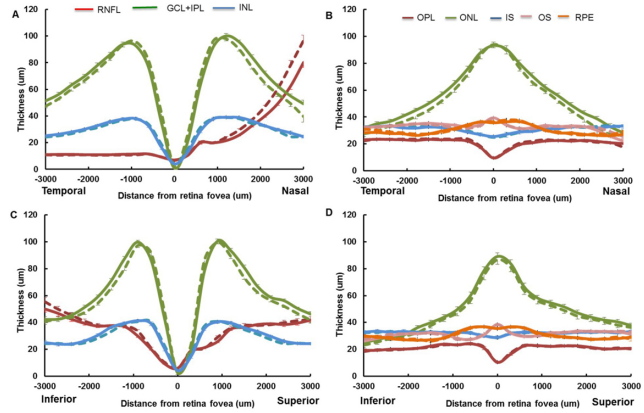

Figure 2 Thickness profiles of eight intra-retinal layers along horizontal (top) and vertical (bottom) scans in myopic eyes. The dashed and solid lines represent the thickness profiles before and after adjustment of the ocular magnification respectively.

120 - P11-11

Improvement of accommodation dynamics in myopic overnight orthokeratology

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Study Group: Ocular Imaging Laboratory

Purpose: To investigate the changes in accommodation dynamics in myopic children with overnight orthokeratology(OK) lens wear.

Methods: Ten children aged from 11 to 18 years were enrolled in this study. OK lenses were performed to correct the refractive errors after measurements of refraction, BCVA, corneal topography and axial length. Before lens wear and after 1 and 3 months of overnight lens wear, given the accommodative stimulation of 3D and 5D, the dynamic accommodation response was monitored using auto refractometer (GrandSeiko 5500). The latency, peak velocity and amplitude of accommodation were recorded and calculated (Fig.a).

Results: The latency and amplitude of accommodation did not change before and after orthokeratology (One-way ANOVA analysis, $p > 0.05$, Fig.c and d). Compared with baseline, the peak velocity of accommodation on 3 month increased significantly, both in 3D (2.40 ± 0.94 D/s versus 3.96 ± 1.11 D/s) and 5D (3.52 ± 1.29 D/s versus 5.58 ± 1.73 D/s) accommodative states ($p < 0.05$, Fig.b).

Conclusions: The function of accommodation improved in myopic children with wearing OK lenses. Changes in accommodation dynamics were mainly embodied in the accelerating of peak velocity. The improvement of accommodation dynamics may contribute to the control of myopia with overnight OK lens wear.

Commercial Relationships: Meixiao Shen, None; Yilei Shao, None; Yimin Yuan, None; Weigen Zhu, None; Fan Lu, None

Support: National Natural Science Foundation of China (81400441), Zhejiang Provincial Natural Science Foundation of China (Y13H180030)

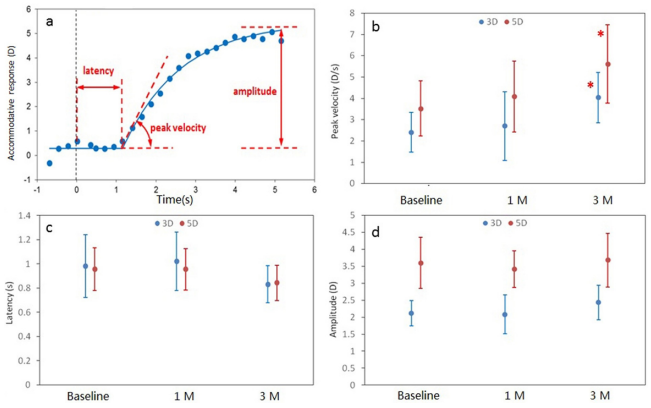

Fig. Curve of accommodative response and parameters of accommodative dynamic (a), changes in peak velocity(b),latency(c) and amplitude(d) of the subjects during 3 months. Error bars represent the standard error of the mean and "*"indicates a statistically signficant change from baseline.

121 - P12-12

Use of atropine for prevention of childhood myopia progression in clinical practice, efficacy and safety during treatment and when tapering off medication

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Purpose: To assess the efficacy and safety of daily 0.125% topical atropine solution for the prevention myopia progression in children in the setting of a real life clinical practice with the need to eventually taper off treatment.

Methods: This was a retrospective interventional case series between July 1st 2011 to June 30th 2014. Patients aged 6-12 years old with spherical equivalent of -1.00D or more were offered treatment with daily 0.125% Atropine solution. Cyclopegic refraction was noted at baseline and then 6 monthly afterwards. If patients were shown to have slow myopic progression (less than -0.5D progression over 12 months), the frequency of application was reduced by one step (daily to alternate day to once weekly to off).

In patients who were being tapered off atropine solution, those who demonstrated fast myopia progression (equal to or more than -0.25D progression over 6 months) at follow-up were resumed on their previous application frequency. The primary efficacy outcome measure was change in spherical error and primary safety outcome measure was occurrence of adverse effects or patient intolerance. For comparison between groups, continuous variables were evaluated with Student's t test or Mann-Whitney U tests, while categorical variables were assessed using a chi-square or Fisher exact test.
Results: 27 patients with 54 eyes were treated with topical 0.125% atropine solution for at least one year during the study period. 14 of them were boys and 13 were girls. Average age upon starting treatment was 7.68 years and the mean duration of follow-up during the study period was 19.6 months. Baseline spherical error was -4.07D. Mean myopia progression was -0.29 ± 0.27(SD). There was no significant difference in myopia progression between genders or between age groups. There was also no significant difference in myopia progression between eyes with low baseline myopia and high baseline myopia. 10 patients were able to initiate weaning off treatment during the study period. Amongst them, only 1 patient required stepping up treatment due to rebound effect.
Conclusions: Daily application of atropine 0.125% was well tolerated and effective in clinical practice. More than one-third of patients were able to able to begin reducing treatment frequency during the study period with only one patient having significant rebound effect.
Commercial Relationships: Kendrick Shih, None; Dorothy Fan, None

122 - P13-13

Detection of mutations in LRPAP1, CTSH, ZNF644, and SCO2 in 298 families with early-onset high myopia by exome sequencing

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Purpose: To evaluate variants in the *LRPAP1*, *CTSH*, *ZNF644,* and *SCO2* genes in 298 unrelated patients with early-onset high myopia (eoHM).
Methods: Genomic DNA from 298 patients with eoHM was analyzed by whole exome sequencing. Variants in *LRPAP1*, *CTSH*, *ZNF644,* and SCO2 genes were selected and analyzed with bioinformatics. Potential candidate variants were confirmed by Sanger sequencing and then validated in available family members and 192 normal controls.
Results: A total of seven variants predicted to affect the functional residues were detected, including a homozygous frameshift mutation (c.197delC, p.S67Qfs*8) in *LRPAP1*, four heterozygous missense mutations in *ZNF644* (c.1648G>A, p.A550T; c.2014A>G, p.S672G; c.2048G>C, p.R683T, and c.2551G>C, p.D851H), and two heterozygous missense variants (c.334C>T, p.R112W and c.358C>T, p.R120W) in *SCO2*. The c.1648G>A (p.A550T) in *ZNF644* was not cosegregated with high myopia in the family. The other six variants were detected in six patients, whose family members were not available for further co-segregation analysis of the variants. The six variants were predicted to be damaging by both Polyphen-2 and SIFT, whereas the previously reported p.S672G mutation in *ZNF644* was predicted to be damaging by SIFT but benign by Polyphen-2. All the six variants were absent in 192 normal control individuals. No homozygous or compound heterozygous variants were found in *CTSH*.
Conclusions: Our results provide additional evidence to support that mutations in *LRPAP1*, *ZNF644,* and *SCO2* were associated with high myopia.
Commercial Relationships: Xueshan Xiao, None; Dan Jiang, None; Shiqiang Li, None; Xiaoyun Jia, None; Jiali Li, None; Wenmin Sun, None; Xiangming Guo, None; Qingjiong Zhang, None
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123 - P14-14

Bcl-2 associated athanogene 3 protects against hyperthermic stress by modulating nuclear factor-kappa B and extracellular signal-regulated kinase activities in human retinoblastoma Y79 cells

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Purpose: Bcl-2 associated athanogene 3 (BAG3), a co-chaperone of HSP70, is a cytoprotective and anti-apoptotic protein that acts against various stresses, including heat stress. Here, we examined the effect of BAG3 on the sensitivity of human retinoblastoma Y79 cells to hyperthermia (HT).
Methods: We examined the effects of BAG3-knockdown on the sensitivity of Y79 cells to HT (44° C, 1 h) by evaluating apoptosis and cell proliferation using western blotting, real-time quantitative PCR (qPCR), flow cytometry, and WST-8 assay. Furthermore, we examined the effects of activating nuclear factor-kappa B (NF-κB) and extracellular signal-regulated kinase (ERK) using western blotting and real time qPCR.
Results: HT induced considerable apoptosis along with the activation of caspase-3 and chromatin condensation. The sensitivity of Y79 cells to HT was significantly enhanced by BAG3-knockdown. Compared to HT alone, the combination of BAG3-knockdown and HT reduced the phosphorylation of inhibitor of kappa B α (IκBα) and p65, a subunit of NF-κB, and degraded IκB kinase γ (IKKγ) during the recovery period after HT. Furthermore, BAG3-knockdown transiently increased the HT-induced phosphorylation of ERK after HT treatment, and the ERK inhibitor U0126 significantly improved the viability of the cells treated with a combination of BAG3-knockdown and HT.

Conclusions: The silencing of BAG3 seems to enhance the effects of HT, at least in part, by maintaining HT-induced inactivity of NF- κ B and the phosphorylation of ERK. These findings indicate that BAG3 may be a potential molecular target for modifying the outcomes of HT in retinoblastoma.
Commercial Relationships: Tatsuya Yunoki, None; Yoshiaki Tabuchi, None; Atsushi Hayashi, None; Takashi Kondo, None

124 - P15-15

A case of ciliary body leiomyoma with prominent accumulation of fluorodeoxyglucose (FDG) in FDG-PET/CT

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Purpose: To report a patient with ciliary body benign tumor (i.e., leiomyoma), nevertheless showing high accumulation of fluorodeoxyglucose (FDG) in FDG-PET/CT.
Methods: A 42-year-old female visited to our hospital with a complaint of floaters. She showed a brown elevated lesion, measuring 6 x 8 mm, in the inferotemporal region of the ciliary body associated with serous retinal detachment in her left eye. The tumor showed high accumulation of ^{18}F-FDG in PET/CT with the SUVmax value of 22.0, whereas ^{123}I IMP-SPECT revealed nothing of note. 5-S cysteinyldopa, a marker for malignant melanoma, in aqueous humor was not detected. These results suggested the possibility of benign tumor, while malignant melanoma was not completely denied. With her strong request, local resection was performed instead of enucleation of the globe. The tumor surface appeared rough, and its color was white with translucency.
Results: Histopathologically, the tumor consisted of spindle-shaped cells with swollen nucleus. Neither mitosis nor necrosis was observed within the tumor tissue. Immunohistochemistry showed that the tumor cells were positive for Desmin and alpha-smooth muscle actin and negative for S100 protein. Ki-67 labeling index was less than 1%. In this case, the ciliary body tumor was histologically diagnosed with leiomyoma.
Conclusions: We experienced a rare case with leiomyoma in the ciliary body showing a prominent accumulation of FDG in PET-CT, which arouses a great caution in order to avoid misdiagnosis.
Commercial Relationships: Kan Ishijima, None; Satoru Kase, None; Hiroshi Yoshikawa, None; Shigenobu Suzuki, None; Yasuhiro Shinmei, None; Kenichi Namba, None; Mayo Nozaki, None; Mika Noda, None; Susumu Ishida, None

Glaucoma - Poster

125 - P16-1

Protective effect of Etanercept, an inhibitor of tumor necrosis factor- α (TNF- α), in a rat model of retinal ischemia

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Purpose: Tumor necrosis factor- α (TNF- α) is an inflammatory cytokine which mediates several neurodegenerative diseases, and Etanercept (Enbrel®) is a commercialized TNF- α inhibitor. This study was conducted to assess the neuroprotective effect of Etanercept on axonal injury in acute ischemic animal model.

Methods: Acute ischemia was induced by elevation of intraocular pressure in 36 rats. Treatment groups had subcutaneous injection of Etanercept three times a week from the day of retinal ischemia; 0.3 mg/kg for 4 weeks (n= 6) or 1.0 mg/kg for 3 days (n= 3) or 2 weeks (n= 6) or 4 weeks (n= 6). The control groups were treated in the same manner as the same amount of phosphate buffer saline (PBS) for 3 days (n= 3) or 2 weeks (n= 6) or 4 weeks (n= 6). The optic nerve damage was evaluated with number of axons counted in electron microscopic photographs. Microglial cell activity was accessed using Iba1 and CD 68.

Results: After ischemia, the preserved axon ratio was greater in 2-week 1.0 mg/kg Etanercept treated group than PBS treated group with statistically marginal significance (p= 0.062). The 4-week 0.3 mg/kg and 1.0 mg/kg Etanercept treated group also showed significantly higher ratio than that of PBS treated group (p= 0.021 and 0.003, respectively). Expressions of Iba1 and CD 68 in optic nerve were reduced in Etanercept treated group. For immunohistochemical staining using rabbit anti-Iba1 antibody, the amount of microglia at optic nerve head decreased noticeably in Etanercept treated group compared with that in PBS treated group.

Conclusions: Etanercept significantly suppressed the optic nerve injury in a rat model of acute ischemia. This in vivo study suggests that Etanercept might be a novel neuroprotective treatment for the TNF- α related diseases.

Commercial Relationships: Hyoung Won Bae, None; Yun Ha Lee, None; Sang Jin Seo, None; Sang Yeop Lee, None; Yoo Kyung Song, None; Samin Hong, None; Gong Je Seong, None; Chan Yun Kim, None

126 - P17-2

Induction of Heat Shock Protein 70 (HSP70) by Geranylgeranylacetone is Critical for Retinal Ganglion Cell Protection

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Purpose: To investigate the cytoprotective effect of geranylgeranylacetone (GGA) on retinal ganglion cell (RGC) degeneration using a normal tension glaucoma (NTG) mouse model, which lacks glutamate/aspartate transporter (GLAST) and demonstrates spontaneous RGC and optic nerve degeneration without elevated intraocular pressure (IOP).

Methods: Three-week old GLAST+/- mice were given oral administration of GGA (100, 300, and 600 mg/kg/day, respectively) or vehicle alone, and littermate control (CTR) mice were given vehicle alone for 14 days, respectively. At the 5th week, the number of RGC was counted in paraffin sections of retinal tissues stained with hematoxylin and eosin. In addition, retrograde labeling technique was also used to quantify the number of RGC. Expression and localization of heat shock protein 70 (HSP70) in retinas were evaluated by reverse transcriptional polymerase chain reaction and immunohistochemistry, respectively. Activities of caspase-9 and -3 in retinas were also assessed.

Results: The number of RGC of GLAST+/- mice significantly decreased, as compared to that of CTR mice. However, RGC loss was suppressed by administration of GGA in a dose-dependent manner. Following GGA administration, HSP70 was significantly up-regulated, and activities of caspase-9 and -3 were suppressed.

Conclusions: HSP70 is a favorable target to suppress RGC degeneration, and thus GGA may be applicable for NTG as a promising therapeutics.

Commercial Relationships: Yasuhiro Shinmei, None; Zhenyu Dong, None; Kousuke Noda, None; Nobuyoshi Kitaichi, None; Atsuhiro Kanda, None; Takeshi Ohguchi, None; Takayuki Harada, None; Shinki Chin, None; Susumu Ishida, None

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127 - P18-3

An in vitro analysis of the induction mechanism of the autophagy by a component chemical of citrus peel and its application to whole animal system

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Purpose: Optineurin (OPTN) has been reported as a causative gene for not only glaucoma but also ALS (Amyotrophic Lateral Sclerosis). For both diseases, various phenomena relating to the accumulation of abnormal protein have been reported. Such phenomena include the "OPTN granules" (foci) formation in cultured cells in which OPTN with the glaucomatous mutation E50K was expressed, and the existence of OPTN-containing intracytoplasmic inclusions in nerve cells of ALS patients. Additionally, we found that a specific OPTN interaction protein generates the "vacuole-like abnormal intracellular structure" (vacuole) when it is coexpressed with OPTN in cultured cells.
Recently, among these phenomena, we found that "foci" and "vacuole " were suppressed by "SiTraRen (STR)" (a tentative name) which is a component of citrus peel through the induction of autophagy. An mTOR inhibitor also showed the similar suppressive effect. Here we report the result of *in vitro* analysis for the mTOR signaling pathway as a possible action mechanism of STR and the autophagy-inducing effect of STR in living mice.
Methods: 1. The activation state of mTOR and related proteins with STR or the mTOR inhibitor in HeLaS3 cells was assayed by the western blotting (WB) using antibodies specific to phosphorylated form of each mTOR pathway protein. 2. To evaluate the autophagy induction effect with STR or the mTOR inhibitor in the living body, those chemicals were intraperitoneally administed (i.p.) to C57BL/6J mice. The proteins were extracted from skeletal muscle, brain, spinal cord, and eyes, and then the antibody specific to LC3 (an autophagy marker protein) was used for WB.
Results: 1. The state of the mTOR and related proteins, The mTOR inhibitor suppressed the phosphorylation of mTOR and p70S6K, which is a down stream protein of mTOR signal. On the other hand, STR did not affect these phosphorylation. 2. The autophagy induction by STR in mice, In brain, spinal cord and eyes, an increase of autophagy specific marker protein LC3 were recognized by STR or mTOR inhibitor. It was not recognized in the skeletal muscle.
Conclusions: It was found that STR induces autophagy in the living bodies by the i.p. administraion. To determine the relevant signal molecules, additional examinations are necessary because the STR action did not depend on mTOR inhibition.
Commercial Relationships: Masafumi Ohtsubo, None; Yasushi Ohizumi , None; Yoshihiro Hotta, None; Shinsei Minoshima, None
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128 - P19-4

Correlation of corneal, scleral and optic nerve elasticity, and intraocular pressure on optic nerve damages

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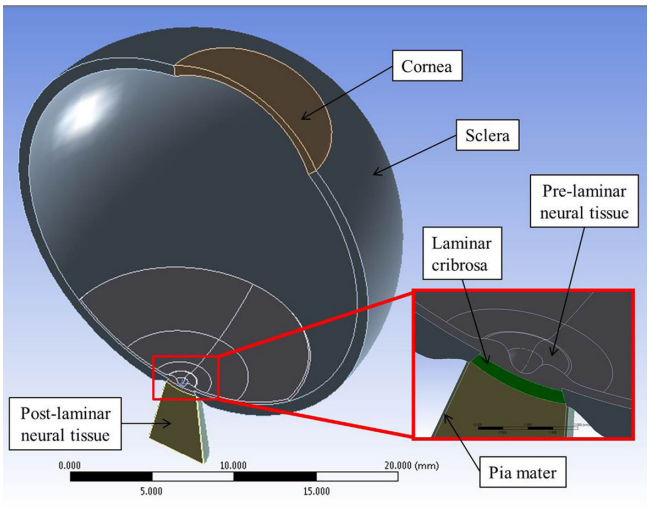
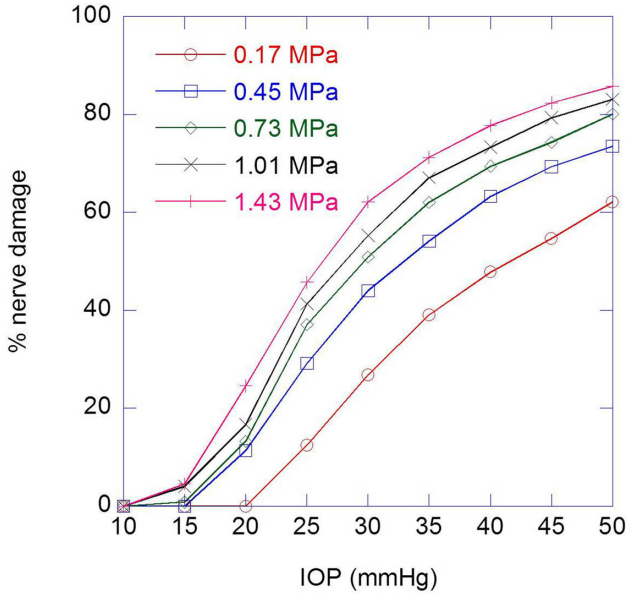
Purpose: Optic neuropathy in glaucoma causes visual field loss and blindness. The optic nerve damage in the lamina cribrosa (LC) of the sclera, the primary site of glaucoma, is correlated with the intraocular pressure (IOP). Literatures show that the optic nerves are sheared at high IOP and the scleral biomechanical properties play an important role in the development and progression of glaucomatous damage to the LC and ganglion cell axons with the optic nerve head (ONH). The aim of this study is to determine and characterize the correlation between the corneal, scleral and ONH elasticity, and intraocular pressure on the optic nerve damages.
Methods: The influence of corneal, scleral and ONH elasticity on the shear stress in lamina cribrosa were modeled using computational finite element analysis. To examine the effects of ocular tissue biomechanical properties, the tangent modulus of the cornea, sclera and lamina cribrosa were varied. The intraocular pressure exerted on the inner surface of the pre-laminar neural tissue, the sclera and the cornea were varied from 10 to 50 mmHg.
Results: The simulation results showed that the shear stresses in LC increase with increase of ocular tissue elasticity even at the same IOP. Classical Tresca shear failure criterion was adopted for the determination of the vision loss. Using this damage criterion, the results showed that percentage nerve damage increases logarithmically with the corneal elasticity, and also increases with the IOP. The percentage nerve damage at 20 mmHg IOP showed a logarithmic increase with the corneal tangent modulus and showed a maximum up to 25%.
Conclusions: This finding implies that, the clinical general guidance for the risk assessment of glaucoma development and progression based on the IOP is not enough. The parameter of corneal elasticity should include also in the diagnosis stage. The corneal elasticity can be clinically assessed by various commercially available medical devices like, ORA and Corvis ST, while the clinical measurement of scleral and LC elasticity have been obfuscated by the lack of instruments. The corneal elasticity can be used as an independent parameter or to form a combined parameter with IOP, for the risk assessment of glaucoma development and progression.
Commercial Relationships: Match KO, None



Three-dimensional human eyeball model used in the study



Optic nerve damage as a function of IOP with corneal tangent modulus vary from 0.17 to 1.43 MPa

129 - P20-5

Corneal biomechanical parameters in subjects with glaucoma

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Purpose: To evaluate the biomechanical parameters obtained by noncontact tonometer with a high speed Scheimpflug camera (Corvis –ST) of eyes with glaucoma and factors associated with the corneal displacement.
Methods: One hundred and forty nine eyes with glaucoma were included in this study. The IOP was measured and the corneal displacement was recorded using Corvis-ST. The biomechanical parameters of the subjects with primary open angle glaucoma (POAG) and subjects with primary angle-closure glaucoma (PACG) were obtained using Corvis-ST. The central vertical corneal displacements at the IOP reading time, applanation time 2, and maximum displacements were analyzed using MATLAB R2014a. The parameters were compared using Mann Whitney-U test.
Results: The applanation 2 velocity, highest concavity time, and peak horizontal distance were significantly different between subjects with POAG and PACG. There were no significant differences of the central vertical corneal displacements at the IOP reading time, applanation time 2, and maximum displacements between those with POAG and PACG. Age affected the corneal displacements at the IOP reading time.
Conclusions: Our results indicate that glaucoma types changed the biomechanical parameters at late phase measured using Corvis-ST. Age affects the amount of corneal displacement.
Commercial Relationships: Yoshiaki Kiuchi, None; Ulfah Rimayanti, None; Yoshitaka Nakao, None; Makoto Kanako, None

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130 - P21-6

Genetic associations of primary open angle glaucoma – a systematic review and meta-analysis

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Purpose: To systemically review and meta-analyze the genetic associations of reported gene variants with primary open angle glaucoma (POAG).
Methods: We searched MEDLINE, EMBASE and the Cochrane Library databases for POAG genetic association studies published from January 1946 to January 2014. All gene variants with complete genotype data reported in at least two cohorts were included for meta-analysis. Odds ratio (OR) and 95% confidence interval (CI) for each variant were estimated using the fixed effects or random effects model based on the heterogeneity test.
Results: A total of 7440 records were identified from the databases, of which 148 studies were eligible for meta-analysis, involving 44 loci/genes and 111 polymorphisms. We identified 18 variations in 11 genes (*CAV1/CAV2*, *EDNRA*, *ELOVL5*, *LMX1B*, *MMP1*, *MYOC*, *OPA1*, *OPTN*, *SIX1*, *SRBD1* and *TLR4*) to be significantly associated with POAG. The median risk OR for POAG was 1.56 (range, 1.23-2.85) and protective OR 0.7 (range, 0.36-0.83), with p values between 3.9×10^{-11} and 0.045. Seven genes (i.e., *ADRB2*, *ATOH7*, *CYP1B1*, *LOXL1*, *MTHFR*, *TP53*, and *WDR36*), reportedly associated with POAG, showed no statistically significant association in the meta-analysis. Sensitivity analysis confirmed the associations. Funnel plots and Egger' s test revealed minimal publication bias.
Conclusions: This systemic review and meta-analysis confirmed positive associations between 18 variants of 11 genes and POAG and thus provide a list of genes and variants for further investigations of their roles in the pathogenesis of POAG.
Commercial Relationships: Shi Song Rong, None; Li Ma, None; Shumin Tang, None; Chi Pui Pang, None; Li Jia Chen, None

131 - P22-7

Clinical characteristics and autonomic function in juvenile ocular hypertension

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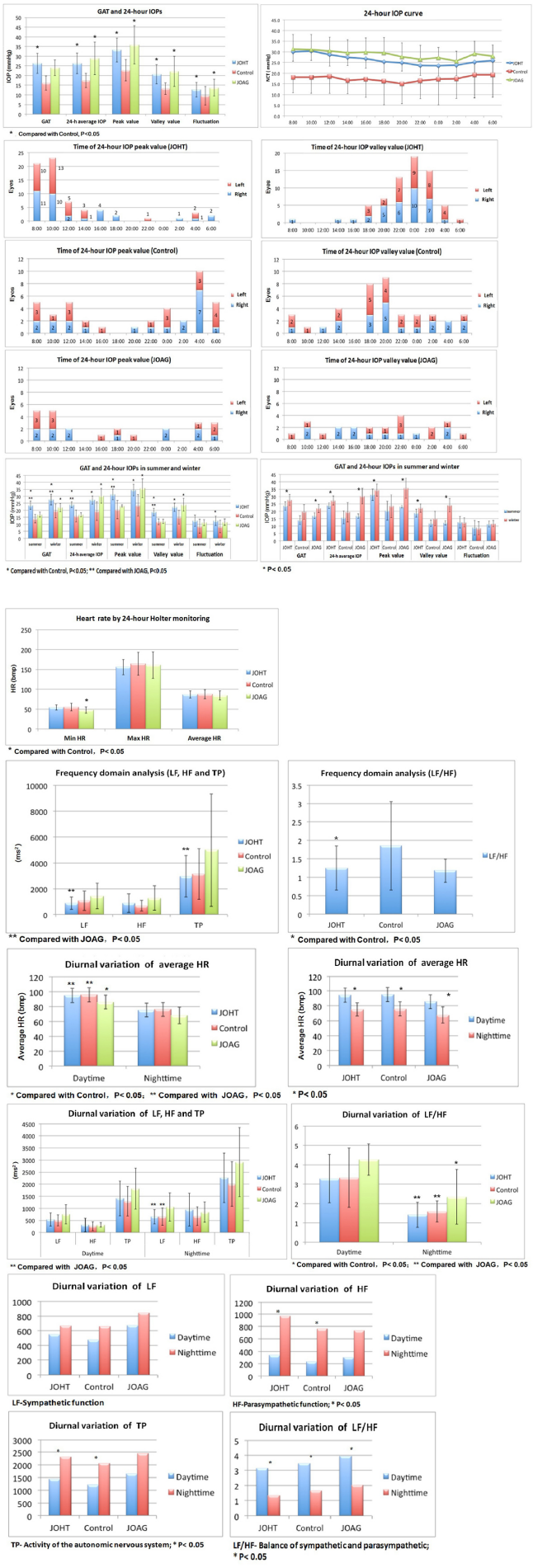
Purpose: To evaluate the clinical characteristics and autonomic function in juvenile ocular hypertension(JOHT),normal control and juvenile onset open angle glaucoma(JOAG)

Methods: Records of follow-up JOHT gathered from outpatients,Mar 2010 to Mar 2012,and healthy volunteers(Control) and JOAG with matched sex,age and BMI were enrolled.Goldmann applanation tonometry(GAT),central corneal thickness(CCT) and corneal curvature were examined,accompanied with 24-hour IOP measurements in hospital by NCT.Frequency domain analysis(LF,HF,TF,LF/HF) of heart rate variability(HRV) were used in 24-hour Holter monitoring to evaluate autonomic function.

Results: 34 subjects were included in JOHT,accordingly 20 in control and 12 in JOAG. No significant differences in CCT and corneal curvature among groups.GAT of JOHT was26.2 ± 5.2(21~42)mmHg,24-hour average IOP26.3 ± 5.3mmHg,peak value33.2 ± 6.2mmHg, appeared at8~10(44/68,64.7%,P=0.00),valley value20.6 ± 5.0mmHg,appeared at 22~2(47/68,69.1%,P=0.00),IOP fluctuation was12.6 ± 3.8mmHg.Variance of IOPs in JOHT were similar to JOAG,which were higher than those of Control(P<0.01).IOPs of JOHT and JOAG were of seasonal variability(P<0.05),but only considering IOP fluctuation variance in summer and winter was observed.Following more than 2-year' observation without medicine,IOPs of 4/34 in JOHT declined to below 21mmHg(11.8%),3 were diagnosed as JOAG(8.8%).Average HR of groups were similar(P=0.812),but the minimum HR of JOAG was lower than that of Control (P<0.05).LF and TP of JOHT were lower than those of JOAG and LF/HF lower than Control (P<0.05).HF,TP of JOHT and Control were of diurnal variability(P<0.05),while diurnal variation of HF,TP in JOAG were weaker(P>0.05).Average HR of JOAG was low at daytime while LF and LF/HF were high at night,variance was significant compared with those of JOHT and Control(P<0.05).24-hour IOP of JOHT and LF/HF were of negative correlation(P<0.05).Ages of JOHT and LF,TP,LF/HF were of positive correlation(P<0.05).

Conclusions: Conclusions, In JOHT,peak value of 24-hour IOPs appeared to be aggregated in the mornings and valley value in the evenings, fluctuation is obvious and significant with season changes;in 2 years without medicine,11.8% IOPs declined to normal after puberty. The study indicated that the immature autonomic function,especially sympathetic and parasympathetic imbalance,might play a role in the pathogenesis of JOHT and there was autonomic dysfunction in JOAG.

Commercial Relationships: Xin Wang, None; Xinghuai Sun, None; Liangcheng Wu, None; Gang Yang, None; Shaolin Wang, None



132 - P23-8

Comparison of intraocular pressure measurements between goldmann applanation tonometry, icare tonometry and non-contact tonometry in a normal population

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Purpose: To compare the intraocular pressure (IOP) measurements between the Goldmann applanation tonometry (GAT), Air puff tonometer and Icare PRO tonometer in a normal population and to examine the effect of central corneal thickness (CCT) on the IOP measured by the three instruments.
Methods: A total of 259 subjects fulfilling the inclusion criteria (no prior history of ocular illness, trauma or surgery, non contact lens wearers)were recruited. In each individual the IOP was measured by the three tonometers in the following order, Icare PRO, Air puff tonometer (NCT-NT 2000), GAT(by three masked examiners. Subsequently the CCT of all subjects were measured using ultrasonic pachymeter (tomey SP 100)
Results: The Icare PRO generally measured significantly higher IOP values compared to both GAT and Air puff tonometers (Mean GAT 16.05mmHg, mean Icare PRO IOP 16.56mmHg, mean Air puff IOP 15.99mmHg, $p<0.017$). The difference in IOP readings between GAT and Air puff tonometer was not statistically significant. The IOPs determined with GAT correlated strongly with Air puff (Spearman r 0.61, $P < 0.001$) and moderately with Icare PRO tonometer (Spearman r 0.55, $P < 0.001$). There was also a strong correlation between Air puff and Icare PRO tonometer. (Spearman r 0.68, $P < 0.001$). The 95% limits of agreement between Icare PRO and GAT were within ± 5.76 mmHg, between Air puff and GAT were within ± 5.42 mmHg and between Icare PRO and Air puff measurements were within ± 5.18 mmHg. All the tonometers showed a weak but stastically significant relationship with the CCT. The difference between GAT and Air puff tonometer measured IOP differed significantly between the thinnest (460-526μm) and thickest CCT(554-618μm) range however GAT-Icare PRO tonometer IOP measurements did not differ significantly across the ranges of measured CCT.
Conclusions: Both Air puff tonometer and Icare PRO tonometer measured IOP correlated significantly with the GAT measurement. Both the tonometers showed similar agreement with GAT across the range of measured IOP. Although the three tonometers showed a weak correlation with CCT the Air puff measured IOP was most affected by CCT.
Commercial Relationships: Fathimath Ahmed, None; Mimiwati Zahari, None; Norlina Ramli, None

133 - P24-9

Fluctuations of intraocular pressure during the day in angle closure glaucoma patients

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Purpose: Intraocular pressure (IOP) is a well-known treatable risk factor of glaucoma. Recent studies showed one time IOP measured at routine office visit may not represent the whole disease course or treatment effect. IOP fluctuations are associated with enlargement of visual field defect in open angle glaucoma. Studies on circadian rhythm and IOP fluctuations in eyes with angle closure are limited. The purpose of this study is to investigate the fluctuations of IOP during the day in primary angle closure glaucoma (PACG) and primary angle closure(PAC) patients.
Methods: Consecutive patients over 40 years-old with PACG or PAC presenting to the Taipei City Hospital in 2013 were enrolled. Exclusion criteria were eyes with corneal opacity or central scar which may affect IOP measurement. All patients received comprehensive ophthalmic examinations, including a series of daily IOP measurement from 8:00 am to 18:00, 2.5 hours apart, with air-puff pneumotonometry.
Results: Forty-two eyes with primary angle closure or angle closure glaucoma met inclusion criteria were enrolled. The mean age of patients was 67.7+/-7.75 (range 50-80); Diagnosis were primary angle closure(19%) and primary angle closure glaucoma (81%). The mean IOP recorded in clinic by pneumotometry versus applanation tonometry was 13.37+/-3.1mmHg versus 15.83 +/-2.48 mmHg, respectively. Circadian rhythms were classified based on the timing and presence of peak and trough IOPs. Almost half patients (47%) in this study showed relative morning peak pattern. The second common pattern was afternoon peaks with an incidence of 35.3%. Incidences of the timing of trough IOP were similar in each time point between late morning through afternoon. But only 5% of patients showed trough IOP in the early morning. Patients diagnosed as PAC had less IOP fluctuations and lower baseline measurement compared to PACG patients. A plateau pattern of IOP measurements and smaller IOP fluctuations were more common in post-iridectomy angle closure glaucoma patients.
Conclusions: Daily IOP fluctuations in patients with primary angle closure glaucoma and primary angle closure showed a tendency of morning peak and second afternoon peak. Higher morning and lower evening IOP readings reported in this study paralleled published studies in open angle glaucoma. Laser iridectomy showed some effects on flattening the IOP fluctuations of circadian rhythm in angle closure glaucoma patients.
Commercial Relationships: Ya-Chuan Hsiao, None; Tsung-Lin Yang, None
Clinical Trail: TCHIRB-1020412-E

134 - P25-10

In vivo confocal microscopy in glaucoma patients after laser peripheral iridotomy

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Purpose: Purpose of this work was to assess how changes in intraocular pressure, the use of topical antiglaucoma treatment and performing laser peripheral iridotomy (LPI) influence the corneal structure in glaucoma patients.
Methods: The study presents an analysis and evaluation of 58 patients (74 eyes). Study group consisted of 19 patients with glaucoma (35 eyes) who underwent laser peripheral iridotomy and a control group of 39 healthy patients (39 eyes). Keratocyte density in the anterior and posterior stroma, as well as the endothelial cell density, pleomorphism and polymegatism were evaluated in the in vivo confocal microscope (CS4 Nidek Technologies, Italy).
Results: Patients in the study group showed statistically significant lower keratocytes density in the anterior (812 ± 35,32 cells/mm ^{2} versus 1126.27 ± 33.20 cells/mm^2 in the control group) and posterior stroma (610,25 ± 16.79 cells/mm^2 versus 687,35 ± 21.27 cells /mm^2 in the control group). Comparative analysis of the endothelial cell density, polymegatism and pleomorphism in both groups was statistically insignificant. In the 6-month follow-up in patients after LPI procedure there was a decrease in the mean number of topical antiglaucoma drugs (from 1,29 ± 0.43% to 0,82 ± 0.37%) observed, while there were no statistically significant changes in the assessment of keratocytes and endothelial cells. There was no significant correlation between keratocyte density in the anterior and posterior stroma, endothelial cell density, pleomorpism and polymegatism with the patients' age and number of topical antiglaucoma treatment used.
Conclusions: Glaucoma as a chronic disease as well as the use of topical antiglaucoma treatment can affect the cornea by reducing the density of the keratocytes in the anterior and posterior stroma. Laser peripheral iridotomy in patients with angle-closure glaucoma in terms of it's impact on the morphology of the cornea has proved to be a safe procedure which may be beneficial for reducing the amount of antiglaucoma drugs used.
Commercial Relationships: Sabina Sapeta, None; Ewa Mrukwa-Kominek, None

135 - P26-11

Relationship between Bruch's membrane opening-minimum rim width and circumpapillary retinal nerve fiber layer thickness and cutoff point of structural marker

Natsuyo Yoshida-Hata [1] Yumi Matsushita [2] Naomichi Katai [1] Shigeko Yashiro [1] Aya O'hira [1] Yumi Sekine [1]

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Purpose: To determine the structure-function relationships between Bruch's membrane opening-minimum rim width (BMO-MRW) and circumpapillary retinal nerve fiber layer thickness (RNFL) using spectral-domain optical coherence tomography (OCT) and also between the BMO-MRW and the mean deviation (MD) of Humphrey field analyzer (HFA) in Japanese glaucoma patients.
Methods: Cross-sectional study of patients with glaucoma and healthy controls. Radial B scans and circular peripapillary B scans of the right optic nerve head of 38 patients with non-endstage glaucoma and 40 controls were determined by SD-OCT. The correlations between the OCT parameters and MD were calculated using the Steiger test. Receiver operator characteristic (ROC) analyses were used to determine the cutoff value for each OCT parameter and BMO-MRW and MD.
Results: The median age was 70 (36-86) years in the glaucoma and 68 (38-83) years in the normal patients. The average MD in the glaucoma patients was -9.22 (0.04-10.03) dB. The mean BMO-MRWs in the temporal inferior sector in the glaucoma patients was 146.2 μm and was 262.3 μm in the control patients. The mean temporal superior BMO-MRW in glaucoma and control was 173.3 μm and 280.7 μm, respectively. The cutoff points for BMO-MRW in the temporal superior and temporal inferior were 196.5 μm and 202.0 μm, and the sensitivity and specificity was 65.9% and 94.1%, and 79.5% and 82.4%, respectively. The cutoff point of RNFL the in temporal superior and temporal inferior was 105.0 μm and 107.0 μm. The sensitivity and specificity of those was 68.2% and 88.2%, and 81.8%, 100%, respectively. The relation between MD and BMO-MRW was higher than that of MD and RNFL.
Conclusions: The significant correlation between BMO-MRW and MD suggests a possibility that BMO-MRW can detect the presence of glaucoma. Although this study is comparatively small study, the cutoff points of structural markers are reasonable.
Commercial Relationships: Natsuyo Yoshida-Hata, None; Yumi Matsushita, None; Naomichi Katai, None; Shigeko Yashiro, None; Aya O'hira, None; Yumi Sekine, None

136 - P27-12

Effect of Pilocarpine on Lens Parameters in Primary Angle Closure before and after Peripheral Iridotomy

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Purpose: Primary angle closure glaucoma (PACG) is a significant cause of irreversible visual impairment in Asia, and Asians are expected to represent 87% of those worldwide with angle closure glaucoma by the year 2020. Pilocarpine is commonly used in the treatment of this condition; this study looked at the effect of this drug on lens parameters before and after peripheral iridotomy (PI). These parameters include Lens Vault (LV) which has recently been independently associated with angle closure after adjusting for age, gender, anterior chamber depth (ACD), and Lens Thickness (LT).
Methods: Patients with Primary Angle Closure, including suspect and glaucoma statuses (PACS, PAC, and

PACG) were recruited from the Ophthalmology Clinic of University Malaya Medical Centre, Kuala Lumpur between April-June 2014. Visual acuity and slit lamp examination (including fundus examination, gonioscopy and intraocular pressure (IOP) measurement) was performed. Subsequently, patients were evaluated with Lenstar biometry to measure ACD and LT; and Anterior Segment Ocular Coherence Tomography to measure LV. These parameters were taken before pilocarpine instillation and 45 minutes after instillation. Subsequently, a PI was done, following which the parameters before and after pilocarpine instillation were re-measured one week later.

Results: We investigated 1 eye each from 14 patients (6 male and 8 female). Mean age was 67.6 ± 14.3 years. LV increased by 0.08 ± 0.06 mm and 0.04 ± 0.04 mm respectively after pilocarpine instillation, before and after PI (Wilcoxon Signed Rank test, P<0.01 in both groups). There was no statistically significant difference when these two groups were compared (Mann-Whitney U test, p=0.70). ACD decreased by 0.02 ± 0.10 mm with pilocarpine instillation before PI and increased by 0.03 ± 0.05 mm after PI, the former being statistically significant (Wilcoxon Signed Rank test, P=0.04 and P=0.09 respectively). IOP reduction post-pilocarpine instillation was 1.1 ± 1.5 mmHg before PI and 0.5 ± 1.1 mmHg after PI, the former being statistically significant (Wilcoxon Signed Rank test, P=0.02 and P=0.11 respectively).

Conclusions: LV increases after pilocarpine. Pilocarpine reduces ACD and IOP in non-PI eyes, but it does not affect these parameters in post-PI eyes. The role of pilocarpine in post-PI eyes appears to be less significant.

Commercial Relationships: N Suchitra M Nadarajah, None; Mimiwati Zahari, None; Norlina Ramli, None; Amir Samsudin, None; Mohammadreza Peyman, None

137 - P28-13

Surgical results of eyes with aniridic glaucoma

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Purpose: To report the surgical results of eyes with aniridic glaucoma.

Methods: This study is a retrospective review of medical records for aniridic glaucoma patients who underwent glaucoma surgeries at Hokkaido University Hospital from 1997 to 2014. Seven eyes of four patients were included. Of four patients, two were siblings, one had a family history of developmental glaucoma, and one was diagnosed with WAGR syndrome complicated by Wilms tumor, genitourinary anomalies and mental retardation. A conventional trabeculotomy (CT) was performed on six eyes, and a modified 360-degree suture trabeculotomy (ST) was performed on one eye. One experienced ophthalmic surgeon (S.C.) performed all surgeries under general anesthesia.

Results: The mean age of patients at the first visit was 21.5 ± 33.8 months (range, 1 month to 6 years). Ocular complications during follow-up included buphthalmos in four eyes of two patients, congenital cataract in five eyes of three patients, and foveal hypoplasia and nystagmus in all eyes. The mean preoperative intraocular pressure (IOP) for the first operations was 33.0 ± 9.4 mmHg (range, 24 to 48 mmHg) and the mean IOP after the primary surgery was 19.8 ± 9.0 mmHg (range, 15 to 40 mmHg). Of seven eyes, three eyes treated first with CT needed reoperations, i.e., two eyes with two CTs and one eye with three CTs in total. The mean IOP at the final visit was 13.6 ± 2.2 mmHg (range, 9 to 16 mmHg). The average decimal best-corrected visual acuity (BCVA) at the final visit was 0.23 (range, 0.05 to 0.5).

Conclusions: In our case series, CT as well as ST effectively controlled IOP for aniridic glaucoma, although the final BCVA was poor because of corneal opacity, cataract and foveal hypoplasia. Further long-term observation is necessary to verify these surgical procedures.

Commercial Relationships: Takeshi Ohguchi, None; Yasuhiro Shinmei, None; Tomoe Uno, None; Mayo Nozaki, None; Ryo Ando, None; Takuya Nitta, None; Shinki Chin, None; Susumu Ishida, None

138 - P29-14

Conservation of Primary Cilia in Trabecular Meshwork

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Purpose: Intraocular pressure is a major risk factor for glaucoma pathogenesis. Trabecular meshwork (TM) is the site of resistance for aqueous outflow and the target for IOP lowering therapy. The mechanism of mechanosensation in the TM is not well understood.

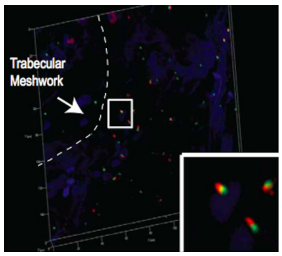
Methods: The presence of primary cilia in trabecular meshwork was determined by immunohistochemistry, immunofluorescence, and electron microscopy using both cultured and explanted trabecular meshwork tissue from human, porcine, bovine, and rodent eyes. The presence of cilia in the TM cells was verified by immunostaining using anti-IFT-88, Arl13b, acetylated alpha-tubulin, and gamma-tubulin antibodies. The expression level changes of TGFb and TNFa in TM cells responding to the pressure similar to organ perfusion in eyes were examined by RT-PCR.

Results: We showed a conserved presence of primary cilia in human, porcine, bovine, and rodent TM cells. Immunofluorescence staining of IFT-88, Arl13b, acetylated alpha-tubulin, and gamma-tubulin also confirmed the presence of primary cilia in cultured TM cells. Electron microscopy showed an abundance of cilia in human TM removed during trabeculectomy surgery. In addition, we found the presence of two inositol 5-phosphatases, OCRL and INPP5B, within the cilia, suggesting a role of phosphoinositides in ciliogenesis and ciliary mechanosensation function.

Conclusions: Primary cilia in TM cells are implicated in how the eye senses intraocular pressure changes and highlights OCRL as an attractive therapeutic target for the treatment of glaucoma.

Commercial Relationships: Yang Sun, NIH (R); Xinyao

Hu, None; Cathleen Wallmuth, None; Na Luo, None
Support: NIH/NEI EY022058, Lowe Syndrome Association, American Glaucoma Society



139 - P30-15

Superior segmental optic nerve hypoplasia in young Japanese subjects

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Purpose: Tajimi eye health care project using fundus photography found that the prevalence of superior segmental optic hypoplasia is about 0.3% in the Japanese population, aged 40 years or older. The purpose of this study is to investigate the prevalence of superior segmental optic nerve hypoplasia using optical coherence tomography (OCT) in young Japanese subjects.
Methods: A prospective observational cross-sectional study comprised 134 right eyes of 134 young Japanese college students. All participants (mean age 26.1 ± 4.1) underwent comprehensive ophthalmologic examination, including TOPCON 3D OCT-1000 MARK II RNFL 3.4 mm circle scan. The superior segmental optic nerve hypoplasia was screened by the RNFL thickness and confirmed by fundus photographs and kinetic perimetry.
Results: Superior segmental optic nerve hypoplasia was detected in three eyes of three subjects, which was 2.2 % of the 134 subjects. All three eyes showed a corresponding visual field defects.
Conclusions: In comparison to the earlier report, superior segmental optic nerve hypoplasia might not be a rare condition in young Japanese.
Commercial Relationships: Takehiro Yamashita, None; Yuya Kii, None; Minoru Tanaka, None; Kumiko Nakao, None; Taiji Sakamoto, None
Clinical Trail: UMIN000006040

140 - P31-16

Diagnostic ability of spectral-domain versus time-domain optical coherence tomography for assessing glaucoma in high myopia

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Purpose: To compare the diagnostic abilities of the spectral-domain optical coherence tomography (SD-OCT; Spectralis OCT) and time-domain optical coherence tomography (TD-OCT; Stratus OCT) to assess the changes of macular parameters in high myopic eyes with perimetric or suspected glaucoma.
Methods: We enrolled 144 high myopic eyes (spherical equivalent ≤ -6.0 D), of which 82 had perimetric glaucoma and 62 with suspected glaucoma formed a control group. All eyes underwent imaging with SD-OCT and TD-OCT. Areas under the receiveroperating characteristic curves (AUROCs), and the sensitivity and specificity for macular volume and thickness parameters between two groups were calculated and compared.
Results: The AUROCs for the best parameter of both macular thickness and volume from the TD-OCT (outer inferior region, AUROC = 0.911 for thickness and 0.909 for volume) were higher than that of the SD-OCT (outer inferior region, AUROC = 0.836 for thickness and 0.834 for volume) but showed no significance (P = 0.141 for thickness and 0.138 for volume). The macular outer inferior average thickness and macular outer inferior average volume on the TD-OCT had the highest sensitivity of 88.2% with a specificity of 80.4% and that of the SD-OCT had the highest sensitivity of 78.6% with a specificity of 82.1%.
Conclusions: Using TD-OCT and SD-OCT, the outer inferior macular thickness and volume are the only two independent predictors of good sensitivity to detect perimetric glaucoma in high myopia with the specificity fixed at 80%. Both TD-OCT and SD-OCT have similar diagnostic abilities according to our results. These parameters may provide a complementary glaucomatous diagnosis in high myopic eyes.
Commercial Relationships: Kuo-Chi Hung, None; Pei-Chang Wu, None; Hsueh-Wen Chang, None; Yi-Chieh Poon, None; Mei-Ching Teng, None

Physiology/Pharmacology - Poster

141 - P32-1

Multifunctional PEG Retinylamine Conjugate Provides Prolonged Protection against Retinal Degeneration in Mice

Guanping Yu [1] Xueming Wu [1] Akiko Maeda [2,3] Song-Qi Gao [2] Marcin Golczak [2] Krzysztof Palczewski [2] Zheng-Rong Lu [1]

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Purpose: Controlled oral delivery of retinylamine (Ret-NH_2) to treat retinal degenerative diseases, including Stargardt disease (STGD) and age-related macular degeneration (AMD).

Methods: A polyethylene glycol (PEG) retinylamine (Ret-NH_2) conjugate with a glycine-phenylalanine-leucine (GFL) spacer (PEG-GFL-NH-Ret) was synthesized for sustained release of the drug by digestive enzymes in the gastrointestinal tract.

Results: The pharmacokinetics experiments showed that the PEG conjugate could control the sustained drug release after oral administration and had much lower non-specific liver drug accumulation than the free drug in wild-type female C57BL mice. At mean time, the conjugate maintained the same concentration of Ret-NH_2 in the eye as the free drug. PEG-GFL-NH-Ret at a Ret-NH_2 equivalent dose of 0.5 mg/mouse produced complete protection of *Abca4*$^{-/-}$Rdh8$^{-/-}$ mouse retinas against light-induced retinal degeneration for at 3 days after oral administration as revealed by OCT retina imaging, whereas free Ret-NH_2 did not provide any protection under identical conditions.

Conclusions: The polymer conjugate PEG-GFL-NH-Ret has great potential for controlled delivery of Ret-NH_2 to the eye for effective protection against retinal degenerative diseases.

Commercial Relationships: Guanping Yu, None; Xueming Wu, None; Akiko Maeda, None; Song-Qi Gao, None; Marcin Golczak, None; Krzysztof Palczewski, None; Zheng-Rong Lu, None

Support: NIH Grant R24EY0211260 and Knights Templar Eye Foundation pediatric opthalmology career-starter research grant

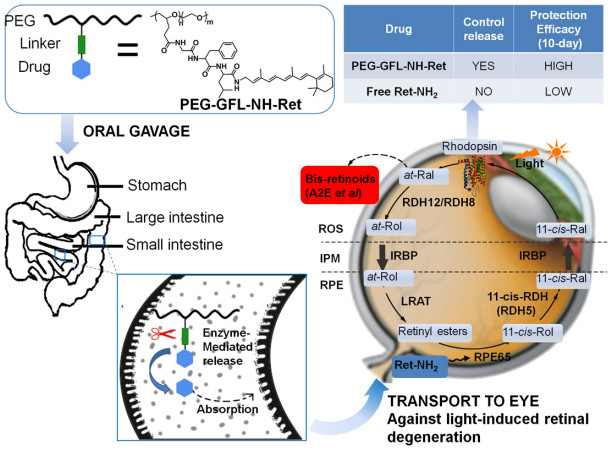

Fig. 1. An oral PEG retinylamine conjugate for prolonged protection against light-induced retinal degeneration in *Abca4*$^{-/-}$Rdh8$^{-/-}$ mice. After oral administration of conjugate, Ret-NH_2 should be gradually released in the small intestine and colon to maintain a relatively stable effective drug concentration in the circulation and a sufficient amount of the drug in the eye for an extended period.

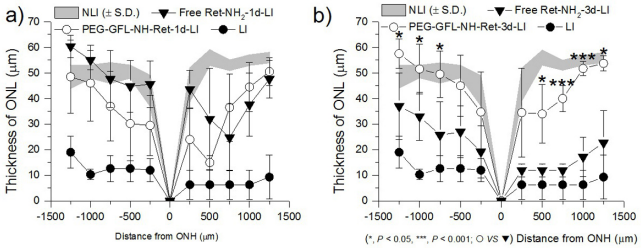

Fig. 2. Protecting effects of conjugate PEG-GFL-NH-Ret against light-induced acute retinal degeneration in 4-week-old *Abca4*$^{-/-}$Rdh8$^{-/-}$ (DKO) mice. DKO mice were given either free Ret-NH_2 or conjugate by gavage at an equivalent dose of 0.5 mg Ret-NH_2 per mouse. Mouse were illuminated with 10,000 lux light for 30 min either 1 day, 3 days after the gavage, mice then were kept in the dark for 7 days. The ONL thickness was measured from *in vivo* OCT images obtained along the vertical meridian from the superior to inferior retina of mice gavaged either 1 day (**a**) or 3 days (**b**) after light exposure(NLI = No light illumination; LI = Light illuminated).

142 - P33-2

Protective role of ZnS nanoparticles on ER stress mediated macular degeneration in Mouse Retinal Pigment Epithelial Cells

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Purpose: Age related macular degeneration (AMD) is the leading cause of irreversible visual impairment affecting 30-50 million individuals. The low level of zinc would be at a greater risk of oxidative stress-induced injury which can lead to AMD. Oxidative stress altered Endoplasmic reticulum stress response in Retinal Pigment Epithelial cells (RPE) behave like double edge sword, which may lead choroidal neovascularization or dry atrophic without neovascularization. Current treatments do not focus

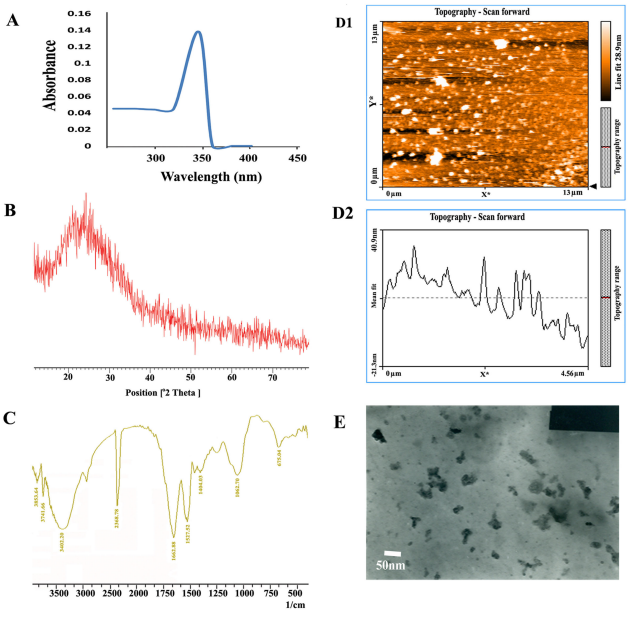
on underlying stimuli responsible for the disease. The aim of this present study is to develop a novel molecule which has anti-oxidant property that would suppress the condition of the AMD.

Methods: Zinc Sulphide (ZnS) nanoparticles were synthesized biologically and characterized using TEM, FTIR and XRD. The inhibitory effect of ZnS nanoparticles on reactive oxygen species (2,7-dichlorofluorescein diacetate, DCF-DA) elevation and RPE cell death induced by Hydrogen peroxide and thapsigargin (TG) was analysed in primary mouse RPE cells. The ability of ZnS nanoparticles control MRPE cell damage against oxidative stress mediated apoptosis was assessed by EtBr-AO, DAPI stain and flow cytometry analysis. The preventive role of ZnS nanoparticles on ER stress inducer TG stimulated expression of GRP-78 and CHOP was determined by western blot analysis.

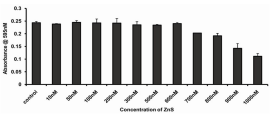
Results: Oxidative stress mediated ROS production and MRPE cell death was significantly blocked by ZnS nanoparticles. GRP-78 and CHOP protein expression was upregulated by the treatment of H2O2 and TG. Also it was found that the presence of ZnS nanoparticles on MRPE cells abrogate apoptosis and expression of GRP-78 and CHOP induced by H2O2 and TG.

Conclusions: These results concluded that ZnS nanoparticles reduce ER stress in RPE cells through the stabilization of reactive oxygen species. Therefore ZnS nanoparticles will be used as a therapeutic drug target for maintaining normal functioning of ER stress regulated proteins in RPE cells.

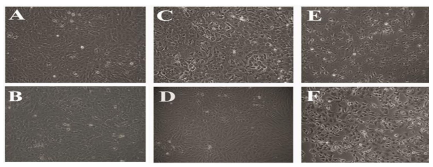
Commercial Relationships: Karthikeyan Bose, None; Harini Lakshminarasimhan, None; Kathiresan Thandavarayan, None



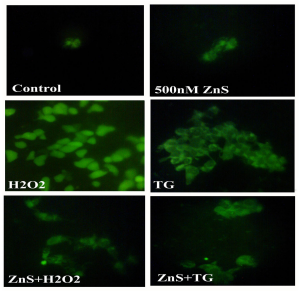
ZnS nanoparticles Characterized by A)UV-Vis spectrum wavelength of 220 to 350 nm B) shows the XRD pattern of the synthesized ZnS nanoparticles C) FTIR spectra was recorded in the range of 500 cm^{-1} to 4000 cm^{-1} D1 & D2) AFM analysis of ZnS NPs and E)The particle's size and morphology of the sample were investigated by TEM size is around (10nm – 50nm)

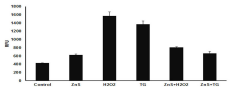


Dose dependent effect of ZnS nanoparticles on MRPE cells



A) Confluent MRPE cells B) RPE cells treated with ZnS-NP C) RPE cells treated H_2O_2 D) Cells treated with 500nM ZnS-NP and H_2O_2 E) cells treated with TG F) Cells treated 500nM ZnS-NP and TG





MRPE cells were incubated with ZnS and H2O2, Thapsigargin. Then labeled with 20 uM of 2',7'-dichlorofluoresceindiacetate (2,7-DCFH-DA; Sigma-Aldrich) for 30 minutes at 37°C. DCF fluorescence was analysed by Flourescence microscope and RFU value of excitation emission settings of 495 and 525 nm, respectively

143 - P34-3

Initial Experience and Early Outcome of Using Intravitreal Aflibercept in Non-AMD Cases in Hong Kong

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Purpose: To report the initial experience and early outcome of using intravitreal Aflibercept in indications other than age-related macular degeneration (AMD) in Hong Kong

Methods: Our centre was one of the first centre in Hong Kong to inject intravitreal Aflibercept. A retrospective study of all the injected cases in the initial 6-month period since June 2013 was performed. Indications other than AMD included diabetic macular edema, macular edema following central or branch retinal vein occlusions, choroidal neovascularization (CNV) secondary to polypoidal choroidal vasculopathy (PCV), myopic maculopathy, angiod streak and idiopathic CNV. CNV cases were given 3 monthly injections followed by as-needed injection. Macular edema cases followed an as-needed regime. Short-term treatment outcome were determined by the changes in visual acuity (VA) and OCT parameters, any recurrence of disease during the follow up (FU) period, and the safety profile. Cases with less than 3 months of FU were excluded.

Results: A total of 21 non-AMD cases received Aflibercept in the study period. Average age was 60.5 with a male predominance (14 out of 21 cases). Average FU duration was 5 months. When comparing the latest FU VA with initial presenting VA, 67% of cases (14 cases) showed improvement, 29% (6 cases) had maintained VA, and only

1 case had worsened VA. For OCT, the average central macular thickness reduced from 439.8μm to 284.6μm (p=0.02) and macular cube volume reduced from 9.49 mm^3 to 7.80 mm^3 ($p < 0.0005$). Qualitatively, complete resolution of sub-retinal fluid and macular edema was found in 16 cases (76%) and partial resolution in 4 cases (19%). No intraocular complications or systemic thromboembolic events were encountered. 10 cases had recurrence of disease during the follow-up period. 2 were recurrent PCV cases and 8 were macular edema from DME or retinal vein occlusions (in which 6 cases had chronic recurrent edema).

Conclusions: Aflibercept was effective in the treatment of a wide range of indications other than AMD with good VA and OCT outcome. All cases had improved or at least maintained VA except 1 case. There was also significant reduction in central macular thickness and macular cube volume with resolution of sub-retinal fluid and macular edema. Majority of macular edema cases with recurrence were chronic edema cases with prior history of repeated anti-VEGF injections every few months.

Commercial Relationships: Lap Ki Alex Ng, None; Wai Man Chan, None

144 - P35-4

Albumin-binding of diclofenac in aqueous humor of cataract patients and development of an administration method utilizing inhibition of protein-binding

Saya Ishii [1, 2] Mineo Ozaki [1, 2] Takashi Osaki [3] Norito Takamura [4] Kenji Ogata [4] Jin Tokunaga [4] Nao Setoguchi [4] Kazuhiko Arimori [5]

1. Ozaki Eye Hospital, Hyuga, Japan. 2. Ophthalmology, Faculty of Medicine, University of Miyazaki, Miyazaki, Miyazaki, Japan. 3. Tsudanuma Eye Hospital, Narashino, Chiba, Japan. 4. Second Department of clinical Pharmacy, School of Pharmaceutical Sciences, Kyusyu University of Health and Welfare, Nobeoka, Japan. 5. Department of Pharmacy, University of Miyazaki Hospital, Miyazaki, Japan.

Purpose: Diclofenac instillation has been widely used to patients with cataract surgery to prevent intraoperative miosis, inhibit postoperative inflammation, and prevent macular edema. Since diclofenac strongly binds to albumin in circulation, if there are patients in whom diclofenac administered by instillation strongly binds to albumin in the aqueous humor, the effect would not be fully exhibited. Therefore, it is necessary to suppress the binding of diclofenac to increase its free-form level. We investigated albumin-binding of diclofenac in the aqueous humor of each patient treated with diclofenac instillation and the influence of binding inhibitors.

Methods: In the experiment to assess albumin-binding of diclofenac in the aqueous humor of each diclofenac-treated patient, diclofenac was extracted from a specific volume of the aqueous humor and the total level was measured using UHPLC. The free-form diclofenac level was measured using ultrafiltration and UHPLC.

Results: The albumin-binding rate of diclofenac was 80% or higher in some aqueous humor samples. The binding of diclofenac to albumin site II was significantly inhibited by ibuprofen in simulated aqueous humor, but not significantly inhibited in pooled aqueous humor. This difference may have been due to the weak binding of diclofenac to albumin site II in the aqueous humor. Thus, we investigated the inhibitory effect of ibuprofen on albumin-binding of flurbiprofen, which also strongly binds to the same site bound by diclofenac, in pooled aqueous humor. Ibuprofen significantly inhibited (substituted) albumin-binding of flurbiprofen in pooled aqueous humor.

Conclusions: For clinical application, an effective diclofenac administration method may be established by the instillation of an appropriate albumin site II binding inhibitor before diclofenac instillation in patients with strong albumin-binding.

Commercial Relationships: Saya Ishii, None; Mineo Ozaki, None; Takashi Osaki, None; Norito Takamura, None; Kenji Ogata, None; Jin Tokunaga, None; Nao Setoguchi, None; Kazuhiko Arimori, None

145 - P36-5

Relationship of Ocular Microcirculation, measured by Laser Speckle Flowgraphy, and Silent Brain Infarction in Primary Aldosteronism

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Purpose: Recent studies have shown that the risk of *cerebro- and cardiovascular* events (CVEs) is higher in patients with primary aldosteronism (PA) than in those with essential hypertension (EH), and that silent brain infarction (SBI) is a risk factor and predictor of CVEs. Here, we evaluated the relationship between findings from laser speckle flowgraphy (LSFG), a recently introduced non-invasive means of measuring mean blur rate (MBR), an important biomarker of ocular blood flow, and the occurrence of SBI in patients with PA.

Methods: 87 PA patients without symptomatic cerebral events (mean 55.1 ± 11.2 years old, 48 male and 39 female) were enrolled in this study. We measured MBR in the optic nerve head (ONH) with LSFG and checked the occurrence of SBI with magnetic resonance imaging. We examined three MBR waveform variables, skew, blowout score (BOS) and blowout time (BOT). We also recorded clinical findings, including age, blood pressure, and plasma aldosterone concentration.

Results: PA patients with SBI (15 of 87 patients; 17%) were significantly older and had significantly lower BOT in the capillary area of the ONH than the patients without SBI ($P = 0.02$ and $P = 0.03$, respectively). Multiple logistic regression analysis revealed that age and BOT were independent factors for the presence of SBI in PA patients (OR, 1.15, 95% CI 1.01 - 1.38; $P = .03$ and OR, 0.73, 95% CI 0.45 - 0.99; $P = .04$, respectively).

Conclusions: PA patients with SBI were older and had lower MBR BOT than those without SBI. Our analysis showed that age was a risk factor for SBI, and that BOT was a protective factor, in patients with PA. This suggests that BOT, a non-invasive and objective biomarker, may be a useful predictor of SBI and form part of future PA

evaluations and clinical decision-making.
Commercial Relationships: Naoko Aizawa, None; Hiroshi Kunikata, None; Masataka Kudo, None; Shunji Mugikura, None; Fumihiko Nitta, None; Hidetoshi Takahashi, None; Toru Nakazawa, None

146 - P37-6

Role of the carrier frequency in Doppler OCT

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Purpose: To implement direction sensitive Doppler OCT system to study flows with complex geometry by registering Doppler shift of the carrier itself.
Methods: The experimental results were obtained using Michelson interferometer with scanning reference arm. In linearly scanning optical delay lines classical Doppler shift of the carrier frequency appears due to the moving mirror. Increasing the scanning velocity of the mirror leads to increase in the carrier frequency, up to tens of megahertz. Hence it is not possible to detect the Doppler frequency shift having the order of a few kilohertz, corresponding to the velocities of a few millimeters per second. In our experiment the frequency of 1 kHz corresponds to the velocity of about 1.5 mm/s. Spectral Domain (SD) and Swept Source (SS) OCT enable fast data acquisition, but do not care about dispersion compensation. Application of two acousto-optics modulators in conjunction with SD-OCT and SS-OCT can shift the carrier to the lower part of the spectrum, but can't compensate dispersion in the dense tissue. Double pass Rapid Scanning Optical Delay (RSOD) line on the basis of the diffraction grating and the scanning mirror placed in the Fourier plane can do both. RSOD allows compensation of envelope dispersion and decoupling the group and phase delay. To realize wide range tuning of the carrier certain modifications were added in the RSOD. They enable simultaneous application of two wavelengths (1.3 and 1.5 nm) to perform differential imaging as well as data processing in frequency domain. Tuning of the carrier in the lower part of the spectrum allows registration of Doppler shift of the carrier and enables to study flows with complex geometry. Developed data processing algorithm can be implemented in low coherence systems and in ultrasound imaging devises.
Results: Application of the modified RSOD allows decoupling the group and the phase delay and shift the carrier frequency to the range of 20 to 30 kHz. This provides an opportunity to perform the measurements in the region with the minimal noise, between the decreasing noise and the increasing white noise, and allows recording the Doppler shift of the carrier frequency with good enough accuracy $\Delta V/V \sim$ 5-10 % in the data processing mode.
Conclusions: Application of the modified RSOD gives an opportunity to compensate dispersion, use two wavelengths simultaneously, move the carrier to 20-30 kHz range with minimal noise and highest signal to noise ratio.
Commercial Relationships: Tatiana Avsievich, None; Anton Potlov, None; Sergey Proskurin, None

147 - P38-7

Effects of alpha-1-antitrypsin administration in a murine model of diabetic retinopathy

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Purpose: Diabetic retinopathy is the most common cause of blindness in the working age population and involves inflammatory processes. The classic tools available to the ophthalmologist to limit the inflammatory reaction are circumscribed to corticosteroids and non-steroids anti-inflammatory drugs. Corticosteroids prolonged application generates undesirable local and systemic effects. In this work, we seek to assess the effect of alpha-1-antitrypsin (AAT), an endogenous anti-inflammatory agent, on diabetic retinopathy.
Methods: Rats Wistar with four weeks of postnatal life, received an intraperitoneal injection of streptozotocin (STZ) 90mg/kg. Animals that after 1 week of application of STZ had higher blood glucose 200 mg/dl were classified as diabetic. In the experimental group, an intravitreal injection of AAT (1 μl Prolastin C) was applied at 25 week of life. Intravitreal buffer was applied to the contralateral eye in the same group of animals. Western blot expression of NFkB, MMP-9 and MMP-12 was analyzed in samples of retinal tissue. Zymography assays were performed to evaluate the activity of MMP-2 and MMP-9. Nondiabetic animals receiving treatment were used as control. The three groups made up of 6 animals each were sacrificed at week 26 of life.
Results: No significant differences in the expression of NFkB p50 and p100 from the different experimental conditions were observed. Diabetic retinas treated with AAT showed less gelatinase MMP-9 activity and increased expression of MMP-12 compared to diabetic retinas of eyes treated with buffer. Comparison with nondiabetic animals treated with or without AAT showed not significant differences.
Conclusions: Our results suggest that the expression of MMP-9 and MMP-12 would be modulated by AAT, being important for the remodeling of the extracellular matrix, a process present in the development of diabetic retinopathy. Understanding the mechanisms that regulate this process will allow us to identify new therapeutic targets and develop drugs that reduce the inflammatory process associated with diabetic retinopathy.
Commercial Relationships: Gustavo Ortiz, None; Juan Salica, None; Lopez Emiliano, None; Jorge Mancini, None; Juan Gallo, None

148 - P39-8

Oxidative Stress in Patients with Diabetic Retinopathy using A Specific Biomarker, 8-Hydroxydeoxyguanosine (8OH-dG)

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Purpose: Several researches suggest the main pathways induced diabetic retinopathy (DR) through oxidative stress(OS), angiogenesis and inflammatory response. 8-hydroxydeoxyguanosine (8-OHdG) is highly sensitive biomarkers for angiogenesis and OS. To evaluate the levels of 8-OHdG in aqueous, vitreous humor, plasma and urine samples from DR patients, the relationship between the stages of DR and the 8-OHdG levels.

Methods: Study groups are 21 patients with diabetes mellitus (DM) and 16 patients without DM, which scheduled to do cataract surgery or vitrectomy or intravitreal injection. DM patients are classified into three DR groups, proliferative DR, non-proliferative DR, and no DR. Samples from blood, urine, aqueous and vitreous humor (0.1~0.5mL for each), will be analyzed by ELISA method. Student *t* test and One-way analysis (ANOVA test) were used for statistical analysis.

Results: Patients with DM had higer 8-OHdG concentration (Mean ± SD) of serum, aqueous/vitreous humor fluid and urine (0.14 ± 0.049, 0.21 ± 0.19, 6.50 ± 7.21) than the controls without DM (0.12 ± 0.029, 0.18 ± 0.10, 5.51 ± 9.86). The levels of 8-OHdG of all 3 kinds of samples had shown in descending tendency by three DR subgroups, PDR, NPDR, and no DR. Among 3 study subgroups, the levels of 8-OHdG of serum were 0.22 ± 0.92, 0.33 ± 0.16, and 0.14 ± 0.42 (p = .021); of aqueous/vitreous humor fluid were 0.51 ± 0.21, 0.18 ± 0.061 and 0.20 ± 0.17 (p = .0002); of urine were 4.06 ± 4.02, 9.53 ± 11.98 and 5.90 ± 8.63 (p = .57).

In additions, the 8-OHdG concentration had statistically significant correlation with comorbidity of DM, hypertension, hyperlipidemia, and nephropathy, but without a statistically significance of patients' age and gender.

Conclusions: The severity of DR may have highly correlate with oxidative stress. The 8-OHdG is likely to be as a sensitive biomarker to predict the exent of oxidative damage to patients with DR. In our study, the levels of 8-OHdG of serum and intraocular fluids have signficant correlation with the severity of DR. So the biological indicator of 8-OHdG concentration of serum is a potential for being used as a reference for DR classification in clinical practice.

Commercial Relationships: Ching-Ju Hsieh, None; Chung-Jun Lai, None; Wei-Chung Lu, None; Lin-Chung Woung, None

149 - P40-9

Cyclosporin A modified intraocular lens prevent posterior capsular opacification in rabbit eyes

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Purpose: To investigate the safety and efficacy of cyclosporine A sustained release from modified intraocular lens in the prevention of posterior capsular opacification (PCO) in rabbits.

Methods: 45 New Zealand albino rabbits undergoing phacoemulsification in their right eyes were randomly and equally divided into three groups. Group A was implanted with the original IOLs, group B was implanted with the PLGA-IOLs(IOLs coated with polylactide-glycoli acid), and group C was implanted with CsA-PLGA-IOL(CsA loaded PLGA-IOL). All the 45 eyes were examined by a slit-lamp microscope. The intraocular pressures were recorded. Anterior chamber flare and aqueous humor cells were graded at different time point after surgery. The concentrations of CsA in the aqueous humor and blood were determined by high performance liquid chromatography. Anterior segment tissue was histologically examined. Wet posterior capsules were weighed. PCO was graded Six months later.

Results: The mean concentrations of CsA in group C at 2 hours,1d,3d,7d,14d,30d,60d after operation were 11.47 ± 2.42mg/L, 10.30 ± 2.15mg/L, 6.71 ± 1.45mg/L, 4.81 ± 1.16 mg/L, 6.11 ± 0.84 mg/L, 2.53 ± 0.77 mg/L, 0.86 ± 0.28mg/L. The concentrations of CsA in blood were undetectable. During the early days after operation, the reactions of the anterior chamber in group A and B were more severe than group C. The initial appearance of PCO in group C was much later than in the other two groups, and the grade of PCO in group C was much lower than the other two groups. The mean weights of wet posterior capsules in group A and B were 312.86 ± 52.91mg,310.64 ± 62.42mg,much heavier than that of group C(56.93 ± 24.24mg).Histological observation showed that there was remarkably less accumulation of lens materials on the posterior capsules in group C than in the other two groups. No toxic actions were found in intraocular tissues in group C.

Conclusions: Our study suggest that Cyclosporin A modified intraocular lens can effectively and safely prevented the formation and development of posterior capsular opacification in rabbit eyes for a relatively long period.

Commercial Relationships: Teng He, None; Tian Fang, None; Zhang Hong, None

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150 - P41-10

Controlled Release of Antiglaucoma Drugs from Injectable Hydrogel Depots

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Purpose: Due to low bioavailability of eye drops, intracameral administration is considered to be a more efficient route that allows direct entry of therapeutic agents into the intraocular cavity and achieves higher drug levels at the site of action. This study aims to investigate the role of gel strength of gelatin in the design of an injectable depot formulation.

Methods: By means of carbodiimide chemistry, the carboxylic end-capped poly(*N*-isopropylacrylamide) brushes were grafted onto aminated gelatin molecules with different Bloom values (i.e., degrees of gel strength). The obtained in situ gelling systems were characterized by thermo-responsiveness, degradability, drug release profile and activity, and cytocompatibility to determine their potential use as pilocarpine carriers. The therapeutic efficacy was also evaluated in a rabbit glaucoma model. All animal procedures were approved by the Institutional

Review Board of Chang Gung University and were carried out in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.
Results: With increasing Bloom value of gelatin, the lower critical solution temperature of in situ gelling systems significantly increased, indicating faster temperature triggered gelation of the copolymeric drug carriers. The hydrogel samples with higher gel strength had a slower degradation rate, thereby exhibiting a sustained drug release pattern over a prolonged period of 6 weeks. In addition, the end-point concentration of released pilocarpine remained at therapeutic level. All the studied biomaterial hydrogel depots were compatible with cultured corneal endothelial and lens epithelial cells. Results of in vivo tests showed that a topically applied eye drop fails to relieve the symptoms associated with glaucoma. In contrast, the pilocarpine delivered by copolymer carriers with higher Bloom values could more effectively improve ocular bioavailability and extend the pharmacological responses (i.e., miosis and intraocular pressure lowering effect and preservation of corneal endothelial cell density).
Conclusions: We have tested the hypothesis that intracameral administration of antiglaucoma medications using injectable hydrogel depots gives an increase in performance over eye drop instillation. The present study suggests that the gel strength of gelatin may have a profound influence on the characteristics of biodegradable in situ gelling systems for glaucoma therapy.
Commercial Relationships: Jui-Yang Lai, None

151 - P42-11

Fabrication and evaluation of voriconazole solid lipid nanoparticles for effective ocular delivery

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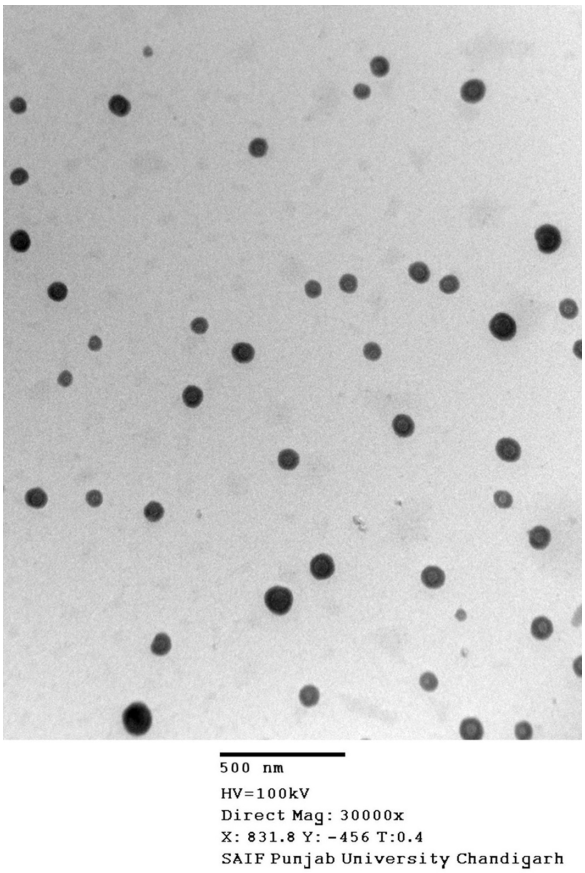
Purpose: Preparation of voriconazole (VCZ) solid lipid nanoparticles (SLNs) for effective ocular delivery for the treatment of fungal keratitis.
Methods: VCZ SLNs were prepared by solvent emulsification technique using Compritol (lipid), Pluronic F-68 (surfactant) and sodium taurocholate (co-surfactant). Characterization of SLNs was performed by size measurement, zeta potential, in-vitro release, ex-vivo corneal permeation studies, in-vitro antifungal activity and irritation study.
Results: Particle sizes of SLNs were found below 300 depending upon lipid/S_{mix} ratio with good zeta potential. Entrapment efficiency of SLNs was found between 40-60% with sustained in vitro drug release (>70% in 12h). The ex-vivo corneal permeation studies exhibited good ocular permeation of VCZ from SLNs when compared with drug suspension. Further, in-vitro antifungal activity exhibited the potential of VCZ SLNs. Corneal irritation study confirmed the safety of VCZ formulation.
Conclusions: The sustained release property with good corneal permeation of VCZ from SLNs encourages its application for in-vivo studies and hence could be proposed as an effective carrier for ophthalmic administration.
Commercial Relationships: Rakesh Kumar, None; Vivek Sinha, None



TEM image of VCZ SLNs

152 - P43-12

Evaluation of Safety and Tolerability of Conjunctival Ring

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Purpose: The conjunctival ring is a novel device for drug delivery to the posterior segment of the eye. Previously, we reported that the conjunctival ring efficiently delivers the antibiotics to the retinal and choroidal tissues compared with medicated conventional contact lens (Shikamura Y. et al, ARVO 2010). In this study, we evaluated the safety of the conjunctival ring containing dexamethasone sodium phosphate (DSP) in mice and rabbits.
Methods: All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Conjunctival rings containing 5% DSP or vehicle (borate buffer) were placed on right and left eyes of C57BL/6J mice, respectively (n=6 each). As

a control, contact lenses containing vehicle were used (n=6). Twenty-four hours later, corneal fluorescein staining was graded according to the McDonald-Shadduck scoring system, ranging 0 to 4. Similarly, conjunctival rings immersed in 2% DSP, 0.2% DSP, or vehicle solution were placed on the eyes of New Zealand white rabbits for 12 hours per day (n=4 each). As a control, 0.1% dexamethasone metasulfobenzoate sodium was topically administered to the eyes of rabbits 4 times a day (eye-drop group, n=4). Eight days after, the ocular injury was scored with the McDonald-Shadduck method.

Results: In mice, corneal fluorescein staining scores were 1 or less in each eye in all groups, and there were no significant differences between conjunctival ring groups and contact lens group. In rabbits, the scores were 1 or less in all but 1 eye in the groups. There were no significant differences between the DSP groups and vehicle group. Also, there was no increase of ocular injury in the conjunctival ring groups compared with the eye-drop group, in which all eyes were scored as 0.

Conclusions: No remarkable side effect of the conjunctival ring was observed in both animal experiments. The current data suggest that conjunctival ring is a safe device for intraocular drug delivery.

Commercial Relationships: Satoshi Kinoshita, None; Kousuke Noda, None; Ikuyo Atsumi, Senju Pharmaceutical Co., Ltd. (E), Senju Pharmaceutical Co., Ltd. (P); Haruka Obata, SEED Co., Ltd. (E), SEED Co., Ltd. (P); Toru Matsunaga, SEED Co., Ltd. (E), SEED Co., Ltd. (P); Takeshi Ohguchi, None; Atsuhiro Kanda, None; Susumu Ishida, Senju Pharmaceutical Co., Ltd. (F)

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153 - P44-13

Ocular Tissue Distribution of Unoprostone and Retinal Toxicity after Transscleral Administration using a Sustained Drug Delivery Device

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Purpose: To evaluate the tissue distribution of unoprostone ispropyl (UNO) and retinal toxicity after implantation of a sustained drug delivery device on the sclera of rabbits.

Methods: The device consists of a reservoir, controlled-release cover, and drug formulation, which were made of photopolymeized poly(ethyleneglycol) dimethacrylates (PEGDM). UNO, a prostone and BK channel activator for antiglaucoma eyedrops marketed in Japan, was loaded in the device at a concentration of 500 mg/mL in PEGDM. High-performance liquid chromatography was used to evaluate the release amount of UNO *in vitro*. The UNO metabolite, unoprostone-free acid (M1), concentrations in the aqueous humor, retina, choroid, and plasma were determined by liquid chromatography-tandem mass spectrometry (LC/MS/MS) at 4, 12, and 24 weeks after implantation in rabbits. Retinal toxicity was evaluated by electroretinogram.

Results: The UNO released from the device *in vitro* showed zero-ordered kinetics for 12 weeks, then the release gradually decreased to 24 weeks. The UNO concentrations in the retina were comparable to those after topical administration of 0.12% UNO eye-drop. However, the UNO concentration in the plasma was 40 times lower than that after the UNO eye-drop administration. No substantial toxic reactions were observed by electroretinogram.

Conclusions: The device could be a useful carrier for intraocular sustained delivery of UNO without producing severe retinal toxicity.

Commercial Relationships: Nobuhiro Nagai, None; Hirokazu Kaji, None; Matsuhiko Nishizawa, None; Takahito Imagawa, R-tech Ueno Ltd. (E); Akiko Morikawa, R-tech Ueno Ltd. (E); Toru Nakazawa, None; Yukihiko Mashima, R-tech Ueno Ltd. (E); Toshiaki Abe, R-tech Ueno Ltd. (F)

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154 - P45-14

Specific Delivery of Systemically Injected Compounds to the Retina Using Artificially Expressed Retinal Receptors

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Purpose: Intravitreal (IV) injection is a simple method to deliver drugs and nanoparticles specifically to the retina. However, frequent IV injection can cause severe complications such as retinal detachment and endophthalmitis. Here we suggest a novel strategy to deliver intravenously injected compounds to the retina specifically using the azide-DBCO reaction (copper free click chemistry).

Methods: Previously, we reported an optimized liposome which can deliver the desired compound to whole retinal layers. This time, we conjugated DBCO on phospholipids of optimized liposomes and performed intravitreal injection on the left eye (liposomes without DBCO were injected into the right eye as a control) of wild type mice (C57BL6) and choroidal neovascularization (CNV) induced mice. Distribution of DBCO on the retina was evaluated using histological analysis. Three days after IV injection, free azide labeled with Cy3 or liposomal azide was injected intravenously, and delivery efficiency of the azide complex to the retina was analyzed.

Results: DBCO was expressed on whole retinal layers of wild type mice and the CNV model after intravitreal injection of DBCO conjugated liposomes. A greater amount of free azide injected systemically was detected on the DBCO liposome treated retina than the control retina in wild type mice. In the case of the CNV model, both free azide and liposomal azide were delivered in larger quantities to the DBCO liposome treated retina than the control retina.

Conclusions: Copper free click chemistry using azide and DBCO is a safe, specific, and biocompatible reaction. DBCO could be expressed on whole retinal layers by IV injection of DBCO conjugated liposomes, and thus systemically

delivered azide could be delivered more efficiently to the DBCO liposome treated retina. We believe that this work offers a promising pathway for reducing the frequency of intravitreal injection by enhancing systemic delivery efficiency to the retina.
Commercial Relationships: Junsung Lee, None; Ji-Ho Park, None

155 - P46-15

Tyrosine-mutated AAV2 mediated BDNF rescued inner retina in rat retinal ischemic injury mode

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Purpose: The loss of retinal ganglion cell (RGC) has been reported in ischemia, glaucoma, diabetic retinopathy, age-related macular degeneration, and optic neuritis. Because RGCs communicate the retina with higher visual centers, loss of RGCs inevitably encompass the impairment of visual function. Brain-derived neurotrophic factor (BDNF) is a secreted protein of the "neurotrophin" family of growth factors, which is related to the canonical "nerve growth factor", NGF. In this study, we investigated the neuroprotective effects of BDNF in rat ischemia reperfusion injury using triple mutant adeno-associated viral (AAV) type 2 vector, which has the excellent ability of transduction by intravitreal injection.
Methods: We generated triple mutant AAV type 2 vector encoding BDNF (scAAV2/BDNF) and GFP (scAAV2/GFP). Sprague–Dawley rats were intraviteally received 3 µl of scAAV2/BDNF or scAAV2/GFP (1×10^{13} gv/ml). Two weeks later, retinal ischemia was induced in rats by raising intraocular pressure (IOP) to 110 mm Hg for 60 mins. One week after retinal ischemia, we examined the expression of BDNF in RNA level and protein level. In addition, the neuroprotective effects of BDNF were evaluated by determining the preservation of the inner retina thickness.
Results: High level of RNA expression was detected in scAAV2/BDNF injected mice (Ratio; No treatment; 1, Control; 0.72, AAV-GFP; 2.51, AAV-BDNF; 8.41). ELISA analysis showed efficient BDNF protein was observed in scAAV2/BDNF injected mice (No treatment; 161.2 ± 68.6 pg/mg Protein, Control; 176.6 ± 100.3 pg/mg Protein, AAV-GFP; 69.8 ± 9.9 pg/mg Protein, AAV-BDNF; 972.8 ± 9.9 pg/mg Protein). Significantly improvement of the retinal thickness was detected in scAAV2/BDNF injected mice compared with scAAV2/GFP injected mice ($p<0.01$) (No treatment; 45.5 ± 3.0 µm, Control; 26.6 ± 4.0 µm, AAV-GFP; 30.1 ± 3.7 µm, AAV-BDNF; 45.4 ± 4.2 µm).
Conclusions: Triple mutant AAV type 2 vector mediated BDNF extremely protected rat retina from ischemia-reperfusion injury. *In vivo* gene therapy using BDNF appears to be a feasible approach to the clinical management of neuroprotection in conditions such as glaucoma and retinal artery occlusion.
Commercial Relationships: Tsutomu Igarashi, None; Koichi Miyake, None; Maika Kobayashi, None; Noriko Miyake, None; Osamu Iijima, None; Kenji Nakamoto, None; Yukihiko Hirai, None; Takashi Shimada, None; Takashi Okada, None; Hiroshi Takahashi, None

156 - P47-16

Lineage negative stem cell transplantation in a mouse model of transient retinal ischemia-reperfusion injury

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Purpose: There is no treatment available for ischemic disorders of retina resulting in visual impairment. Stem cell intervention in treatment of retinal ischemia has not been attempted so far. Thus, the aim of our study was to test the efficacy of mouse bone-marrow derived lineage-negative stem cells in pterygopalatine artery ligation induced retinal ischemia.
Methods: All the experiments conducted were approved by the Institutional ethical committee. Age-matched and sex-matched C57BL/6J mice were subjected to PPA ligation. The external carotid artery and the pterygopalatine artery were ligated for 3.5 hours which reduces the ocular blood flow. The cerebral blood flow was assessed using the Laser Doppler blood flow meter and the retinal blood supply was measured using fluorescein angiography. The retinal damage was assessed after 5 days of reperfusion against contralateral eye using histological, molecular and immunohistochemical techniques. The stem cells were isolated from mouse bone-marrow and lineage negative population was separated using magnetic associated cell sorter (MACS). These cells were further characterised for Sca-1, CD34 and CD117 population by flow-cytometry. The stem cells were isolated from mouse bone marrow and lineage-negative population was transplanted intravenously 24 hours after injury. The efficacy of these transplanted cells was assessed at day 10 post-transplantation through immunofluorescence and real-time PCR for neurotrophic factors and cell markers.
Results: The histological damage was observed as thinning of retinal layers in the cryosections. The immunohistochemical analysis demonstrated an increase in GFAP expression in retinal cryosections as compared to the control sections, whereas decrease was observed after 10 days of transplantation. Similarly an increase in GFAP mRNA levels was manifested in real-time PCR. The expression of stem cell marker Nestin, BDNF, FGF2 was found to be increased after 3.5 hrs of PPA ligation and 5 days of reperfusion in the ischemic retina and as well as after transplantation immunohistochemically and in real-time PCR.
Conclusions: The study shows neuroprotective effect of lineage-negative population of stem cells in the retinal ischemia and presents a useful model for validating therapies for ischemic disorders of the retina in future.
Commercial Relationships: Gillipsie Minhas, None; Akshay Anand, None

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157 - P48-17

Protective Effect of Genistein on Glucose Induced Toxicity in Cultured Human RPE cells

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Purpose: Genistein, an isoflavinoid has been shown to possess many biological activities including anti-inflammatory, antioxidant and anti-angiogenic property. It has been shown to be protective in dampening diabetes induced retinal inflammation in vivo. Therefore, the purpose of the present study is to investigate the effect of genistein on glucose- induced toxicity in cultured human RPE cells (ARPE-19).

Methods: ARPE-19 cells were challenged with normal glucose (NG 5mM) and high glucose (HG1 25 mM & HG2 50 mM) concentrations under normoxia with or without genistein (20 µM) for 24 hours. The mRNA expression of aldose reductase (ALR) and VEGF were measured by real-time PCR using SYBR green. Inhibitory effects upon ALR activity were performed. The VEGF levels of cell supernatant were estimated by sandwich ELISA. Cellular viability and mitochondrial function upon genistein treatment were assessed using dye exclusion method and MTT assay respectively.

Results: Genistein at the studied concentration showed 93% of cell viability and no marked toxicity was observed on cell growth. ARPE-19 cells challenged with HG1 and HG2 showed (2.32) and (2.48) fold increase in ALR expression as compared to NG. Significant increase in $VEGF_{165}$expression was observed in ARPE-19 cells with HG1 and HG2 as compared to NG. The genistein treated cells significantly reduced the mRNA expression of both ALR and $VEGF_{165}$. No significant increase in VEGF level was observed in ARPE-19 cell supernatant with HG1 and HG2 (220.68 ± 5.24 and 228.96 ± 7.19 pg/ml) as compared to NG (220.30 ± 2.04 pg/ml) however significant reduction was observed in treated of genistein in HG1 AND HG2 (155.51 ± 9.33 and 122.85 ± 4.76 pg/ml). Also ALR activity significantly reduced in genistein treated cells when compared with HG1 and HG2 concentrations.

Conclusions: The results of the present study clearly demonstrate that genistein protects RPE cells from glucose toxicity. genistein could be a prospective potent agent for treating complications linked with diabetes mellitus, such as diabetic retinopathy.

Commercial Relationships: Shirish Dongare, None; Rajani Mathur, None; S Senthilkumari, None; R Sharmila, None; S K Gupta, None; Sushma Srivastava, None; Dr. Rohit Saxena, None
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158 - P49-18

Green tea extract reduces inflammation and restores retinal pigment epithelial cell dysfunction in a sodium iodate rat model

Tsz Kin Ng [1] Jian Xiong Wang [1] Yaping Yang [1] Kai On Chu [1] Sun On Chan [2] Chi Pui C. Pang [1]

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Purpose: Selective retinal pigment epithelium (RPE) degeneration by sodium iodate is a well-established oxidative stress animal model. Green tea extract has been shown to possess anti-inflammatory and anti-oxidative effects in different disease models. Here, we hypothesized that green tea extract could protect the sodium iodate-induced RPE degeneration in rats. This study aims to determine the protective effect and to identify the mechanism of green tea extract against the sodium iodate-induced RPE degeneration.

Methods: Sprague-Dawley rats were treated with single intravenous injection of sodium iodate (40 mg/kg) or saline (control). For treatment, the sodium iodate-treated rats were fed intragastrically with green tea extract (550 mg/kg) or saline. The eyeballs were enucleated and fixed at 14 days after sodium iodate injection. Markers for RPE (RPE-65), photoreceptors (Rhodopsin), macrophages (CD68), tight junction (ZO-1) and apoptosis (caspase-3) were evaluated by immunohistochemistry analysis. The study protocol was approved by the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong, which is in accordance with the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Results: The sodium iodate-treated rats showed the photoreceptor rosettes along the whole retina. RPE65 expression was drastically reduced in RPE cells of sodium iodate-treated rats. Moreover, ZO-1 was also downregulated in both RPE and photoreceptor cells. In contrast, caspase-3 and CD68 expressions were increased in the outer segment of photoreceptor cells after sodium iodate treatment. In reverse, the disorganization of outer nuclear layer was not observed after green tea extract treatment. Furthermore, RPE65 and ZO-1 expressions in RPE were also restored. In addition, caspase-3 and CD68 expressions were reduced in the outer segment.

Conclusions: Sodium iodate induces photoreceptor cell disorganization, inflammation and apoptosis through RPE disintegration and dysfunction. Green tea extract treatment restored the RPE function and integrity as well as reduced photoreceptor cell death and disorganization. In addition, green tea extract also reduced the sodium iodate-induced retinal inflammation. Our results demonstrated the therapeutic effect and the possible mechanism for green tea extract against sodium iodate-induced retinal changes.

Commercial Relationships: Tsz Kin Ng, None; Jian Xiong Wang, None; Yaping Yang, None; Kai On Chu, None; Sun

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Visual Neuroscience - Poster

159 - P66-1

Multifocal electroretinogram in Diabetic Retinopathy

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Purpose: To observe the prognostic prediction ability of multifocal electroretinogram (mfERG) in diabetic retinopathy (DR)
Methods: This prospective study was carried out on 100 consecutive patients of DR at its different stages. All subjects underwent full ophthalmic assessment. DR and Diabetic macular edema (DME) was be defined by the clinical appearance. Visual Evoked Response Imaging System (VERIS ™), electro diagnostic imaging inc. CA, USA was used to do the investigation in dilated eyes. mfERG response were recorded simultaneously from both eyes. The first-order mfERG response namely the P1 latency & P1 amplitude were analyzed. Statistical analysis was carried out using SPSS 18.0.
Results: The correlation coefficient between mfERG amplitude and logMAR of visual acuity is statistically significant (p value of 0.000068). There is no statistically significant correlation between mfERG latency and logMAR of visual acuity (p value is 0.996). Mean mfERG amplitude is 34.21 ± 17.77 without DME and 24.05 ± 10.88 with DME. This difference is statistically significant (p value of t – test is 0.027). Mean mfERG P1 latency is 32.26 ± 15.50 without DME and 29.61 ± 13.89 with DME. There is no statistically significant difference between them (p value of t – test is 0.543). Mean mfERG amplitude is 28.53 ± 14.51 for NPDR cases and 31.38 ± 14.16 for PDR cases. No statistically significant difference between them (p value of t – test is 0.614). Mean mfERG P1 latency is 31.42 ± 13.96 for NPDR cases and 30.31 ± 20.22 for PDR cases. There is no statistically significant difference between them (p value of t – test is 0.849). Present study shows statistically significant correlation of mfERG amplitude with visual acuity logMAR and DME, although no correlation with type of diabetic retinopathy.
Conclusions: The present study shows that mfERG can be an useful tool for prognostic prediction in diabetic retinopathy.
Commercial Relationships: Satyen Deka, None

160 - P67-2

Systematic and local responses of modified volvox Channelrhodopsin-1 gene therapy

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Purpose: Our originally developed modified *Volvox*-derived channelrhodopsin1, mVChR1, has a broader, red-shifted action spectrum that is useful for restoring vision. We previously reported that the transduction of the mVChR1 gene into the retinal ganglion cells could restore the visual function in the genetically blind rats. The purpose of this study is to reveal the influence of exogenous expression of mVChR1 to systemic and local responses.
Methods: Adeno-associated virus(AAV) vector encoding mVChR1 gene fused with venus was intravitreously injected into both eyes of aged dystrophic Royal College of Surgeons (RCS) rats. As a control, phosphate buffered saline (PBS) or AAV vector encoding venus was injected. Peripheral blood was collected from caudal vein at pre- and post-injection at 1, 2, 4, 6 and 10 months. Blood test and biochemical examination were performed. Visually evoked potentials (VEPs) were periodically recorded at 9 months after the injection. After 10 months of injection, every organ was enucleated and RNA was isolated. Dissemination of the transduced gene to organs was studied by polymerase chain reaction. Histological study was also performed.
Results: AAV-mVChR1 injection recovered the light response against Blue (4865nm) and Green light (525nm) in RCS rats, whereas RCS rats with PBS or AAV-Venus injection recorded no response. To study the dissemination of the transgene to organs, the expression was analyzed. Expression of the transgene was no detectable in all organs except for retina. The results of the blood test indicated that severe dysfunction in organs did not elicited by the transduction of mVChR1. Histological studies also showed no inflammatory responses such as leukocyte migration that observed in LPS-injected group.
Conclusions: The administration of mVChR1 gene to the eye appears well tolerated. These data demonstrated mVChR1 transgene into retina with AAV vector is an applicable method for restoring vision.
Commercial Relationships: Eriko Sugano, None; Kitako Tabata, None; Maki Takahashi, None; Namie Murayama, None; Hiroya Shimizu, None; Masatoshi Sato, None; Fmiaki Nishiyama , None; Makoto Tamai, None; Hiroshi Tomita, None
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161 - P68-3

Visual properties of photoreceptor degenerated rat with dual channelrhodpsin genes

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Purpose: We previously reported that visual responses in blind rats were restored by gene transfer of *Chlamydomonas* channelrhodopsin-2 (ChR2). However ChR2-expressing rats with the photoreceptor degeneration could only detect wavelengths less than 540 nm because of the limitation of the ChR2 action spectrum. We generated modified *Volvox* channelrhodopsin-1 (mVChR1) which had a broad spectrum. We showed that the blind rats transduced mVChR1 gene could record visually evoked potentials with stimulation at 450-600 nm and optomoter responses were elicited with the all of color stripes, blue, green and red. To investigate effects dual gene expression (ChR2 and mVChR1) on the visual function of blind rats, we transduced mVChR1 gene into Thy-I ChR2 transgenic rat with the photoreceptor degeneration and recorded VEPs using various wavelengths of

Methods: The experiments were conducted on 3-month-old Thy-I ChR2 transgenic rats. The rats were kept under cyclic light conditions (12 h on/off). At least 2 weeks before AAV-mVChR1 vector administration, photoreceptor degeneration (PD) was induced by intraperitoneal injection of N-methyl-N-nitrosourea (MNU; 60mg/kg). Then AAV-mVChR1 vector was injected into the right eye to transduce mVChR1 gene. The contralateral eye was used as a control. Visual properties were evaluated by the recording of VEPs with a stimuli of various wavelengths LED.

Results: The thickness of photoreceptor layer was gradually shortened by MNU application and photoreceptor cells disappeared at a week after the MNU-injection under the OCT imaging. Amplitudes of ERGs (a- and b-wave) were also decreased by the PD. However in the VEP recordings, PD-Thy-I ChR2 transgenic rats still responded to only a stimuli with blue LED. After the transduction of mVChR1, mVChR1-transduced eyes became to respond to a stimuli with all of the color LED.

Conclusions: The action spectrum of ChR2 peaks at 460 nm. In comparison to ChR2, mVChR1 has a broader spectrum and peaks at 550nm. Transduction of dual channelrhodopsin genes possibly give the majority of the visual spectrum to blind patients.

Commercial Relationships: Hiroshi Tomita, None; Eriko Sugano, None; Kitako Tabata, None; Masatoshi Sato, None; Maki Takahashi, None; Kei Sannohe, None; Namie Murayama, None; Fmiaki Nishiyama , None; Takehiko Saito, None; Makoto Tamai, None

Support: Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (nos. 24390393 and 25462747, Translational Research Network program) and the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (no. 10-06, NIBIO).

162 - P69-4

Isolated cilioretinal artery occlusion treated with prostaglandin E1

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Purpose: To report two cases of isolated cilioretinal artery occlusion that treated with systemic prostaglandin E1 (PGE1) according to the standard protocol.

Methods: Case report. The protocol included intravenous injection of 40 μg PGE1 twice daily (80 μg per day) for 5 days then oral PGE1 three times daily (30 μg per day) for at least 1 month. The best-corrected visual acuity (BCVA) and visual field testing with Goldman perimeter were recorded before and after the therapy.

Results: Case 1. A 62-year-old woman referred to our hospital due to sudden visual loss in her right eye. She had a history of hypertension, hyperlipidemia, and percutaneous coronary intervention for acute myocardiac infarction 7 year ago. Fundus examination of affected eye revealed an isolated retinal edema that localized in the perfusion area of cilioretinal artery; two plaques were observed in the column of cilioretinal artery. She had started systemic PGE1 3 hours after the onset. BCVA of 0.6 at baseline was improved to 1.2 until 1 week; paracentral scotoma at baseline improved until 1 month. Case 2. A 51-year-old woman referred to our hospital due to sudden visual loss in her left eye. Although she had no remarkable medical history, she was found an untreated severe systemic hypertension (250/150 mmHg) at the referral to our hospital. Fundus examination of affected eyes revealed retinal vessel sclerosis, retinal hemorrhages, cotton wool spots, and retinal edema in the perfusion area of cilioretinal artery; compression by dense cotton wool spots was likely a cause of cilioretinal artery occlusion in this case. She had started systemic PGE1 24 hours after the onset. BCVA of 0.8 at baseline was improved to 1.2 until 1 week leaving paracentral scotoma observed at baseline unchanged until 1 month.

Conclusions: Though each case was caused by different mechanism of artery occlusion, i.e., embolic or hemodynamic, recovery of visual acuity was observed after the PGE1 monotherapy in both cases. The difference in time to treatment and mechanism of occlusion might explain a reason for difference in visual field improvement in our cases.

Commercial Relationships: Ichiya Sano, None; Masaki Tanito, None; Yoshifumi Ikeda, None; Yasuyuki Takai, None; Kazunobu Sugihara, None; Akihiro Ohira, None

163 - P70-5

Case of binasal hemianopia determined to be psychogenic by multifocal visual evoked potentials

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Purpose: Psychogenic visual disturbances are difficult to diagnose because they are 'real' for the patient and can be detected by subjective testing procedures. Multifocal visual evoked potentials (mfVEPs) are used to objectively evaluate the functioning of focal areas of the visual fields. The purpose of this study was to analyze the visual fields in a patient with binasal hemianopia using mfVEPs.

Methods: A 36-year-old woman who complained of visual disturbances was analyzed by Goldmann perimetry, static automated perimetry, and mfVEPs. mfVEPs were recorded with the VERIS Junior Science recording system (Mayo, Inazawa, Japan). The mfVEPs were elicited by pattern reversal stimuli with a dart board pattern. The pattern consisted of 60 elements subtending approximately 20 degrees. The frame rate was 75 Hz, and the pseudo-random m-sequence stimulation was presented at 2^{15}-1. Each mfVEP was divided into eight equal segments with a total recording time of about 8 minutes. Responses from the 60 sites were divided into the superior and inferior temporal quadrants, and the superior and inferior nasal quadrants. In each quadrant, 15 responses were grouped and averaged.

Results: Initial examination showed that her visual acuity was 24/20 OU. The intraocular pressure was 14 mmHg OD and 16 mmHg OS (CT-80; Topcon,Tokyo,Jpn). Slit-lamp examination showed that the anterior segment of both eyes was normal, and ophthalmoscopy showed the fundus of both eyes was normal. Both Goldmann perimetry and static automated perimetry showed binasal hemianopia. Magnetic resonance imaging (MRI) of the brain including the optic chiasm showed no abnormalities. From these findings, she was diagnosed with binasal hemianopia. However, the amplitudes and latencies of the mfERGs were within normal limits in all quadrants in both eyes. She was followed without treatment, and 3 years later, the subjective symptoms and the visual field defects disappeared.

Conclusions: Based on the clinical findings, we diagnosed this patient with psychogenic visual disturbances, and we conclude that mfVEPs were invaluable in making this diagnosis.

Commercial Relationships: Mariko Yamashita, None; Eiichi Yukawa, None; Tomo Nishi, None; Nahoko Ogata, None

Visual Psychophysics/Physiological Optics - Poster

164 - P50-1 Withdrawn

The effects of a near tasking on ocular dominance in different viewing conditions

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Purpose: To investigate whether ocular dominance (OD) can be changed, in terms of refraction, under different binocular viewing conditions.

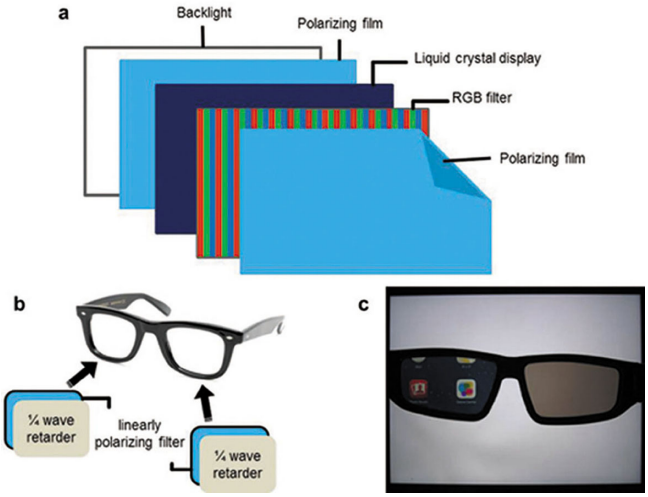
Methods: Fourteen subjects without any organic ophthalmologic disease were required to play video games on tablet computers for one hour while wearing polarizing glasses. Five measurements of far-distance refraction were taken in each eye with a binocular open-view autorefractor (WR-5100, Grand Seiko) before and 30 and 60 min after the near tasking. For this tasking, a modified liquid crystal display screen and polarizing glasses with different polarizing filters were used in given viewing conditions. The measured eyes were divided into 3 groups, binocularly used eyes (BUE), monocularly used eyes (MUE) and monocularly non-used eyes (MNUE). Intra-group refraction differences were compared among the above 3 time points (Paired t-test), and inter-group differences at each time point were compared. (ANOVA)

Results: In BUE, no statistically significant difference of refraction between pre- and post-tasking was found ($p>0.05$). In MUE, significant differences of refraction were found among the 3 time points ($p<0.05$). In MNUE, there were no significant changes occurred during 1 hour of tasking ($p>0.05$) except for a slight hyperopic shift within the first 30 min ($p=0.010$). More marked myopic shifts in MUE than in BUE and MNUE were found at 60 min after tasking ($p=0.016$).

Conclusions: OD may be reversed when monocular visual input is blocked for a certain time. Further quantitative studies about the magnitude of OD for adults in monocular use are needed.

Commercial Relationships: Ruiqing Wang, None; Tomoya Handa, None; Hitoshi Ishikawa, None; Kimiya Shimizu, None; Guanfang Su, None

Support: A-STEP (H25 Sen Ⅱ -566)



(a) shows the liquid crystal display (LCD) architecture and modification, exfoliation of the anterior polarizing film from the LCD. (b) shows the production of polarizing glasses, circularly polarizing filters, comprised of an 1/4 wave retarder and a linearly polarizing filter, were attached to the glasses. (c) shows one of three viewing conditions. The left eye can perceive video information because the left polarizing filter matches the output of the LCD screen, but the right eye can perceive only white backlights without any information because the right polarizing filter does not match the screen output.

165 - P51-2

Implantation of Hydrogel Corneal Shape Changing Inlay in Pseudophakic Subjects

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Purpose: To provide pseudophakic patients with improved near vision by implanting a hydrogel corneal inlay with and without concurrent LASIK correction.

Methods: 35 monofocal pseudophakic subjects were implanted with a 2.0 mm diameter hydrogel corneal inlay (ReVision Optics, Inc.)* under a femtosecond corneal flap, in the non-dominant eye. Pre-op MRSE varied between-1.13 to +1.00 D. When necessary, LASIK was used in the non-dominant eye targeting +0.75 D and targeted emmetropia in the dominant eye. Twenty-two subjects had an inlay-only procedure in the non-dominant eye, of which four had LASIK in the dominant eye for residual refractive error. Thirteen subjects had an inlay and LASIK in the non-dominant eye, with seven subjects receiving LASIK in the dominant eye. The Optec® 6500 Vision Tester was used to record visual acuities. Ability to perform everyday tasks (five tasks for each of three distance ranges) without additional visual aid was ascertained using a questionnaire. The study was performed at 3 sites under IRB-approved protocols.

Results: At the six month postoperative exam, 88% of

the patients had an uncorrected near visual acuity of 0.2 logMAR [20/32]or better, with 66% 0.1 logMAR [20/25] or better. On average there was a four line improvement in uncorrected near visual acuity. At day one post implant, uncorrected near VA improved by 2 lines and increased to 4 lines at one week. In the same eyes, 78% of the subjects had an uncorrected distance visual acuity of 0.3 logMAR [20/40] or better, with 69% 0.2 logMAR [20/32] or better. Binocularly, 100% of subjects achieved an uncorrected distance VA of 0.3 logMAR [20/40] or better with 96% at 0.1 logMAR [20/25] or better. 100% of patients were "neutral, satisfied or very satisfied" with overall visual function, with 88% "satisfied or very satisfied". With visual symptoms scoring "moderate, marked or severe", there were no reports of glare or halos, and only 6 % reported dryness issues. Trace haze was observed at the periphery of the inlay in five cases; three resolved with a round of steroids and two remain in treatment.
Conclusions: Implantation of a corneal shape changing inlay with or without concurrent LASIK is potentially an effective tool to improve near vision in bilateral pseudophakic patients.
Commercial Relationships: Hai Yen Tran, Revision Optics (C); Arturo Chayet, Revision Optics (C); Enrique Barragan Garza, Revision Optics (C); Dan Tran, Revision Optics (C)
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Clinical Trail: 3920/QD-BYT

166 - P52-3

The improvement of spoke-wheel pattern foveoschisis in a patient with X-linked retinoschisis treated with topical dorzolamide observed by high-resolution adaptive optics retinal camera

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Purpose: X-linked juvenile retinoschisis (XLRS) is the common inherited retinal dystrophy, with an estimated prevalence at 1:5000 to 1, 25000. Mutations within the retinoschisin (*RS1*) gene are responsible for the disease. Typical clinical features include foveomacular cavities in inner retina and spoke-wheel pattern retinoschisis in their macular area. Topical and oral carbonic anhydrase inhibitors have been demonstrated to cause an improvement in the macular cystic cavities in XLRS.
The purpose of the study is to report the improvement of foveomacular cavities and spoke-wheel pattern retinoschisis observed by spectral-domain optical coherence topmography (SD-OCT) and high-resolution adaptive optics (AO) retinal camera in a patient with XLRS treated with topical dorzolamide.
Methods: A 42 y.o. man with XLRS underwent detailed ophthalmic examinations. A mutation of *RS1* gene was detected earlier. Fundus images with microscopic resolution were obtained using the flood-illuminated AO retinal camera (rtx1™, Imagine Eyes, France). He was treated with topical dorzolamide three times a day. Transverse foveomacular cavities were observed by SD-OCT and the *en-face* images of spoke-wheel pattern foveoschisis was observed by AO retinal camera during a follow-up period.
Results: His decimal BCVA was 0.15 in the right eye and 0.3 in the left eye. The right eye showed atrophic macular degeneration and left eye showed spoke-wheel pattern foveoschisis. The dark-adapted 3.0 full-field ERG showed electronegative b-wave. SD-OCT showed the thinning of retinal thickness in the right eye and cystoid foveoschisis in the left eye. AO images showed spoke-wheel pattern retinal fold in the left eye. The spoke-wheel pattern in AO was sharper compared to the images obtained by fundus photography and autofluorescence imaging. After 14 month of treatment with topical dorzolamid, improvement of foveomacular cavities in SD-OCT was observed. The spoke-wheel pattern retinal fold in AO become obscure after treatment, however still detectable even after foveomacular cavities in SD-OCT was almost disappeared.
Conclusions: AO imaging showed detailed microstructure of spoke-wheel pattern foveoschisis and their improvement during a follow-up period. AO imaging may be helpful in clarifying the pathology of the foveoschisis in XLRS.
Commercial Relationships: Kiyoko Gocho, None; Keiichiro Akeo, None; Shuhei Kameya, None; Kunihiko Yamaki, None; Atsushi Mizota, None; Hiroshi Takahashi, None

167 - P53-4

Sensor-less Correction of Defocus and Astigmatism in an Optical Coherence Tomography System with a 3.0 mm Beam Diameter

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Purpose: As newly developed (swept-source) OCT engines become faster, practical use of OCT is becoming limited by the amount of light that can be safely sent into the eye. By increasing the $1/e^2$ imaging beam diameter from 1.2 mm (now typically used in OCT) to a beam size of 3 mm ($1/e^2$), and adding automated aberration correction, a ~2.5 times smaller speckle size, a ~2.5 times higher lateral resolution and a ~6 times higher number of photons collected returning from the retina (+ 8 dB), since light is collected over a larger angle. The extra photons that are collected can be used to offset the loss in sensitivity occurring in faster OCT engines.
Methods: We designed and built a sensor-less adaptive optics system built around a motorized Badal optometer for defocus correction and a liquid crystal lens for correction of linear and oblique astigmatism. A beam size of 3 mm was used. The normalized variance in the retinal OCT was used as a measure for the correction signal sent to the motorized Badal optometer for correction of defocus. After the best performance was reached, the linear astigmatism was optimized, followed by optimization

of oblique astigmatism. So far, the performance was tested on a model eye, consisting of a 30 mm achromatic lens, a trial lens with 1D of astigmatism and a cardboard retina.
Results: Prior to correction, a signal-to-noise ratio (SNR) of 37.5 dB was measured in the artificial retina. After automatic defocus correction, an SNR of 51.4 dB was measured, which increased to 56.4 dB and 59.6 dB after automatic correction of linear astigmatism and oblique astigmatism, resulting in a total gain of 22.1 dB. It took approximately 2 minutes to automatically correct these aberrations. Due to scattering, the liquid crystal lens has a one-way optical loss of ~40%.
Conclusions: The sensor-less OCT system was able to correct for defocus, linear- and oblique astigmatism in a model eye, and a gain of 22.1 dB was measured. The liquid-crystal unit for astigmatism correction can easily be implemented in current commercial OCT sample arm designs, as it is compact and can easily be inserted in an existing optical design. It runs on a low-power 5V signal, and does not require any sensors for feedback except for the existing OCT image.
Commercial Relationships: Reddikumar Maddipatla, None; Kingshuk Bose, None; Raju Poddar, None; Ayano Tanabe, None; Noboyuki Hashimoto, None; Barry Cense, Topcon (F), Utsunomiya University (P)

168 - P54-5

Clinical benefits in color vision after phacoemulsification surgery combined with intraocular lens implantation

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Purpose: To detect clinical benefits in terms of color vision at different illumination levels in patients with senile cataract after phacoemulsification surgery.
Methods: Twenty-two senile cataract patients (44 eyes) scheduled for binocular phacoemulsification surgery combined with ultraviolet-only absorbing intraocular lens (IOL) implantation were enrolled for color perception test by FM 100-Hue under photopic (1000 lux) and mesopic (40 lux) condition, preoperatively and 3 months postoperatively. Nineteen senile individuals with clear crystalline lens were recruited as control group.
Results: Preoperatively, total error scores (TES) in the patient group were 128.4 ± 60.7 at 1000 lux and 193.0 ± 71.9 at 40 lux, exhibiting worse color perception than the control group ($TES_{1000lux}$=72.2 ± 38.7, $t_{1000lux}$ =3.466, P <0.01 and TES_{40lux}=108.6 ± 36.5, t_{40lux} =4.829, P <0.01). Postoperatively, TES in the patient group was 96.6 ± 63.4 at 1000 lux and 123.4 ± 93.1 at 40 lux, both of which were better than preoperative phase ($t_{1000lux}$ =3.599, t_{40lux} =4.632; P<0.01) and comparative to the control group ($t_{1000lux}$ =1.454, t_{40lux} =0.688 ; P>0.01). Analysis on advances in color vision gained from the surgery showed that better hue perception occurred on consecutive color bands including yellow to yellow-green (Y-GY), yellow-green to green (GY-G) and green to blue-green (G-BG) at 1000 lux (t= 3.803, 3.437, 3.057 ; P<0.01). While at 40 lux, progresses occurred on consecutive bands including GY-G, G-BG and blue-green to blue (BG-B) (t=4.378, 4.521, 3.637 ; P<0.01).
Conclusions: Patients with senile cataract would suffer from significant deterioration to color perception. Phacoemulsification could rebuild these patients' hue discrimination and more benefits would be gained on consecutive color bands from Y-GY to G-BG under photopic condition and GY-G to BG-B, which shifted to short-wave bands, under mesopic condition.
Commercial Relationships: Mingxin Ao, None; Wei Wang, None

169 - P55-6

The Effect of the Longitudinal Chromatic Aberration on Color Visual Acuity

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Purpose: To investigate the relation between the CVA (Color Visual Acuity) and longitudinal chromatic aberration.
Methods: Four normal eyes of 4 subjects enrolled in this study. All the subjects had no ocular disease or color anomaly. The mean age was 35.5 ± 6.7 years old (30~47 years). The spherical equivalent was 3.19 ± 2.56 [D] (-1.25~-7.5 [D]). They had best corrected visual acuity of 20/13 or above. We utilized a laptop personal computer and Liquid Crystal Display (ColorEdge Cg245W, EIZO Corp., Ishikawa, JPN) to display the Landolts ring. The Landolt rings were colored with four color (R, Red, GY, Green-Yellow, BG, Blue-Green, BP, Blue-Purple) in the 15 colors (Chroma 6) of NEW COLOR TEST. The background was colored with white point D65 (achromatic color). The luminance of the background and Landolt ring color was equiluminance (30 [cd/m^2]) based on colorimetry. The CVAs of four colors were measured from undercorrection of +1.0 [D] to over-correction of -2.0 [D] in 0.5 [D] increments on the best correction. The visual distance was 3 [m]. The displaying time of Landolt ring was 5 [s] and the interval (displaying of background color) was 3 [s] or more. This experiment was conducted in the dark room.
Results: The figure shows the results. The loads of the lens which achieved best visual acuity (LogMAR) with the most positive side diopter were R, ± 0 [D] (CVA = 0.2081) , GY, -1.5 [D] (CVA = 0.7704), BG, -0.5 [D] (CVA = 0.2157), BP, -0.5 [D] (CVA = 0.6777).
Conclusions: The CVA of R which is the long-wavelength component of light, showed the best results with the best correction lens. In contrast, the CVA of BG and BP which is the short-wavelength component, showed the best results with the load of -0.5 [D] lens. With regard to the CVA of GY, although the best visual acuity was achieved with the load of -1.5 [D] lens, there was no significant difference in visual acuity between the load of -0.5 [D] and -1.5 [D]. As we did not consider the age of the subjects in this study, it is considered that the factor of accommodation had a huge effect on the results. In the future, it is necessary to verify the relation between CVA and longitudinal chromatic aberration in details considering the ages of subjects and by using cycloplegic

and IOL eyes.

Commercial Relationships: Yoshiki Tanaka, None; Sho Yokoyama, None; Hideki Nakamura, None; Kazuo Ichikawa, None; Shoko Tanabe, None; Yukihito kato, None; Rie Horai, None; Kiyoshi Tanaka, None

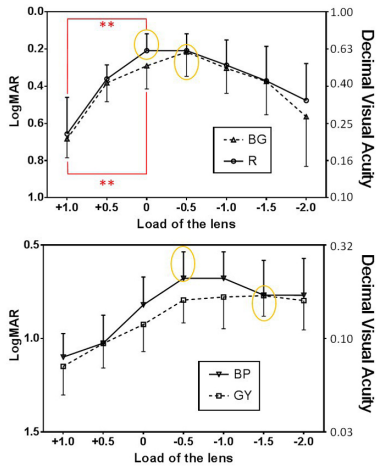

Figure: The changes of CVA by the load of the lens. there was significant difference in CVA between best correction and each load of the lens in R: +1.0 (**: p<0.01), BG: +1.0 (**: p<0.01) (Friedman test followed by Dunn's multiple comparisons test.).

The changes of CVA by the load of the lens.

170 - P56-7

Functional MRI Visualization of the Murine Visual Cortex Activity Evoked by Light Stimulation

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Study Group: Ophthalmology

Purpose: Visual response in experimental animals has been examined by various methods such as electoretinography, visual evoked potential, open field, visual discrimination, and optokinetic tests. These methods have been already modified and established to use for experimental animals such as mice. To understand the visual activity in more details, however, other methods are desired. In recent years, the use of functional magnetic resonance imaging (fMRI) is attempted to visualize cerebral cell activity in mice. In this study, we examined fMRI visualization of the visual cortex activity in mice by light stimulation.

Methods: All animal experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by Institutional Animal Care and Use Committee of the Keio University School of Medicine. To test awake mice using fMRI, we performed an operation to fix a 2 × 3 × 20mm plastic plate on parietal bone of C57BL6/j mice with local anesthesia. These mice were adapted for head fixing and MRI sounds for a couple of days after the operation till fMRI examination. To obtain the visual cortex activity, light stimulation was repeated for 5 times (10 seconds duration, 60 seconds interval) on right eye with 5 Hz blue light. Analysis was performed with SPM8 (Wellcome Trust Centre for Neuroimaging, UCL Institute of Neurology, London, UK) and tailored software in MATLAB.

Results: Bilateral occipital lobe response in the C57BL6/j mice by 5 Hz blue light stimulation with fMRI was observed. V1M area, V1B area in striate cortex and V2MM area, V2ML area in extrastriate cortex activity was observed.

Conclusions: We tested the visual cortex activity in awake mice by light stimulation with fMRI, and obtained responses from primary and secondary visual cortex successfully. These data indicated that fMRI may be effective to examine the murine visual response.

Commercial Relationships: Kinya Tsubota, None; Yuji Komaki, None; Norio Takata, None; Tetsu Yoshida, None; Kenji Tanaka, None; Hideyuki Okano, None; Hiroshi Goto, None; Kazuo Tsubota, None; Toshihide Kurihara, None

171 - P57-8

Intraocular lens position, refractive status, and visual acuity during the cataract postoperative period

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1. Shinseikai Toyama Hospital Eye Center, Toyama, Japan.

Purpose: To evaluate one-piece aspheric intraocular lens (IOL) position using anterior segment optical coherence tomography (AS-OCT) effect on refractive change and uncorrected distance visual acuity (UDVA) in the early postoperative period.

Methods: In 50 eyes of 42 patients, small-incision cataract surgery was undertaken with implantation of 2 types of aspheric IOL, TECNIS one-piece ZCB00V and Acrysof IQ SN60WF. Before cataract surgery, the axial length was measured and keratometry was performed using a partial coherence interferometer. The predicted postoperative refractions were obtained using the SRK/T formula. The target of refraction was myopia up to -0.50D. The refractive error was the difference obtained by subtracting the predicted postoperative refraction for the implanted IOL power calculated using the SRK/T formula from the spherical equivalent of subjective refraction, which was measured during the corrected distance visual acuity (CDVA) examination at 5 m. An AS-OCT instrument was used to image the postoperative anterior chamber depth at 1 week, 2 weeks, and 1 month after surgery. Refraction and UDVA were performed at each visit.

Results: The ACD in the ZCB00V were 4.14 ± 0.27, 4.14 ± 0.29, and 4.19 ± 0.30 mm at 1 week, 2 weeks, and 1 month after surgery. Those in the SN60WF were 3.90 ± 0.22, 3.90 ± 0.25, and 3.95 ± 0.24 mm. For each IOL, the value of ACD did not change significantly during the following period. The refractive errors in the ZCB00V were 0.02 ±

0.58, -0.02 ± 0.50, and -0.12 ± 0.46D at 1 week, 2 weeks, and 1 month after surgery. Those in the SN60WF were -0.04 ± 0.35, -0.05 ± 0.34, and 0.10 ± 0.39D. For each IOL, the value of refractive error did not change significantly during the following period. The UDVA of logMAR unit in the ZCB00V were 0.19 ± 0.24, 0.20 ± 0.25, and 0.17 ± 0.23 at 1 week, 2 weeks, and 1 month after surgery. Those in the SN60WF were 0.22 ± 0.19, 0.22 ± 0.19, and 0.23 ± 0.19. For each IOL, the value of UDVA did not change significantly during the following period.
Conclusions: The 2 types of IOL produce stable lens position, refraction, and good visual function early after cataract surgery.
Commercial Relationships: Kohei Utsuki, None; Naoko Tachi, None; Yoshiki Ueta, None; Yasuhiro Okamoto, None; Takao Fukutome, None; Koji Nagae, None; Kayoko Yoshimura, None; Yoshihiro Hashimoto, None

172 - P58-9

Higher-order aberrations in different types of posterior capsular opacification

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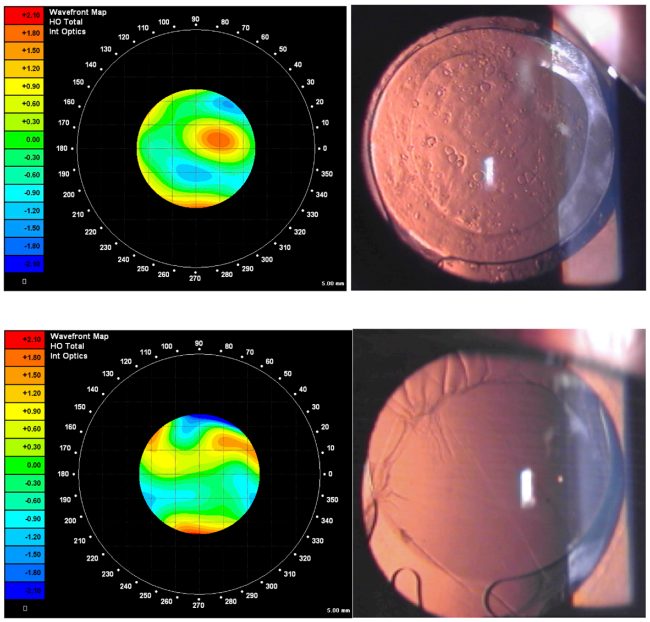
Purpose: Combined posterior capsular opacification (PCO) analysis system EPCO 2000 software and Tracey-iTrace visual function analyzer to evaluate the influence of two different types of PCO on the higher-order aberrations in patients with good best corrected visual acuity (≥0.8).
Methods: This prospective study comprised 50 senile cataract patients (68.0% females, 67 eyes), aged 69.51 ± 8.73 yrs (53 ～ 85 yrs), who had phacoemulsification and intraocular lens (IOL) implantation. These psudophakic eyes were divided into PCO group and no PCO group (22 cases, 38 eyes). Two groups were age and gender matched. According to different PCO types, the PCO group was divided into two subgroups, fibrosis-PCO group (19 cases, 20 eyes) and Elschnig-peal-type PCO group (9 cases, 9 eyes). Higher-order aberrations were measured with Tracey-iTrace visual function analyzer in all eyes. Retroillumination photographs were taken and PCO was scored using EPCO 2000 software in PCO groups. The relationship of PCO score and higher-order aberrations in two types of PCO subgroups was analyzed with Pearman' s correlation coefficient.
Results: Follow-up time was 23 months ～ 75 months. PCO score was 0.328 ± 0.180 in fibrosis-PCO group and 0.356 ± 0.232 in Elschnig-peal-type PCO group, P=0.729. Total higher-order aberrations and total coma aberration were higher in Elschnig-peal-type PCO group than that in fibrosis-PCO group, P<0.05. The correlations between PCO score in fibrosis-PCO group and total higher-order aberrations (r = 0.578, P = 0.01) and total spherical aberration (r = 0.610, P = 0.00) were significant. The correlations between PCO score in Elschnig-peal-type PCO group and total higher-order aberrations (r = 0.667, P = 0.05) and total trefoil aberration (r = 0.678, P = 0.05) were significant.
Conclusions: Our results showed that before the high contrast Snellen visual acuity loss, Elschnig-peal-type PCO and fibrosis-PCO both increased higher-order aberrations and then impaired the image quality in retina. The effects on visual acuity and vision performance were more serious in Elschnig-peal-type PCO than in fibrosis-PCO. Positive correlation between PCO score and total higher-order aberrations as well as total trefoil aberration further proved that forward light scatter and irregular astigmatism induced impaired retina image quality and vision performance in Elschnig-peal-type PCO.
Commercial Relationships: Wenjie Wu, None



173 - P59-10

Comparison of SRK/T and Haigis formulae for Predicting Corneal Astigmatism Correction by Toric Intraocular Lens

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Purpose: The aim of this study was to compare the accuracy of the SRK/T and Haigis formulae for predicting corneal astigmatism correction by toric intraocular lens (IOL).
Methods: A total of 73 eyes from 73 patients implanted with an AcrySof toric IOL were enrolled. The corneal plane effective cylinder power of toric IOLs (target induced astigmatism vector [TIA]) predicted by the SRK/T and Haigis formulae was compared with the postoperatively achieved cylindrical correction (surgically-induced astigmatism vector [SIA]). Magnitude of error was defined as difference between the magnitudes of the SIA and TIA. The median absolute magnitudes of error (MedAMEs) predicted by the SRK/T and Haigis formulae were compared. The median absolute errors (MedAEs) predicted by the two formulae were also compared.
Results: The postoperatively achieved SIA was 1.80 ± 0.55 D. Magnitude of error predicted by the Haigis formula (0.23 ± 0.40 D) was significantly smaller than that predicted by the SRK/T formula, 0.31 ± 0.40 D (P < 0.001). The MedAME predicted by the Haigis formula was significantly smaller than that predicted by the SRK/T formula (P < 0.001). The MedAE predicted by the Haigis

formula (0.35 D) was also significantly smaller than that predicted by the SRK/T formula (0.43 D) (P = 0.003).
Conclusions: The Haigis formula was more accurate than the SRK/T formula both in predicting refractive outcome and in predicting corneal astigmatism correction by toric IOL.
Commercial Relationships: Youngsub Eom, None; Jong Suk Song, None; Yong Yeon Kim, None; Hyo Myung Kim, None

174 - P60-11

Understanding Causes of Ring-Shaped Dysphotopsia Associated with Posterior Chamber Phakic Implantable Collamer Lenses with a Central Hole

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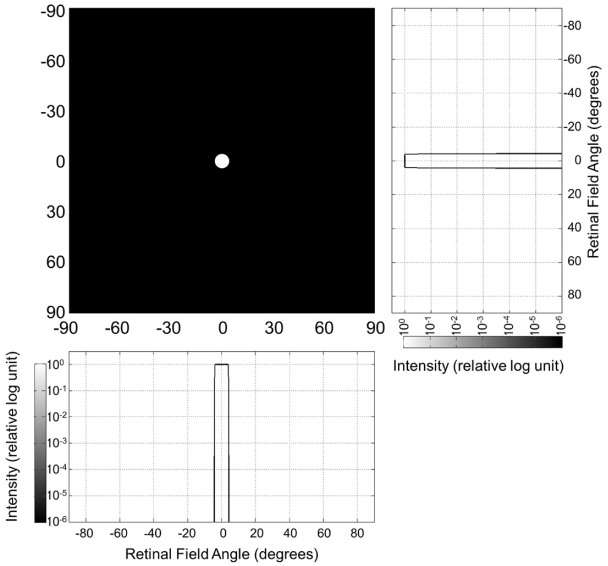
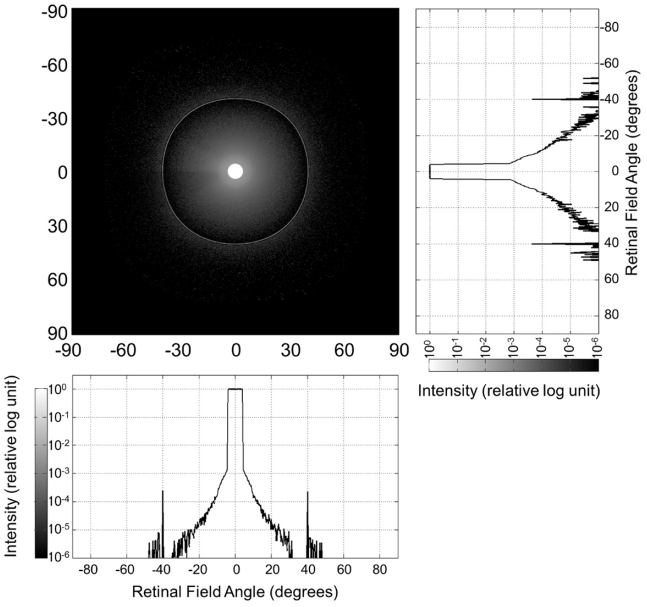
Purpose: The aim to this study was to compare the vision quality of posterior chamber phakic implantable collamer lenses (ICLs) with and without a central hole (*i.e.* conventional vs. Hole ICL) and to determine the causes of central hole-induced dysphotopsia.
Methods: Non-sequential ray tracing was used to construct a myopic human eye model with conventional and hole ICLs. Simulated retinal images measured in log-scale irradiance were compared between the two ICLs for an extended Lambertian light-emitting disk object 20 cm in diameter placed 2 m away from the corneal vertex. To investigate the causes of hole-induced dysphotopsia, a series of retinal images were simulated using point sources at infinity with well-defined field angles (0 to -20°) and multiple ICL models with varying ICL thickness and radius of curvature of the hole edge.
Results: From the case study using an extended Lambertian source, hole ICL-evoked ring-shaped dysphotopsia was formed at a retinal field angle of ± 40°. Component level analysis using a well-defined off-axis point source from infinity revealed various hole-induced stray light patterns in addition to the nominal point spread image on the retina. Ring-shaped dysphotopsia was generated by stray light refraction from the inner wall of the hole and the posterior ICL surface. Hole-induced ring-shaped dysphotopsia was successfully simulated and the mechanism of development was established.
Conclusions: Hole ICL-evoked ring-shaped dysphotopsia was related to light refraction at the central hole structure. Surgeons should explain to patients the possibility of glare and ring-shaped dysphotopsia after Hole ICL implantation.
Commercial Relationships: Hyo Myung Kim, None; Youngsub Eom, None; Dae Wook Kim, None; Dongok Ryu, None; Seul Ki Yang, None; Jong Suk Song, None; Sug-Whan Kim, None
Support: The SRC program (2010-0027910) of the Center for Galaxy Evolution Research and the R&D program (Project No. 2014-9-710-03) supervised by the Ministry of Science, ICT and Future Planning, South Korea.



Retinal images measured as normalized log-scale irradiances of the myopic human eye model (spectacle plane refraction of -10 diopters at a vertex distance of 12 mm) corrected with a conventional posterior chamber phakic implantable collamer lens (ICL) in non-sequential ray tracing using an extended Lambertian light-emitting disk object 20 cm in diameter and 2 m away from the corneal vertex. The conventional ICL showed a well-demarcated round image at the foveal center.



In non-sequential ray tracing using an extended Lambertian light-emitting disk object 20 cm in diameter and 2 m away from the corneal vertex, the hole ICL produced a glare and ring-shaped dysphotopsia at a retinal field angle of ± 40° .

175 - P61-12

Effects of sustained accommodation on the optics of human eye

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Purpose: Myopia often presents and progresses throughout the school years and it has been hypothesized that high levels of near work may contribute to its development. The optics change during the near work for example accommodation lag might be related to myopia progression. This study is to investigate the possible changes of optics of the eye during the sustained near work.

Methods: A commercial Shack-Hartmann aberrometer was modified to be an open field instrument in order to achieve unrestricted visual field. 21 low myopes were recruited in the study with left eye measured only. Subjects were instructed to watch a 15 minutes long movie displayed on a computer screen which placed 50 cm in front of the eye. Measurements were taken every 1 minute when subjects were directed to fixate on a central Maltese Cross target. Up to six-order Zernike coefficients were recorded and analyzed. Small sample non-parametric sign test and paired t-test were performed in data analysis.

Results: Averaged mean spherical equivalent (MSE) decreased in the first 9 minutes at the rate of 0.06 D per minute. After 9 minutes, MSE showed more fluctuation with time. Changes of 3rd-order coma had more effect than other higher order aberration terms. Spherical aberration consistently decreased during the first 7 minutes. Compared to defocus change, higher order aberrations had no significant impact to the changes of the optics of the eye within 15 minutes of accommodation ($p > 0.05$).

Conclusions: The accommodation of the eye decreases first and shows more fluctuation later with sustained near work. Optical changes in the eye with prolonged near work mainly caused by the changes in the defocus term rather than the other optical characteristics in the eye.

Commercial Relationships: Jason Shen, None; Frank Spors, None; Don Egan, None

176 - P62-13

Comparison of Subjective Refraction under Binocular and Monocular Conditions

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Purpose: To compare subjective refraction under binocular and monocular conditions, and to investigate the clinical factors that influence the difference in the spherical refraction between the two conditions in in healthy subjects.

Methods: We examined thirty eyes of 30 healthy subjects (age, 29.9 ± 5.5 years (mean ± standard deviation); gender, 16 men, 15 women). Binocular and monocular refraction without cycloplegia was measured through circular polarizing lenses on both eyes, with Landolt-C chart of the 3D visual function trainer-ORTe (Figure 1). Pupil size was also measured using the FP-10000. Stepwise multiple regression analysis was used to assess the relation between several variables and the difference in spherical refractions under binocular and monocular conditions.

Results: Subjective spherical refraction under monocular condition was significantly more myopic than that under binocular condition ($p<0.001$), whereas no significant differences in subjective cylindrical refraction ($p=0.99$) was found. Explanatory variables relevant to the difference in spherical refraction between binocular and monocular conditions were the binocular spherical refraction ($p=0.021$, partial regression coefficient $B=0.026$) and binocular cylindrical refraction ($p=0.113$, $B=0.082$) (adjusted $R^2=0.421$). No significant correlation was seen with other clinical factors such as age, gender, corrected distance visual acuity, binocular pupil size, change in pupil size from binocular to monocular conditions, and corneal, or ocular higher-order aberrations.

Conclusions: Subjective spherical refraction under monocular condition was significantly more myopic than that under binocular condition. Eyes with higher myopia are more predisposed to show a large difference in spherical refraction between these two conditions, suggesting that eyes with higher myopia might lead to be overcorrected in refractive surgery, since myopic correction is usually determined by monocular refraction.

Commercial Relationships: Hidenaga Kobashi, None; Kazutaka Kamiya, None; Akihito Igarashi, None; Kimiya Shimizu, None; Wakako Ando, None; Tomoya Handa, None

Clinical Trail: UMIN000015182

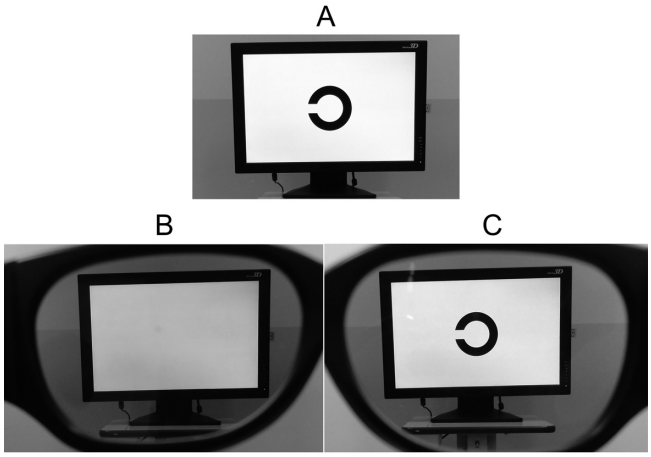

Figure 1. The 3D visual function trainer-ORTe. **A,** Landolt-C chart on the monitor, **B,** Non-testing eye under binocular condition using a circular polarizing lens. No Landolt-C chart was shown by the display. **C,** Testing eye under binocular condition using a circular polarizing lens. The Landolt-C chart was shown by the display.

177 - P63-14

Inter-Rater Agreement of Computerized Visual Acuity (COMPlog) and Refractive Error Measurements (Plusoptix Photorefractor) Among School Children

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1. Department of Optometry, School of Allied Health Sciences, Manipal University, Manipal, Karnataka, India. 2. Department of Public Health, Manipal University, Manipal, Karnataka, India.

Purpose: To study the inter-rater agreement of computerized visual acuity and refractive error measurements using COMPLOG (NHS trust,UK) and PLUSOPTIX photorefractor (S08 Plusoptix,USA) respectively among participants of VARES (Visual Acuity Refractive Error Squint) school eye screening study

Methods: VARES was an epidemiological study to find the prevalence of refractive error and visual impairment in Udupi district among 1774 school children of 5 to 14 years of age. Of these, 50 participants were randomly selected from each of the three subgroups namely primary, upper primary and secondary school level. The study was approved by the institutional Ethics committee, Informed consent was obtained from the parent or principal and assent was given by the student for participation. Study examinations were done in the respective schools and under recommended illumination standards. Visual acuity was measured using computerized software (COMPLOG), Plusoptix, an eccentric photorefractor was used to assess refractive error, interpupillary distance (IPD), pupil size and ocular deviation. Repeated measurements of all the parameters were taken by 2 different examiners within an average time interval of two weeks. JT was a qualified optometrist with two years of experience and MP was an optometry graduate student. Measurements obtained were masked from either examiners and interobserver variability was assessed for both the parameters

Results: Mean age of the 150 students was 10 (2.80) years. Wilcoxon sign rank test did not show significant differences for visual acuity measurements (p, OD=0.069, OS=0.564) and refractive error measurements (p, OD=0.117; OS=0.067) between the 2 independent observers. Bland Altman plot showed good agreement between the two examiners and the limits of agreement were within clinically acceptable range. Mean spherical equivalent refractive error showed a trend of gradual decrease among primary to secondary school, but this was not statistically significant (p>0.05). No significant difference was observed for measurements such as pupil size (p OD=0.821, OS=0.379), ocular deviation (p= 0.323) and IPD (p= 0.530)

Conclusions: This study reports good inter-rater agreement for visual acuity (COMPlog) and photorefraction measurements between examiners with varied clinical experience

Commercial Relationships: Avinash Prabhu, None; Marita Pinto, None; Juthika Taludkar, None; Jyothi Thomas, None; Ramesh Venkatasubbu, None

178 - P64-15

Astigmatism in Chinese primary school children, prevalence, change, and effect on refractive development

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Purpose: To study the prevalence, type, and progression of astigmatism in Chinese primary school children, and its effect on refractive development.

Methods: A cross sectional study of primary school children was carried out in two randomly selected primary school. A cohort study was performed on a subset of children, one year after initial examination. Refractive error (measured by cycloplegic autorefraction) was the main study outcome.

Results: 992 children participated in the study; the mean age was 8.85 y/o (SD 1.34; range 7 to 11 y/o). 24.7% subjects have astigmatism at the initial screening. The mean cylinder reading was -0.64 D (SD 0.68; range 0.00 to -5.25 D), and with the rule astigmatism was predominant (54.2%). In the 462 children studied longitudinally, the mean yearly cylinder reading change was -0.0051 D (SD=0.64, range in -2.44 D to 2.81D). In the non-myopic group (SER, spherical equivalent refraction, >-0.5D), the presence of astigmatism in initial examination have the trend of less myopic shift, but it did not reach statistical significance (p=0.063). In addition, in the myopic group (SER<=-0.5D), the presence of astigmatism in initial examination have no statistical significance in comparison to non-astigmatism subjects (p=0.25). Besides, comparing children with decreased or stable in astigmatism, subjects with astigmatism in progression have more myopic shift (yearly SER change was -0.47 D, SD=0.86, P<0.05).

Conclusions: This study reports the prevalence of astigmatism in Chinese primary school children. The presence of astigmatism, and particularly with increasing astigmatism, appears to predispose the children to progressive myopia.

Commercial Relationships: Shao-en Chan, None; Hsi-Kung Kou, None; Pei-Chang Wu, None

179 - P65-16

Individual difference between static and dynamic motion in depth discrimination training tasks

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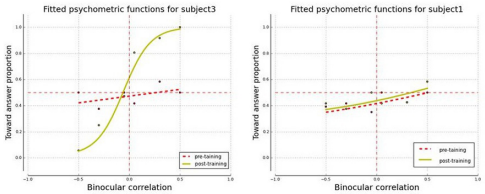
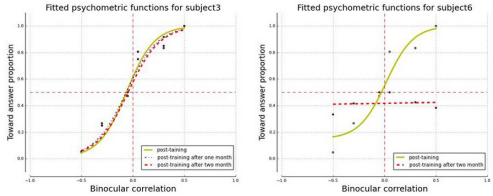
1. Optics Department, Telecom Bretagne, Brest, France.

Purpose: Perceptual learning has been studied and applied in numerous training tasks and treatments in clinical practice to improve stereoacuity. Most of the technologies are based on implementing static random dot stereogram (RDS) to improve the threshold of discrimination sensitivity, there is little information on how dynamic RDS motion in depth could affect our perceptual training. For comprehensive stereo vision training exploration, it is essential to involve training tasks with moving objects in

depth. We investigated how observers will respond during a specific dynamic motion in depth discrimination training.
Methods: Six adults (mean age = 25.6 years) with normal binocular and stereoscopic vision were trained to judge the motion direction through dynamic RDS composed by varied proportion of signal and noise.The total training trails were 2880. Different lighting conditions were used to evaluate whether luminance could be a factor affecting the training. The post-training tests were repeated after two months to explore exposure period effect on observers' memory.
Results: There was a great individual difference on the effect of perceptual training for dynamic RDS. Four observers' performance were significantly improved after the training ($p<0.001$), the other two observers could rarely perceive the RDS motion before and after the training, their answers for motion in depth were almost random. Lighting condition had an impact on motion perception ($p=0.039$). Three of the subjects could easily extract signal motion through RDS after two months, while the other subject performed quite differently ($P<0.001$).
Conclusions: The observers' performances before and after the dynamic motion in depth training suggested that a single perceptual training could not work for all the people, the method designed for training should take specific considerations on individual vision difference and capability.Human perception for dynamic stereo motion discrimination could be improved and last for a long period,so there is a great sense to investigate in more detail.
Commercial Relationships: Di Zhang, None; Yulia Fattakhova, None; Jean Louis de Bougrenet de la Tocnaye, None; R Sharmila, None; S K Gupta, None; Sushma Srivastava, None; Dr. Rohit Saxena, None



Fitted functions before and after traning for subject1 and subject3. '-' on the xaxis:signal dots motion-away; '+' :signal dots motion-toward. Binocular correlation is the proportion of signal dots and the total dots on screen.



after two months, Subject3 could remember how to extract motion in depth after two months but subject6 could not.

Clinical/Epidemiologic Research - Poster

291 - P01-1

Reticular Drusen in Chinese

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Purpose: To find out the association of reticular drusen and macular diseases in Hong Kong Chinese.
Methods: This was a cross-sectional study of patients with reticular drusen recruited from 1 Jan 2013 to 30 June 2013. Optical coherence tomography (OCT) was performed on all subjects to confirm the presence of subretinal drusenoid deposits. Near-infrared photography, red-free photography, fundus fluorescein angiography (FFA), indocyanine green angiography (ICG) and fundus autofluorescence (FAF) were performed on selected patients. Diagnosis of reticular drusen was by an experienced retinal specialist, after which data would be collected and analyzed.
Results: One hundred and five patients were recruited. The mean age was 73.8 ± 3.7 year-old. 21.9% of these 105 patients having non-exudative age-related macular degeneration, 17.1% having exudative age-related macular degeneration (AMD) and 3.8% having polypoidal choroidal vasculopathy (PCV) instead. No other macular pathologies besides reticular drusen were detected in the remaining 57.1%.
Conclusions: The prevalence of reticular drusen is lower in Hong Kong Chinese than other ethnic groups. A relatively higher proportion of PCV patients among AMD patient might be one of the explanation.
Commercial Relationships: Ian Wong, None; Raymond Wong, None; Qing Li, None; Wai Yip, Jacky Lee, None; Shiu Ming, Jimmy Lai, None

292 - P02-2

MMP20 and ARMS2/HTRA1 are Associated with Neovascular Lesion Size in Age-Related Macular Degeneration

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Study Group: Nagahama Cohort Research Group.

Purpose: Age-related macular degeneration (AMD) is the leading cause of severe visual impairment. Despite treatment, a central scotoma often remains. The size of the scotoma depends on the lesion size of the choroidal neovascular membrane and significantly affects the patient's quality of life, and the lesion size of neovascularization also affects response to treatments. In the present study, we sought for genes associated with the neovascular lesion size in AMD.
Methods: To identify genetic polymorphisms associated with lesion size in neovascular AMD, we performed a two-stage genome-wide association study (GWAS) for the lesion size of AMD as a quantitative trait among 1,146(1st stage, 727, 2nd stage, 419) Japanese patients with neovascular AMD. Lesion size was determined by the greatest linear dimension measured with fluorescein angiography examination before treatment. We examined the association between genotypic distribution of each SNP and the trait using an additive model adjusted for age and sex. To evaluate the associations between AMD development and SNPs associated with lesion size, we performed a case-control study by using the genotype data from these 1,146 Japanese patients as case subjects and the fixed dataset from the Nagahama Study as control subjects.
Results: In the discovery stage, rs10895322 in *MMP20* had a *P*-value of 6.95×10^{-8}, and rs2284665 in *ARMS2/HTRA1* had a *P*-value of 1.55×10^{-7}. The associations of these 2 SNPs were successfully replicated in the replication stage ($P = 5.26 \times 10^{-3}$ and 4.31×10^{-3}, respectively), and a combined analysis of both stages showed genome-wide significant *P*-values ($P = 2.80 \times 10^{-9}$, $\beta = 569.8$ and $P = 4.41 \times 10^{-9}$, $\beta = 525.0$, respectively). While *ARMS2/HTRA1* is a major susceptibility gene for AMD, we could not find contribution of *MMP20* rs10895322 for AMD development.
Conclusions: In the present study, we confirmed that *ARMS2/HTRA1*, a well-known susceptibility gene for AMD, is a leading predictor of lesion size in neovascular AMD. Furthermore, our GWAS identified associations between lesion size and *MMP20*. MMP20 would have major roles in the later step of AMD development rather than the onset of AMD.
Commercial Relationships: Yumiko Akagi-Kurashige, None; Kenji Yamashiro, None; Masahiro Miyake, None; Masaaki Saito, None; Masako Sugawara, None; Ching-Yu Cheng, None; Khor Chiea Chuen, None; Wong Tien Yin, None; Nagahisa Yoshimura, None

293 - P03-3

Utilization of Eye Care Services Among Those with Unilateral Visual Impairment in Rural South India – Andhra Pradesh Eye Disease Study (APEDS)

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Purpose: To report on the utilization of eye care services

and its associated factors among those with unilateral visual impairment (VI) in a rural south Indian population.
Methods: A population based cross-sectional study was conducted using a multistage cluster random methodology to select participants from three districts (Adilabad, Mahbubnagar and West Godavari) in the state of Andhra Pradesh, India. A detailed in-depth interview and a comprehensive eye examination were conducted by trained personnel. Among those with unilateral VI, the questions asked were about noticing any change in vision and on utilization of eye care services. Factors affecting noticing a change in vision and utilization of eye care services were assessed using logistic regression. The unilateral VI was defined as presenting visual acuity <6/18 in one eye but ≥6/18 in the fellow eye and included unilateral blindness (<6/60 in one eye but ≥6/18 in the fellow eye).
Results: Among the 4456 participants aged ≥16 years who were administered the questionnaire, 53% were women, and 54.7% had no education. The mean age was 37.1 years (SD:13.5 years). Of the 489 (11%) people with unilateral VI, 399 (81.6%) participants reported noticing a change in their vision over the last five years but only 136 (34%) participants sought eye care consultation. On multiple logistic regression analysis, those in the older age group (>50 years) were more likely to notice a change in their vision compared to those aged ≤ 50 years (OR:2.8; 95% CI:1.7–4.6). Similarly, the residents of Adilabad and Mahbubnagar districts had higher odds for noticing a change (OR, 2.0; 95% CI:1.1–3.6 & OR:2.7; 95% CI, 1.5–5.1 respectively) compared to those from the West Godavari district. Seeking eye care consultation was associated with any education (OR, 1.9; 95% CI:1.1–3.2), blindness category (OR:2.7; 95% CI:1.5–5.2) and cataract as a cause of visual impairment (OR:2.1; 95% CI:1.0–4.3). The participants belonging to Mahbubnagar district had lower odds of seeking consultation compared to their peers in West Godavari district (OR:0.4; 95% CI:0.2-0.7).
Conclusions: Though the rural population with unilateral VI noticed a change in their vision, a large proportion of them did not utilize eye care services. More robust strategies may be needed to address these issues to improve utilization of services in these rural communities.
Commercial Relationships: Srinivas Marmamula, None; Rohit Khanna, None; Giridhar Pyda, None; Rao Gullapalli, None

294 - P04-4

Awareness and health-seeking practices about cataract in urban slum population of Delhi in India

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Purpose: Under the Vision 2020, The Right to Sight Programme, cataract is identified as one of the most common causes of avoidable blindness. Despite achieving a high cataract surgical rate of more than 5000 surgeries per million populations in the country, the load of blindness due to cataract is still high. In this regard, a study was conducted to assess the awareness and health-seeking practices about cataract among the urban slum population of Delhi, India.
Methods: A descriptive cross-sectional study was conducted in the 5 slum clusters from the urban population of South Delhi. A specially designed pre-tested questionnaire schedule was administered. The study sample was comprised of 1552 inhabitants.
Results: The mean age of the respondents was 34 ± 12.1 years. Overall 89.8% of the respondents have heard of cataract. The awareness about the surgical treatment of cataract was known to 44% of the respondents.61.9% people don' t know any symptom of cataract. Out of all who had been advised cataract surgery, only 60.7% had underwent surgery. Education level (p<0.001), age (p<0.001) were found to be the determining factors related to awareness of cataract while occupation (p=0.283) and socio-economic factor (p=0.089) showed no significant association.
Conclusions: The findings highlighted that even though majority of respondents have heard of cataract but the awareness about symptoms and treatment was found low in the community. There is a need for efforts directed to enhance community level counseling and educational programmes.
Commercial Relationships: Vasundhra Misra, None; Praveen Vashist, None; Tanuj Dada, None; Sumit Malhotra, None; Suraj Singh Senjam, None; S N Dwivedi, None

295 - P05-5

Magnitude and Determinants of Ophthalmoplagia among Persons with Diabetes in Saudi Arabia

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Purpose: 56% of Saudi diabetics are suffering from symptomatic diabetic neuropathy. Old age, type 2 diabetes with long duration, poor control diabetes and smoking were risk factors for symptomatic neuropathy. Researchers have notes that cases with acute painful ophthalmoplagia are diabetic. We aim to estimate the prevalence of 3rd, 4th, 6th nerve palsy among registered diabetics in Saudi Arabia and to determine the risk factors of ophthalmoplagia among them. Also recommend improvement of eye care based on study outcome is one of our objectives
Methods: This will be retrospective review of national registry for diabetics in Saudi Arabia. nearly 120,000 diabetics registered till end of April 2014 will be included in this study will be analyzed to estimate the prevalence of 3rd, 4th, and 6th nerve palsy and correlate them to the known risk factors of complications of diabetes and ophthalmic comorbidities. Based on the ICD 10 codes, patients with cranial nerve palsy will be identified from the registry. The rest of the diabetics without ophthalmoplagia will be the comparison group. The incidence of each mononeuropathy and multiple

ophthalmoplagia will be calculated. The demographics of the registered diabetics in both groups (age, sex, location) will be noted. Ocular profile, especially diabetic retinopathy stage will be compared in two groups. Presence of other types of diabetic neuropathies that associated with ophthalmoplagia, as well other known risk factors of diabetes complications that associated with ophthalmoplagia will be noted as well. SPSS software will be used for analysis.
Results: Among the 64,351 patients selected from the Saudi national diabetes registry aged 18-100 years 209 were with ophthalmoplagia, 36.36% with 3rd cranial nerve (CN) palsy, 2.87% with 4th CN palsy, 53.11% with 6th CN palsy, and 7.66% with more than one CN palsy. Age more than 45 years, male gender, BMI more than 30, duration of diabetes more than 15 years, hyperlipidemia, diabetic neuropathy and diabetic retinopathy were all risk factors for ophthalmoplagia
Conclusions: The prevalence of ophthalmoplagia in diabetic patients that has been register in the Saudi National diabetic registry was 0.32%. We found diabetic retinopathy is one of the risk factors beside the age, male gender, BMI, diabetic neuropathy, and hyperlipidemia. Recognizing the risk factors may help to recommend improvement of eye care based on study outcomes.
Commercial Relationships: Eman Kahtani, None

296 - P06-6

VF-14 score and referral decisions for cataract patients made by optometrists at a triaging ophthalmology clinic in Ampang, Malaysia

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Purpose: To assess whether the VF-14 score of cataract patients differed according to referral decisions made by optometrists at a triaging ophthalmology clinic.
Methods: Eight optometrists conducted dilated comprehensive eye examinations on patients who were recruited at a triaging ophthalmology clinic. Patients self-administered a modified Bahasa Malaysia VF-14 questionnaire while waiting for their eye examination. At the end of the examination, optometrists decided on appropriate management, referral, follow-up or non-referral for patients diagnosed with any type of cataract.
Results: A total of 94 patients were assessed for cataract. Of these, cataract of any type was diagnosed in 69 (73.4%) patients. Optometrists decided on referral for only 25 (36.2%) patients and discharged 19 patients (27.5%) after the examination and a follow-up decision was made for the remaining 25 patients (26.6%). The mean rank VF-14 score for referred patients was lowest (25.8), while the mean rank scores were similar for both non-referral (40.1) and follow-up (40.4). A Kruskal-Wallis H test showed that there was a statistically significant difference in VF-14 score between the different patient managements, (χ^2 (2) = 8.295, p = 0.016), with the mean rank score for referred patients being significantly different to the non-referral (U = 138.5, $p < 0.05$) and follow-up (U = 181.5, $p < 0.05$) groups.
Conclusions: Patients with reduced quality of visual function only were referred to the ophthalmologist for further consultation and cataract surgery. Inclusion of the VF-14 score into considerations for referral decisions has great potential to reduce unnecessary referrals to ophthalmologists.
Commercial Relationships: Duratul Hussin, None; Peter Hendicott, None; Andrew Carkeet, None; Philip Baker, None; Ai-Hong Chen, None

Immunology/Microbiology - Poster

297 - P07-1

Loop-Mediated Isothermal Amplication For The Diagnosis Of Herpes Simplex Virus Keratities

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Purpose: To establish a loop-mediated isothermal amplification (LAMP) assay for the detection of herpes simplex virus 1 (HSV-1), and to evaluate the LAMP assay for the diagnosis of herpes simplex viral keratitis.
Methods: We established the LAMP assay to detect HSV-1 using specifically designed primers. The specificity and sensitivity of the assay was tested . To evaluate the application in clinical diagnosis, LAMP was performed to test the samples with herpes simplex viral keratitis and normal samples, to evaluate the accuracy of the test(without DNA extraction). We also collected 13 corneal samples of from the patients with clinical diagnosis of herpes simplex viral keratitis. LAMP assay and real-time polymerase chain reaction (Real-Time PCR) were performed to evaluate the concordance between them.
Results: The positive reaction showed a green colour change, and the negative reaction maintained to be orange. The specific LAMP primers amplified only HSV-1 DNA, no LAMP products were detected with the DNAs of HSV-2 and VZV. LAMP was sensitive with the lowest detection limit being 10^1copies/ul of HSV-1 DNA. 5 out of 8 cases of keratitis patients were detected, the positive detection rate was 62.5% , and no positive result was detected in the 3 normal samples. Of the 13 patients clinical diagnosis of herpes simplex viral keratitis, 11 was tested positive for HSV-1 using LAMP(84.6% positive rate), and 9 positive for HSV-1 using Real-Time PCR(69.2% positive rate). A 84.6% concordance was observed across the two methods. The positive rate for LAMP with and without DNA extraction was 75% (6/8) and 62.5% (5/8) respectively, which showed no difference between them($p>0.05$).
Conclusions: The specific primers can be used to perform the LAMP assay for the detection of HSV-1. LAMP and Real-Time PCR detection results show a higher consistency. What's more, LAMP performed without DNA extraction remains a high positive detection rate.
Commercial Relationships: Zhuoshi Wang, None

298 - P08-2

Human corneal endothelial cells are fully permissive for human cytomegalovirus infection

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Purpose: Since the first case of human cytomegalovirus (HCMV) -induced corneal endotheliitis in which HCMV DNA was detected from the aqueous humor of the patient via PCR, the clinical evidence for HCMV endotheliitis has been accumulating. However, it remains to be confirmed whether HCMV can efficiently replicate in corneal endothelial cells. We, therefore, sought to determine whether primary cultured human corneal endothelial (HCE) cells could support HCMV replication.
Methods: Human foreskin fibroblast (HFF) cells have been shown to be fully permissive for HCMV replication and are commonly used as an *in vitro* model for HCMV lytic replication. Therefore, primary HCE or HFF cells were infected with HCMV TB40E strain, which is highly endothelial cell-tropic, and we compared viral mRNA and protein expression, genome replication, and growth between the TB40E-infected HCE cells and HFF cells. We also analyzed sections of the infected HCE cells by an electron microscope (EM).
Results: When HCE cells were infected with TB40E, rounding and vacuolation resembling owl's eye cells were observed at 2 and 3 days after infection. Synthesis of viral mRNA and protein expression proceeded more efficiently in the TB40E-infected HCE cells at a high multiplicity of infection (MOI) than in the HFF cells. Likewise, viral genome was also effectively replicated with UL44, a viral DNA polymerase processivity factor, foci observed in the nuclei of HCE cells. EM analysis of the infected HCE cells clearly detected cytoplasmic virus particles and HCE cells produced a substantial number of infectious virions after infection with TB40E at both a high and low MOI.
Conclusions: HCE cells could efficiently support HCMV replication after infection at both a high and low MOI. These results suggest that HCMV endotheliitis is caused by HCMV replication in HCE cells and replicated virions are released into the aqueous humor of the patients.
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299 - P09-3

Long-term observation of murine models of anterior scleritis

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Purpose: We have previously established a model of anterior scleritis by modifying a collagen-induced autoimmune arthritis model. In the present study, the long-term observation of the clinical and the immunological findings were performed.
Methods: Male DBA/1J mice (8-week-old) received primary immunization in the back of the neck with 200*ug* of bovine type II collagen (CII) emulsified using equal

volume of complete Freund's adjuvant (CFA, containing 100ug H37Ra *Mycobacterium tuberculosis*). After 3 weeks, CFA-emulsified CII was injected intradermally around the eye for secondary immunization, then the arthritis and eyes were examined. Eyeballs were excised at 3, 5, 8, 12 and 24 weeks after secondary immunization and analyzed histologically and immunohistologically.
Results: Clinical findings comprised severe arthritis and dilation of scleral blood vessels from 3 weeks after secondary immunization. Histological findings revealed anterior scleral thickening, with significantly large number of infiltrating cells as compared to untreated mice. Infiltration of CD4+, CD11b+ cells were present in the Tenon's layer, while deposition of plasma cells (CD138), complement (C3), immunoglobulin (Ig)G and IgM were seen in the anterior sclera in contact with the ciliary body and blood and lymphatic growth (CD31 and LYVE-1) expression was increased in the corneal limbus compared to untreated mice, throughout all observation periods.
Conclusions: T cells, macrophages, plasma cells, complement, immunoglobulins, the outgrowth of blood and lymphatic vessels were persistently found in the sclera of the collagen-induced anterior scleritis model. It is suggested that the involvement of immunocomplex deposition, and blood and lymphatic growth in the sclera is one of immunopathology of this scleritis model.
Commercial Relationships: Hiroko Taniguchi, None; Yuki Kitahara, None; Junko Hori, None
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300 - P10-4

The V-domain Ig suppressor of T cell activation (VISTA) plays an essential role in the acceptance of corneal allografts

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Purpose: V-domain Ig suppressor of T cell activation (VISTA) is a novel and structurally distinct Ig superfamily inhibitory ligand. We have previously demonstrated that survival of allografts treated with anti-VISTA mAb was less than that of the control, and that VISTA plays important role in induction of alloantigen-specific ACAID.
(1) To further investigate the mechanism of VISTA-mediated corneal allograft survival, we examined destruction of corneal endothelial cells (CECs) by allo-reactive T cells in vitro.
(2) As the next, we examined infiltrating T cells in the graft-bearing eyes from the recipients treated with anti-VISTA or control IgG.
Methods: (1) The corneas from C57BL/6 (B6) eyes pre-treated with anti-VISTA mAb or control rat IgG were incubated with CD4+ T cells for 6h. Dead CECs stained with propidium iodide were counted and compared.
(2) Normal corneas of C57BL/6 were transplanted into normal eyes of BALB/c mice. Recipients were administrated with 0.2 mg of anti-VISTA mAb or control rat IgG, three times a week after grafting. For Immunohistochemical staining, graft-bearing eyes were removed.
Results: (1) No significant differences were observed between the number of dead CECs treated anti-VISTA monoclonal antibodies (mAb) and that treated control IgG after incubation with allo-reactive T cells. It is indicated that VISTA does not have protective effect in the cornea from the allo-specific killing by CD4 T cells.
(2) Immunofluorescent staining of the graft-bearing eyes at 3 to 5 weeks after grafting revealed that the numbers of infiltrating CD4+ T cells and CD8+ T cells at graft center and host-graft junction were significantly higher in anti-VISTA treated recipients compared to control.
Conclusions: Taken together of our present and previous data, it is suggested that VISTA plays an role in the acceptance of corneal allografts by inducing allo-specific ACAID which suppress T cell infiltration into the cornea, although VISTA doesn't have a local protective effect in the interaction between CECs and CD4+ T cells.
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Support: Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science.

301 - P11-5

Inhibiting effect of wild rice (Zizania latifolia (Griseb) Turcz) on endotoxin-induced uveitis in mice

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Purpose: One of wild rice (WR) (*Zizania latifolia* (Griseb) Turcz) is native to China, Japan, and Vietnam. Recent animal studies have demonstrated that fed WR can suppress oxidative stress. The purpose of this study was to investigate the possible anti-inflammatory effect on endotoxin-induced uveitis (EIU) in mice.
Methods: EIU was induced by an injection of a lipopolysaccharide (LPS) into the footpad of male C57BL/6J. After daily oral administration of WR for 5 days, EIU was induced. Twenty-four hours later, the eyes were enucleated and infiltrating cells in anterior chamber (AC) and vitreous cavity (Vit) were counted. *In vitro* study, murine macrophages were stimulated with LPS and with or without WR. Interleukin (IL)-1beta and tumor necrosis factor (TNF)-alpha in supernatant were analyzed by ELISA.
Results: The total number of infiltrating cells in the AC and Vit in eyes with WR was significantly reduced than that without WR (0.48 ± 0.77 vs. 2.9 ± 1.7 cells, respectively. $P < 0.00001$). Both concentrations of IL-1beta and TNF-alpha in supernatant treated with WR were significantly suppressed than those without WR (7.2 ± 1.0 vs. 19.8 ± 6.1 and 313.3 ± 13.3 vs. 730 ± 193.4 pg/ml, respectively).

Conclusions: Our findings show that WR is a potent anti-inflammatory agent against the inflammation of EIU, and suggest a potential use in treatment of acute uveitis.
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302 - P12-6

Analysis of PU.1 expression in experimental autoimmune uveoretinitis

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Purpose: PU.1 is an Ets family transcription factor, which is required for the development of the immune system, via the generation of myeloid and lymphoid lineages, including macrophages, neutrophils, dendritic cells (DCs). Recently, we demonstrated that PU.1 regulates the expression of CD80, CD86, and MHC class II in DCs and PU.1 knock down suppresses the function of DCs. In the present study, we analyzed whether PU.1 expression is detected in the retina of mice with experimental autoimmune uveoretinitis (EAU), which is used as a model of human uveitis, and PU.1 expression level is associated with the inflammation in EAU.
Methods: C57BL/6N mice were immunized by hIRBP 1-20 peptide in complete Freund's adjuvant (CFA). Funduscopic examination was conducted in individual mice every 7 days until day 42 after immunization and graded on a scale of 0 to 4. After every funduscopic examination, mice were euthanized and the eyes were enucleated. We examined the expression of PU.1 in the retina and RPE/choroid complex collected from normal mice, CFA-immunized mice (CFA mice), and EAU mice. RNA was prepared from retina and retinal pigment epithelium (RPE)/choroid complex of enucleated eyes, and reverse-transcribed PCR. Total mRNA levels of PU.1, CD11c, F4/80 were quantified Real-time PCR. Immunostaining of retinal flat-mounts was performed using the following primary antibodies, anti-mouse CD11c, and anti-mouse PU.1. Flat-mounts were examined with a confocal microscope.
Results: Quantitative PCR showed that the amount of mRNAs for PU.1, CD11c, and F4/80 in the retina and RPE/choroid complex were significantly increased in EAU mice compared to CFA mice and normal mice at days 21 and 28 after immunization. The changes in clinical scores of EAU over time followed a similar course of changes in retinal expression of PU.1, CD11c and F4/80 molecules. In the immunostaining of retina flat-mounts, most of PU.1-positive cells were stained with anti-CD11c antibodies. These results suggest that the inflammation degree of EAU is associated with the number and/or the PU.1 level of PU.1-expressing cells migrated into the retina, which are probably DCs and macrophages.
Conclusions: The present study demonstrates that PU.1 expression is detected in the retina and RPE/choroid complex of EAU mice especially in severe stage of retinochoroiditis. These findings suggest that PU.1 may play crucial roles for the development and progression of inflammation in EAU.
Commercial Relationships: Akihiko Umazume, None; Takeshi Kezuka, None; Ryusaku Matuda, None; Naoyuki Yamakawa, None; Takuya Yashiro, None; Chiharu Nishiyama, None; Hiroshi Goto, None

303 - P13-7

Effects of intermittent intraocular pressure elevations on the humoral autoantibody repertoire

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Purpose: In glaucoma, the contribution of an autoimmune component is suspected in the pathogenesis since complex changes of the IgG autoantibody (Aab) repertoire were identified in serum samples of glaucoma patients. A highly dynamic intraocular pressure (IOP) comprising pressure peaks and pressure drops is the main risk factor for glaucoma. The aim of the study was to identify the extent of damage on the axon density of the optic nerve set by recurring IOP elevations and to add alterations to the Aab repertoire using an innovative glaucoma animal model.
Methods: In total, 30 loop adjusted oculopression (LAOP, n=12) manipulations were performed unilateral for one hour per day during six weeks. A silicone loop was set around the eye bulbus of Long Evans rats and adjusted to a target IOP level of 35 mmHg. Contralateral eyes and untreated and age–matched animals served as controls (CTRL, n=12). The extent of axonal damage in optic nerve cross-sections was identified semi-automatically via customized macro in ImageJ after paraphenylenediamine staining. Alterations of the Aab repertoire of serum samples collected before (baseline) and six weeks after first IOP elevation (end) were detected against 42 antigens using microarray analysis.
Results: After six weeks, intermittent IOP elevations led to a cumulative IOP exposure of +737 ± 10 mmHg. The axon density of treated eyes (19266 ± 2366 axons/0.05 mm^2) showed a significant reduction compared to CTRL eyes (22528 ± 2127 axons/0.05 mm^2, $p<0.05$). Furthermore, a significant difference was identified between treated and contralateral eyes (23144 ± 1956 axons/0.05 mm^2, $p<0.05$). Distinct alterations of the IgG Aab repertoire were found six weeks after the first IOP elevation compared to baseline time point. Elevated IgG serum immunoreactivities were observed for ubiquitin, actin, MAPK3, MBP and HSP 27 at the end of the study (all $p<0.01$). Additionally, significantly decreased IgG immunoreactivities could be identified for α-synuclein and HSP 10 ($p<0.05$).
Conclusions: This study demonstrates that intermittent IOP elevations lead to significant damages of the optic nerve. Recurring IOP elevations and resulting axonal damage might effect changes of the humoral IgG autoantibody repertoire. In summary, this innovative glaucoma animal model enables the analysis of the pathological impact of intermittent IOP elevations and the detection of changes of the humoral Aab repertoire.

Commercial Relationships: Julia Teister, None; Oliver Gramlich, None; Theresa Lueckner, None; Maren Kriechbaum, None; Norbert Pfeiffer, None; Franz Grus, None

304 - P14-8

Complement synthesis and propagation by monocytes/microglia in AMD

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Purpose: Complement activation is associated with the pathogenesis of retinal dystrophies such as age-related macular degeneration (AMD), though the events leading to complement synthesis and propagation in the retina are unclear. Here we examined the transcriptome of FACS-isolated monocytes/microglia in a light-induced model of retinal degeneration, using high-throughput sequencing. Additionally, the synthesis of complement genes was correlated in human donor retinas with either 'dry' or 'wet' forms of AMD.

Methods: SD rats were exposed to 1000lux light for 24hrs, after which some animals were kept in dim light for either 3 or 7 days to recover. Animals were then euthanized and retinas homogenised to a single-cell suspension. Samples were stained for CD45, with the resultant CD45+ monocytes/microglia isolated using FACS; high-throughput sequencing was then performed from RNA extracted from each sample. Human donor tissue was collected and cryosectioned from patients with either 'wet or 'dry' AMD. Expression of complement genes was assessed by *in-situ* hybridization, while monocytes/microglia were identified with immunoreactivity for IBA1.

Results: In the light-damage model, sequencing data indicated a significant up-regulation of inflammatory genes within the population of CD45+ monocytes/microglia following 24hrs of damage, which included genes from the complement cascade. Persistent increases in C2, C3, C4, and CFD were observed in CD45+ cells, particularly after 7 days post-exposure. In human donor retinas featuring either wet- or dry- AMD, *in-situ* hybridization showed that C3 is also expressed by monocytes/microglia – stained using IBA1 – which were situated in the inner retina, ONL and subretinal space. This was particularly prevalent within the lesion and surrounding edges, and also occasionally among drusen deposits.

Conclusions: Our data demonstrate that monocytes/microglia contribute to prolonged activation of complement in the degenerative retina. Crucially, we also show that these cells are responsible for synthesizing C3 in both 'wet' and 'dry' forms of AMD. These findings have relevance to the cellular events of complement activation underling the pathogenesis of AMD.

Commercial Relationships: Matt Rutar, None; Riccardo Natoli, None; Krisztina Valter, None; Jan Provis, None

305 - P15-9

Transcription Factor Expression by Human Retinal Endothelial Cells in Response to Tumor Necrosis Factor (TNF)- α and Interleukin (IL)-1 β

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Purpose: Biologic drugs targeting inflammatory cytokines are therapeutic for uveitis, but cytokines are required for health, and blockade may have serious complications. An alternative approach would be to inhibit cytokine-effected transcription of key inflammatory molecules. Uveitis is triggered when leukocytes cross the ocular vascular endothelium. The process is coordinated by endothelial adhesion molecules, particularly intercellular adhesion molecule (ICAM)-1. We studied transcription factor expression by retinal endothelial cells stimulated by TNF-α or IL-1 β.

Methods: Confluent monolayers of human retinal endothelial cells were treated for 60 minutes at 37 ° C and 5% CO_2 with TNF-α (10 ng/ml) or IL-1 β (5 ng/ml) or no cytokine (n = 4-5 cultures/condition). Total RNA was isolated, and integrity was determined using the Agilent 2100 Bioanalyzer. After reverse transcription, expression of ICAM-1, normalized to 18S rRNA, was measured by real-time PCR to confirm biological reactivity of stimulated cells. PrimePCR Transcription Factor Assays (Bio-Rad), designed to detect changes in 86 transcription factors, were run on the CFX Connect real-time thermal cycler. Data were normalized to GAPDH, HPRT1 and TBP, and analyzed with CFX Manager software (v3.1).

Results: RNA integrity numbers ≥ 9.8 indicated high integrity. TNF-α or IL-1 β stimulations resulted in significant 5.4- and 7.0-fold, or 3.5- and 3.6-fold up-regulations of retinal endothelial ICAM-1 by the 2 independent primers sets, respectively ($p \leq 0.005$). Profiling analysis - setting $p \leq 0.01$ - indicated significant increases of up to 5.7-fold in 8 transcription factors in retinal endothelial cell cultures treated with TNF-α or IL-1 β (i.e., IRF1, ATF3, JUNB, ETS1, ETS2, REL, RELB and EGR1); TNF-α -stimulated cells up-regulated an additional 12 factors. JASPAR and NCBI database searches indicated that all 8 common transcription factors had the potential to regulate ICAM-1 gene expression.

Conclusions: Transcription factors linked to ICAM-1 gene expression are increased in human retinal endothelial cells exposed to master inflammatory cytokines. Specific inhibition of one or more of these transcription factors may prove effective in treatment of patients with uveitis while avoiding the complications that limit the use of currently available biologic drugs.

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306 - P16-10

HIF-1 α upregulates ANGPTL4 to promote angiogenesis in Uveal Melanoma

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Purpose: The transcriptional response promoted by hypoxia-inducible factor (HIF)-1 α has been associated with angiogenesis and metastatic spread in uveal melanoma (UM). Expression of one HIF-1 target gene, vascular endothelial growth factor (VEGF) correlates with tumor vascularity in UM, as well as other tumors. However, treatment of patients with therapies targeting VEGF has not proven sufficient alone to prevent UM growth or spread. Here we set out to evaluate the potential role of a novel HIF-regulated gene product, angiopoietin-like 4 (ANGPTL4), in the promotion of angiogenesis in UM.
Methods: UM cell lines were examined for expression of HIF-1 α , VEGF, and ANGPTL4. Expression of HIF-1 α , VEGF, and ANGPTL4 were further assessed by immunohistochemical analysis in UM tissue and quantitated using a UM tissue array. Expression of ANGPTL4 was also examined in vitreous biopsies from patients with uveal melanoma. The role of ANGPTL4 in angiogenesis in UM was assessed using endothelial cell (EC) tubule formation (TF) assays. Inhibition of HIF-1 α , VEGF, and ANGPTL4 was performed using RNAi.
Results: Expression of HIF-1 α was detected in all UM cell lines tested. Hypoxic stabilization of HIF-1 α resulted in the promotion of TF in treated ECs in vitro; this affect was inhibited by blocking HIF-1 α translation, but only partially inhibited by blocking VEGF expression, implicating additional HIF-regulated genes in the promotion of angiogenesis in UM. We demonstrate that HIF-1 α stabilization results in ANGPTL4 mRNA and protein expression in UM cell lines. These results were corroborated in tissue samples from patients with UM. Using a UM tissue array, we further observed expression of HIF-1 α in a majority of tumors in the array. Expression of either ANGPTL4 or VEGF was detected in almost all (99%) of the tumors in the UM tissue array. ANGPTL4 expression was increased more than 50 fold in vitreous biopsies from UM patients at levels that were sufficient to promote angiogenesis in vitro. RNAi targeting ANGPTL4 inhibited the promotion of TF induced by UM cell lines; this effect was additive to RNAi targeting VEGF.
Conclusions: We propose that therapies targeting both VEGF and ANGPTL4 will be necessary to effectively inhibit tumor-induced angiogenesis in patients with UM.
Commercial Relationships: Ke Hu, None; Savalan Farrokhran, None; Murilo Rodrigues, None; Brooks Puchner, None; Laura Asnaghi, None; James T Handa, None; Shannath Merbs, None; Charles Eberhart, None; Silvia Montaner, None; Akrit Sodhi, None

307 - P17-11

Diagnostic evaluation of RTVue-100 Fourier-domain optical coherence tomography in herpetic eye disease

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Purpose: To evaluate the morphologic appearance of keratic precipitate (KP) with RTVue image for the diagnosis of herpetic eye disease.
Methods: Eleven eyes of 10 patients suspected of herpetic eye disease were included in this study. RTVue-100 Fourier-domain optical coherence tomography (RTVue) (optovue, USA) was used to investigate the clinical characteristics of KPs in herpetic eye diseases.
Results: Four patients of 10 cases were polymerase chain reaction (PCR)-positive for herpetic virus gene. Among PCR positive cases, 2 cases were positive for cytomegalovirus, 1 case was positive for herpetic simplex virus and 1 case was positive for Varicella zoster virus. In PCR negative cases, 5 cases exhibited clinical feature of herpetic corneal endotheliitis and 1 case exhibited those of herpetic iridocyclitis. In all cases, the slit-lamp examination revealed whitish and/or brownish KPs extended downward from center to inferior part of the retrocornea. Those KPs also showed various morphologies, such as vaguely-outlined, round, and dendritic appearance. In RTVue examination, KPs were observed as relatively high intensity compared with intensity of cornea. They showed various patterns such as dome-shaped, saw-edged, rectangle-shaped appearance protruded from the retrocornea. Before treatment, KPs were diffusely scattered over the corneal endothelium, but, through the course of treatment, they aggregated each other and deposited dispersedly. Concerning of KPs appearance, no significant difference was observed between virus PCR-positive and negative cases. Surprisingly, in PCR-positive corneal endotheliitis cases, the surface of the corneal endothelium was waving and KPs deposition was not sharply recognized.
Conclusions: The morphology of KPs in various herpetic eye diseases showed characteristic images in slit-lamp and RTVue examination. Notably, the RTVue image of PCR positive corneal endotheliitis cases was different from other eye disease. RTVue examination is non-invasive and repeatable methods for evaluating treatment effectiveness.
Commercial Relationships: Reiko Kobayashi, None; Noriyasu Hashida, None; Shizuka Koh, None; Takeshi Soma, None; Kohji Nishida, None
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308 - P18-12

Virulence factors in Pseudomonas aeruginosa isolates associated with keratitis

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Purpose: *Pseudomonas aeruginosa* is a leading pathogen of blinding keratitis worldwide. In this study, bacterial virulence factors in clinical isolates from patients with *P. aeruginosa* keratitis were examined, and the relationship between virulence factors and clinical features were investigated.
Methods: The study involved 25 subjects including 18 contact lens (CL) and 7 non-CL-related *P. aeruginosa* keratitis. Slit-lamp observations of the cornea were performed on all subjects. Twenty-five clinical *P. aeruginosa* isolates from keratitis were assessed for protease production, elastase production, biofilm formation, bacterial swimming and swarming motility, and genes encoding the type III secretion system (TTSS) effectors (ExoU and ExoS).
Results: Slit-lamp observations showed ring abscess in 9 of 18 CL-related *P. aeruginosa* keratitis. Nine CL related keratitis without ring abscess had a smaller size of focus than a quarter of corneal diameter. Seven non-CL keratitis showed irregular, amebic shaped focus. Virulence factors in *P. aeruginosa* isolates from 9 CL keratitis with ring abscess (ring(+)), 9 CL keratitis without ring abscess (ring(-)), and 7 non-CL keratitis were compared. The rate of positive swimming motility in ring(+) or non-CL was higher in ring(-) ($P<0.05$), while the rate of positive swarming motility in ring(+) was higher in ring(-) or non-CL($P<0.05$). Prevalence of an exoS+/exoU- genotype in ring(+) or non-CL was higher than that in ring(-) ($P<0.05$). There was no significant difference in protease production, elastase production and biofilm formation between ring(+), ring(-), and non-CL.
Conclusions: Swimming motility, swarming motility and TTSS ExoS could play a major role in the determination of clinical feature in *P. aeruginosa* keratitis.
Commercial Relationships: Naoko Oka, None; Takashi Suzuki, None; Naoki Hayashi, None; Naomasa Gotoh, None; Yuichi Ohashi, None

309 - P19-13

Demodex infestation as a potential cause of keratitis

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Purpose: To report keratitis in patients with ocular demodicosis.
Methods: This retrospective study reviewed 24 eyes of 16 patients with refractory keratitis.
Results: The patients included 9 males and 7 females aged 16.8 ± 12.1 years. All cases had been diagnosed as bacterail keratitis, viral keratitis, limbits, peripheral infiltration, and superficial punctate keratopathy but hadn't respond well to tradictional therapies. Demodex mites were detected in all cases while other pathogen cultures were negative. The corneal changes included phlynctenular keratitis in 7 eyes, peripheral infiltration in 7 eyes, peripheral ulceration in 5 eyes, central infiltration in 3 eyes, and superficial punctate keratopathy in 2 eyes. Corneal neovascularization was presented in 21 out of 24 eyes. In vivo confocal microscope revealed increased Langerhan cells infiltration. After using tea tree oil lid scrub, all patients showed dramatic resolution of ocular inflammation while Demodex counts dropped from 4.3 ± 2.5 to 0.4 ± 0.6 ($P< .001$). All corneal signs resolved within 2 weeks except for residual stromal scar in 4 eyes.
Conclusions: Demodicosis should be considered as a potential cause of keratitis. Immune reaction may play an important role in the underlying pathogenic mechanism.
Commercial Relationships: Lingyi Liang, None; Hongmin Ke, None
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310 - P20-14

Long-term Result of Tacrolimus Ointment in Ocular Graft versus Host Diseases

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Purpose: To investigate the efficacy of long-term use of topical tacrolimus ointment in chronic ocular graft versus host disease (GVHD) with severe ocular surface inflammation.
Methods: Topical 0.02% tacrolimus ointment was applied in patients with chronic ocular GVHD with severe ocular surface inflammation (at least grade 2 inflammatory score) with or without topical steroid to inhibit inflammation. This study included patients who were treated with tacrolimus ointment for more than 2 months. The outcomes including inflammatory score, corneal/conjunctival staining, number of inflammatory aggravation, and need for steroid therapy after tacrolimus treatment were evaluated.
Results: A total of 21 eyes in 11 patients with severe inflammation were treated with topical tacrolimus up to 20 months (average 9.4 months), as an additional or single agent to inhibit inflammation. One month after beginning the tacrolimus ointment, clinical outcomes including visual acuity, inflammatory score, and corneal/conjunctival staining were significantly improved. After initial improvement, tacrolimus treatment was not ceased, but maintained. Ocular surface inflammation remained stable and the number of aggravation and the need of steroid decreased during or after tacrolimus ointment treatment. At final followup, all patients reported an improvement in symptoms and signs compared to initial findings.
Except for a mild burning sensation, there were no specific side effects related to tacrolimus ointment.
Conclusions: Considering the chronic course of GVHD, long-term maintenance treatment with tacrolimus ointment could be useful and safe to locally treat ocular surface inflammation and maintain stable ocular surface in chronic ocular GVHD.
Commercial Relationships: Ji Won Jung, None; Da Ham Cho, None; Kyoungyul Seo, None

311 - P21-15

Clinical backgrounds and characteristics of the patients with scleritis during the three years from 2011 to 2014

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Purpose: To evaluate the patients' profiles, clinical features, systemic complications, and treatment modalities of scleritis.

Methods: We previously reported the clinical backgrounds and characteristics of the patients with scleritis (2011 ～ 2013) at WOC2014. This time, we further investigated the statistics of the patients with scleritis by extending the observation periods. The clinical data (sex, onset age, monocular or binocular, type of scleritis, administered treatments, and systemic and local complications) of 102 patients, who were referred to the ophthalmology clinic at Jichi Medical University Hospital during the three school years (2011 ～ 2014) and diagnosed with scleritis, were investigated retrospectively.

Results: The series comprised of 43 males (uniocular:31, binocular:12) and 59 females (uniocular:39, binocular:20). The average onset ages of males and females were 52.0 ± 16.9 and 54.6 ± 16.7 years old, respectively. The types of scleritis were diffuse anterior scleritis (DA) in 32.4% (33/102), followed by episcleritis (EP) in 29.4% (30/102), nodular anterior scleritis (NOA) in 19.6% (20/102), necrotizing anterior scleritis (NEA) in 15.7% (16/102) and posterior scleritis (PO) in 2.9% (3/102). Significantly, the average onset age of the patients with EP was younger than those of the other patients, and that of the patients with NEA was older than those of the other patients. The patients with EP were mostly treated only with topical medication (TM). On the contrary, all patients with NEA and PO were treated with systemic corticosteroid (SC). Moreover, 3 patients (DA:2, NEA:1) were treated with SC and cyclosporine. Two patients with DA were treated with SC and cyclophosphamide. Systemic complications included rheumatoid arthritis in 9 patients (EP:4, NOA:1, DA:3, NEA:1), granulomatosis with polyangiitis in 4 patients (NOA:1, DA:1, NEA:2), and the other ANCA-associated vasculitis in 3 patients (EP:1, NEA:2). As for the ocular complications, 15 patients (EP:1, NOA:2, DA:9, NEA:3) had suffered secondary glaucoma and 5 (NOA:1, DA:2, NEA:2) had received cataract surgery.

Conclusions: We could reconfirm the tendencies, i.e. the patients with EP had fairer prognoses and the patients with NEA required stronger medication. It is quite urgent to develop more effective therapeutic modalities for severe cases.

Commercial Relationships: Atsushi Yoshida, None; Akira Ookubo, None; Hidetoshi Kawashima, None

312 - P22-16

Incidence Rate and Risk Factors for Vision Loss among AIDS-related Cytomegalovirus Retinitis Patients in Southern Thailand

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Purpose: To describe the prevalence and incidence of visual acuity loss and to identify risk factors associated with the development of visual acuity loss among patients with AIDS and Cytomegalovirus (CMV) retinitis.

Methods: A total of 110 patients with AIDS and Cytomegalovirus (CMV) retinitis treated at the CMV retinitis clinic, Songklanagarind Hospital between 2008 and 2011 were included. Clinical data were analyzed by charts review. Main outcomes measurement were the occurrence of visual acuity loss to ≤ 20/50 and ≤ 20/200.

Results: At presentation, 93 eyes (55.7%) had a visual acuity of ≤ 20/50 and 56 eyes (33.5%) had a visual acuity of ≤ 20/200. Over a mean follow-up period of 1.1 years, the incidences of visual acuity loss to ≤ 20/50 and ≤ 20/200 were 0.36/eye-year (EY) and 0.24/EY respectively. At 1 year, the cumulative incidence of visual acuity loss to ≤ 20/50 and ≤ 20/200 were 40% and 27% respectively. In multivariate analyses, large retinitis area was the significant factor associated with visual acuity loss to ≤ 20/50 (hazard ratio [HR] = 4.31, P value [P] =0.001). Older age, not received highly-active antiretroviral therapy (HAART) during follow-up and large retinitis area were significantly associated with visual acuity loss to ≤ 20/200 (HR= 3.19, P=0.017, HR=5.6, P=0.031 and HR= 3.43, P=0.008 respectively).

Conclusions: Prevalence and incidence of visual loss among AIDS patients with Cytomegalovirus (CMV) retinitis in our setting were relatively high. Education of AIDS patients regarding the early presenting symptoms of CMV retinitis and the importance of HAART receiving would be the critical managements potentially minimize the risk of visual acuity loss.

Commercial Relationships: Usanee Seepongphun, None; Wantanee Sittivarakul, None

313 - P23-17

Analysis of complications in patients with HTLV-1 uveitis

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Purpose: Human T cell lymphotropic virus type 1 (HTLV-1) is the first retrovirus described as a causative agent of human disease. HTLV-1 infection affects systemic tolerance and causes inflammation. HTLV-1 uveitis (HU) is a sight threading inflammatory ocular disease caused by HTLV-1 infection, but its complications are still not well known. In this study, we investigate ocular/systemic complications in patients with HU and also analyze the serological relationship between HU and its complications

from the viewpoint of the titer of anti-HTLV-1 antibody.
Methods: The clinical records of 80 patients with HU during 1990-2013 were retrospectively reviewed. Particle agglutination method was used to measure the titer of anti-HTLV-1 antibody in all patients with HU.
Results: The survey showed the most common ocular complications in patients with HU was glaucoma (36.3%), and then cataract (18.8%), dry eye (13.8%). Significantly higher anti-HTLV-1 antibody titers were not seen in HU patients who developed theses ocular complications as compared with those who did not. As for systemic complications, hyperthyroidism (16.3%), HTLV-1 associated myelopathy (HAM) (6.3%), rheumatoid arthritis (5.0%), Sjogren' s syndrome (1.3%), adult T cell leukemia (1.3%) were seen in HU patients. Among the systemic complications, significant increased the titers of anti-HTLV-1 antibody were observed in those who developed HAM, as compared with those who did not ($p<0.05$).
Conclusions: In managing patients with HU, ophthalmic attention should be paid to the development of glaucoma. In case high titer of anti-HTLV-1 antibody is detected in HU patients, systemic follow-up is also necessary for observation of HAM.
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314 - P24-18

Rapid detection of Staphylococcus aureus and Staphylococcus epidermidis in infectious endophthalmitis by SYBR Green RT PCR

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Purpose: To design and validate a real-time polymerase chain reaction (PCR) assay using SYBR Green dye capable of screening vitreous tap specimens for *S. aureus* and *S. epidermidis* 16S rDNA in induced rabbit endophthalmitis.
Methods: The analytical specificity of SYBR Green RT PCR was determined with 16 rabbit vitreous samples with a 5-fold dilution of *S. aureus* and *S. epidermidis* DNA. First group (n =8) received intravitreal injection of both *S. aureus* and *S. epidermidis*, and the second group (n=8) act as negative control. Culture and RT PCR were done in all specimens. Primers used were specific for *S. aureus* and *S. epidermidis* target genes. Optimal melting temperature (Tm) for *S. aureus* was 74-74,4 °C and 76-76,5 °C for *S. epidermidis*.
Results: For *S. aureus*, cultures and PCR were positive in 100% of cases, meanwhile for *S. epidermidis*, sensitivity and specificity of PCR were 100% and 76.92%, respectively, with cutoff value of 31 cycles. Lower specificity for *S.epidermidis* may be associated with bacterial interactions in the vitreous via quorum sensing mechanism. With the use of these methods, *S. epidermidis* detection was improved from 37,5% to 75%. Time needed for DNA extraction to RT PCR from analysis was 4 hours, compared to 24 hours for conventional culture. None of the controls were positive for bacterial etiology by culture or PCR.
Conclusions: SYBR Green RT PCR assays provide faster and more sensitive testing for the laboratory diagnosis of *S. aureus* and *S. epidermidis* endophthalmitis.
Commercial Relationships: Ratu Puri Sastradiwirja, None; Iwan Sovani, None

315 - P25-19

Relationship between Choroidal Blood Flow Velocity and Choroidal Thickness during Systemic Corticosteroid Therapy for Vogt-Koyanagi-Harada Disease

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Purpose: To investigate the relationship between circulation hemodynamics and morphology in the choroid during systemic corticosteroid therapy for patients with Vogt-Koyanagi-Harada (VKH) disease.
Methods: This retrospective case series include eighteen eyes of nine patients with initial onset VKH disease (two men and seven women; average age, 40.8 years) received systemic corticosteroid therapy. Laser speckle flowgraphy (LSFG) and enhanced depth imaging optical coherence tomography (EDI-OCT) were performed before treatment and at 1 week and 1 and 3 months after treatment. The average values of the mean blur rate (MBR) at the macula and the central choroidal thickness (CCT) were compared at each stage.
Results: The changing rates of the average MBR significantly increased at 1 week and later compared with the pretreatment value, concurrently with resolution of serous retinal detachment (SRD) (P = 0.0002 for all). The CCT decreased significantly at 1 week and later compared with the pretreatment value (P = 0.0002 for all). Changes in MBR and CCT during the 3-month follow-up period correlated significantly (R = –0.5913, P = 0.0097). The best-corrected visual acuity at pretreatment correlated significantly with the changing rate of the MBR from 0 to 3 months (R = 0.5944, P = 0.0093) but not with CCT. The height of SRD at pretreatment correlated with MBR change (R = 0.4815, P = 0.0431) but not CCT changes.
Conclusions: Our data suggest that circulatory disturbances and increased thickness of the choroid relate to the pathogenesis of VKH disease with link mutually. LSFG is useful as an index for evaluating the choroiditis activity of VKH disease as well as EDI-OCT.
Commercial Relationships: Kiriko Hirooka, None; Wataru Saito, None; Kenichi Namba, None; Yuko Takemoto, None; Kazuomi MIzuuchi, None; Tomoe Uno, None; Yoshiaki Tagawa, None; Michiyuki Saito, None; Yuki Hashimoto, None; Susumu Ishida, None

316 - P26-20

Application of International Revisied Diagnostic criteriea for Diagnosis of Vogt-Koyanagi-Harada (VKH) disease

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Purpose: Application of International revisied diagnostic Criteria for the diagnosis of Vogt-Koyanagi-Harada disease in Bangladesh

Methods: This prospective study was conducted in the National Institute of Ophthalmology and Hospital, Dhaka, Bangladesh during January 2012 to June 2014. All the bilateral uveitis patients attended the Uvea clinic were scrutinized and revised diagnostic criteria of VKH was applied for diagnosis. A total of 44 patients were included in this study, 24 patients attended the uvea clinic within one months of appearing clinical features regarded as Early group and 20 patients those who attended the uvea clinic one month after appearing of clinical features regarded as Late group.

Results: Out of 44 patients 29 (66%) were male and 15(34%) were female. Mean age was 36 ± 12 years ranging from 16-49yrs. After applying the international revised diagnostic criteria 23 patients of the 24 Early group were diagnosed as incomplete VKH and one diagnosed as probable VKH. During follow-up, 2 patients were finally diagnosed as complete VKH disease. In the Late Group, all the 20 patients initially were diagnosed as incomplete VKH and during follow-up period one patients diagnosed as complete VKH. Vitiligo, poliosis, alopecia appeared after 125(average) days from the onset of sign and symptoms.

Conclusions: The Revised Diagnostic Criterias are effective tool for making the final diagnosis of VKH disease

Commercial Relationships: Pankajkumar Roy, None

Lens - Poster

317 - P27-1

Outcome of surgical management in ectopia lentis of Marfan syndrome

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Purpose: To describe the clinical outcome of surgical management of ectopia lentis in patients with Marfan syndrome
Methods: Patients with Marfan syndrome receiving surgical management of ectopia lentis were retrospectively reviewed. The pre- and postoperative best-corrected visual acuity, change of refraction, axial length as well as operation method and additional procedures were reviewed.
Results: Seventeen eyes of 11 patients received surgical management of ectopia lentis by a single surgeon. Average time at surgery was 23.3 years old (range 4-39 years) with 5 patients (8 eyes) younger than 18 years of age. The mean axial length was 26.76 mm (range 22.29-34.40). The lenses were removed by pars plana lensectomy in 4 eyes, intracaspular lensectomy (ICCE) in 2 eyes, and phacoemulsification through clear cornea wound in 11 eyes. Anterior vitrectomy was performed in all the 6 eyes that received pars plana lensectomy and ICCE with concomitant capsular bag removal and in only 1 eye with phacoemulsification. The phacoemulsification of the subluxated lenses was assisted with 2 iris retractors. In eyes with phacolensectomy, the capsular bags were preserved. Intraocular lens (IOL) was then sutured to the sulcus in 13 eyes. Four eyes were left aphakic. 10 eyes of 6 patients received prophylactic retinal laser before surgery. The BCVA improved from logMAR 0.77 to logMAR 0.26 ($p<0.01$) after the surgery. Fifteen eyes (10 patients) had un-measurable high astigmatism preoperatively. After surgery, the pseudophakic eyes had a mean spherical refraction of -1.39D (range -3.75 to +0.25D), -2.07D of cylinder (range -0.75 to -3.5D), and a spherical equivalent of -2.5D (range -1.0 to -4.25D). The aphakic eyes had a mean cylinder of -1.88D (range -1.5 to -2.25D). One eye developed retinal detachment one year after surgery. IOL subluxation was noted in two eyes and received IOL reposition. No other intra- or postoperative complications were reported. There were no cases of glaucoma or increased intraocular pressure in our series.
Conclusions: Surgical management of ectopia lentis in patients with Marfan syndrome remains challenging. Our procedures of clear cornea phacoemulsification with preserved capsular bag were safe without disturbing the anterior hyaloid membrane. Careful examination of the peripheral retina with pre-operative prophylactic laser if there is thinning or breaks could probably explain the low incidence of retinal detachment.
Commercial Relationships: Yu-Yun Huang, None; Fang-Yi Tsai, None; Shih-Jen Chen, None

318 - P28-2

Area Densitometry of Rotating Scheimpflug Images Interchangeable in Quantification of Intraocular Lens Surface Opacification

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Purpose: Surface opacification on implanted intraocular lens (IOL), such as posterior capsular opacification (PCO) and surface light scattering has been quantitatively analyzed with Scheimpflug image densitometry. Scheimpflug image was captured at a fixed angle in EAS-1000 (Nidek), hitherto, that has been obsoleted. Rotating imaging system (Pentacam, OCULUS) is comprehensively used in topographic analysis, but rarely used for analyzing IOL opacification. This prospective assessment is to examine the efficacy and interchangeability in the use of a rotating imaging system for the area densitometry analysis of implanted IOLs.
Methods: The study comprised 74 eyes receiving foldable IOLs 1 to 18 years (mean, 5.9 ± 5.2 years) ago, including 59 eyes with AcrySof IOLs (Alcon) that could increase surface light scattering over time. After full mydriasis, Scheimpflug images were acquired using the Pentacam and EAS-1000. Full-angle images (Rotation mode) and single images (Single mode) were obtained with the Pentacam. For surface light scattering examination, scattering density in the perpendicular images was analyzed in the central 3 mm diameter area on the anterior surface of AcrySof IOLs. For PCO examination, densities in 4-meridian images were analyzed and averaged as done in the EAS-1000. Correlations with the EAS1000 results were evaluated by linear regression analysis.
Results: On the anterior IOL surfaces of the AcrySof IOLs, density due to surface light scattering was associated between the Pentacam and EAS-1000. Significant and strong linear correlation was found when the EAS-1000 result was up to 170 CCT steps in the Rotation and Single modes ($P < 0.001$, $R^2 = 0.81$ and 0.87, respectively). In the PCO analysis on the posterior surface of other IOLs, there was significant correlations ($P<0.001$) with the R^2 values of 0.77.
Conclusions: Area densitometry with the rotating Scheimpflug imaging was effective for quantitative evaluation of IOL surface opacification, and retained interchangeability with the previous EAS-1000 analysis. Deviation from linear correlation was considered as due to saturation in EAS-1000 measurement, so that wider dynamic range is anticipated with the Pentacam measurement.
Commercial Relationships: Keiichiro Minami, Abbot Medical Optics (C), Chuo Sangio (C), Alcon (C); Masato Honbo, None; Yasushi Kataoka, None; Kazunori Miyata, None

319 - P29-3

Outcome of Cataract Surgery from Outreach Eye Camp of Community Ophthalmology Department, Dr. Rajendra Prasad Centre for Ophthalmic Sciences AIIMS, New Delhi, Review for Five Years of Services

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Study Group: Community Ophthalmology Dr. RPC AIIMS

Purpose: To share the concept of Reach in Programme (RIP) outreach eye care model and presents its outcome for the last five years of services (2009-13).

Methods: **Reach in Programme:** RIP is primarily Public-Private partnership model for outreach cataract intervention services with occasional involvement of Panchayati Raj Institution. Operable cataract patients were identified and referred to base hospital for intervention. **Review methods**, Review of the RIP files and registers as primary sources of the data and cataract surgical records as secondary sources was done during June & July of 2013. Data were collected on those patients who had undergone surgery through outreach eye care services only. Data were entered in Microsoft excel and analyzed descriptively. The outcome was presented in terms of quantity and visual acuity status as a dichotomized optotype with a cut off of 6/60.

Results: A total of 8735 (M=47.5%; F=52.5%) patients were operated in either eye during the five year period (2009-13) out of 9729 admitted. Of the total operated, 19% of them were less than 50 years of age. Very few were conventional surgery (2.2%); rests were phaco-emulsification technique. Preoperatively, 60% had visual acuity <6/60 in the operated eye. Six weeks after surgery, 9.5% had poor visual outcome (<6/60) out of total follow up (415/5241) without any correction. Records of 61 patients were available for poor visual outcome that was attributed to surgical complications. Bullous keratopathy (27), posterior capsular opacity (16), uveitis (10) were maximally documented in decreasing numbers.

Conclusions: Quite an amount of cataract surgery was conducted through our outreach eye care services with reasonably good quality. Strategy needs to be developed to improve follow up compliance and records for poor outcome.

Commercial Relationships: Senjam Suraj Singh, None; Praveen Vashist, None

320 - P30-4

Comparison of intra-ocular pressure changes in Ziemer LDV femtosecond laser platforms with liquid or flat applanation interfaces

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Purpose: To determine the effect of the Ziemer LDV Z8 liquid interface femtosecond laser platform on intra-ocular pressure (IOP) during lens fragmentation and capsulotomy and compare it to the flat applanation Z6 contact system used for LASIK flap creation.

Methods: An ex vivo model to determine real-time IOP change with porcine eyes was undertaken. A 30-g anterior chamber IOP catheter sensor was calibrated prior to commencement of surgery. Comparisons were made between the Z8 (n=17) during fragmentation (8 segment, 5.3mm diameter) for different capsulotomy sizes (4, 5 or 6mm diameter). Sub-group comparison between the 5mm Z8 capsulotomy group (n=6) and the Z6 applanation system using standardized LASIK settings (8.8mm diameter and 110mm depth flap) (n=6) was undertaken.

Results: Procedures were completed successfully without complication. Of 17 eyes undergoing femto cataract with the Z8 laser, the total time to completion was 218 seconds (SD 44). The average IOP was 72.7 (17.7) mmHg with peak elevation at docking (144 mmHg). There was no significant difference between IOP for different capsulotomy sizes, however undertaking a 6mm capsulotomy took significantly longer (248.7s; p=0.02). The Z8 liquid interface system demonstrated a significantly lower IOP than for the Z6 curved applanation system (mean 201.9 mmHg; p<0.0001). During applanation, there was no significant difference in IOP with the Z8 interface but greater fluctuation was seen with Z6 (p<0.0001). Time to compete the procedure was considerably shorter with the Z6 (56s; p=0.0001) due to time taken to determine rhexis size with Z8 (15.4s vs 106.8 vs ; p<0.001). There was no significant difference in the overall area under the curve (mmHgâ- s).

Conclusions: The Ziemer LDV Z8 liquid interface generated a significantly lower IOP compared to the Z6 femtosecond laser but the procedure time was predictably longer. The greatest time differential was seen for larger capsulotomy cuts, in part to ensure that the capsulotomy diameter did not encroach on to the iris during laser. This was reflected in higher areas under the curve at this stage, indicating a sustained but lower relative pressure required to undertake Z8 femto-cataract surgery. The time differences may be abrogated with greater experience with this novel platform and the lower pressures offer a potential advantage than for other femto suction devices.

Commercial Relationships: Geraint Williams, None; Heng Pei Ang, None; Benjamin George, None; Xin Yi Seah, None; Yua Chi Liu, None; Gary Peh, None; Donald Tan, Ziemer (C); Jodhbir Mehta, Ziemer (C)

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321 - P31-5

Postoperative Measurement of the Diameter of Femtosecond Laser-created Anterior Capsulotomy by Using the Implanted Intraocular Lens as a Reference Point

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Purpose: Utilizing femtosecond laser in cataract surgery improves the accuracy in anterior capsulotomy by creating a perfect circle. There are *ex-vivo* investigations on the size of femtosecond-laser assisted capsulotomy by extracting the resected capsule disk. However, its size *in vivo* is rarely evaluated. The purpose of this study was to measure the postoperative diameter noninvasively by using the implanted intraocular lens (IOL) as a reference point.

Methods: This retrospective study comprised 22 eyes of 15 patients who received diffractive multifocal IOL (ZMB00, Abbot Medical Optics and SN6AD1 and SN6AD3, Alcon). Anterior capsulotomy of 5 mm diameter was conducted using LenSx® with SoftFit® contact lens system. Pulse energy and spot size of the laser was 6 μJ and 5 μm respectively, with spot separation at 5 μm horizontally and 4 μm vertically. Centration of the capsulotomy was set according to the pupil center. Perfection and diameter of anterior capsulotomy were observed during surgery. Using intraoperative OCT, the tilt of the lens was evaluated by the difference between the equatorial line of the lens and the horizontal line of the OCT display. At one week postoperatively, the pupil was dilated and the diameter of the laser capsulotomy was measured with reference to the first diffraction ring size of the implanted IOL using anterior segment photographs and Photoshop®.

Results: Capsulotomy was performed in all cases; no tears or capsular tags were observed. The average diameter of capsulotomy was 5.27 ± 0.31 mm (from 4.86 mm to 6.00 mm). The diameter was statistically larger (5.37 ± 0.36 mm) when the lens tilt was more than 2° compared with that of less than 2° (5.16 ± 0.20 mm).

Conclusions: We developed a noninvasive method of measuring anterior capsulotomy size without influence by anterior chamber depth or corneal refraction. Femtosecond laser could produce an accurate and ideal capsulotomy with high reproducibility, and the diffraction ring of the multifocal IOL was effective in measuring accurate diameter of the capsulotomy. The precision of the capsulotomy is expected to increase by ideal eye positioning during OCT measurement.

Commercial Relationships: Manabu Hirasawa, None; Keiichiro Minami, None; Riho Yumiyama, None; Shinichi Ooki, None; Hiroko Bissen-Miyajima, None

322 - P32-6

Femtosecond Laser Cataract Surgery, Results of a prospective, contralateral, comparative single-center study

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Purpose: To compare cataract surgery outcomes with one eye performed using a femtosecond laser and the fellow eye with conventional surgery (manual continuous curvilinear capsulorhexis and phacoemulsification) in terms of capsulotomy parameters, phacotime and functional results.

Methods: In this contralateral, comparative, randomized, prospective, single-centre study 60 eyes of 30 patients with a median age of 72.5 years (range 45 – 82 years) underwent cataract surgery. On one eye of each patient capsulotomy and lens fragmentation were performed with the VICTUS Femtosecond Laser Platform (Bausch+Lomb/ Technolas Perfect Vision). In the fellow eye the manual technique was used. Examinations were performed preoperatively and 1 day, 1 week, 1, 3 and 6 months postoperatively evaluating functional results, flare, endothelial cell count, wavefront measurements as well as slit-lamp findings and IOL photos.

Results: The size and centration of capsulotomies performed with the femtosecond laser were closer to the intended size than in the manual group. Median effective phaco time (EPT) was statistically significantly lower (Wilcoxon p = 0.0107) in the laser group compared to the manually performed surgeries, whereas the average phaco time did not show a statistically significant difference between both groups (7.83 vs. 13.71 seconds). The median endothelial cell loss showed no statistically significant differences. Three months postoperatively median UDVA was 0.17 logMAR (range, 0.72 to -0.12 logMAR) in the femtosecond laser group and 0.08 logMAR (range, 0.96 to -0.20 logMAR) in the manual group. Median CDVA was -0.04 logMAR (range, 0.24 to -0.20 logMAR) and -0.05 logMAR (range, 0.32 to -0.20 logMAR), respectively. No statistically significant difference was found between both groups in terms of visual acuity as well as achieved vs. target refraction (+0.20 vs. +0.36 D). One day and one week after surgery the flare values showed no significant difference (one day, 19.40 vs 13.20 p/ms and one week, 13.20 vs. 13.20 p/ms).

Conclusions: In this contralateral comparative study femtosecond laser treated eyes showed a closer to intended capsulotomy size and significant reduction in EPT whereas flare values were not statistically different between laser and manual technique. Visual outcomes were similar in both groups.

Commercial Relationships: Mike Holzer, Technolas PV (F); Mary Attia, Technolas PV (F); Ramin Khoramnia, Technolas PV (F); Tanja Rabsilber, Technolas PV (F); Gerd Auffarth, Technolas PV (F)

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Clinical Trail: NCT01769313

323 - P33-7

Early clinical outcomes after implantation of a fully diffractive trifocal toric intraocular lens for presbyopia correction

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Purpose: To evaluate the early refractive and visual outcomes among eyes undergoing presbyopia correction using refractive lens exchange with a fully diffractive trifocal toric intraocular lens.

Methods: Ongoing prospective, non-randomized, single-center, clinical study comprised 11 eyes of 11 consecutive patients who had cataract surgery with a fully diffractive trifocal toric intraocular lens (FinevisionToric, PhysIOL, Liege, Belgium). Additional results will be presented at meeting. Main outcome measures, preoperative and 1-month postoperative data on the following, uncorrected and best corrected distance visual acuity, uncorrected and best corrected near visual acuity, manifest and corneal astigmatism.

Results: The mean preoperative manifest spherical equivalent refraction (1.75D ± 0.89) decreased to 0.08D ± 0.32 postoperatively (p<0.0001). The mean pre-operative and postoperative manifest astigmatism was -0.23 D ±0.40 and -0.16D ±0.23 respectively (p=0.60). The mean pre-operativeand postoperative corneal astigmatism on the other hand was -1.0D ± 0.72 and -1.0D ± 0.39 respectively. The mean surgical induced astigmatism was 0.18D ± 0.4.

The preoperative uncorrected distance acuity (UCDA) improved from 0.37 ± 0.28 LogMAR improved to -0.03 ± 0.11 LogMAR postoperatively(p=.0016) while the preoperative uncorrected near acuity (UCNA) improved from 0.80 ± 0.23 LogMAR to 0.06 ± 0.11 LogMAR postoperatiely (p< 0.0001).

The preoperative best corrected distance acuity (-0.03± 0.08 LogMAR) improved to -0.07± 0.08LogMARpostoperatiely(p=.01) while the preoperative best corrected near acuity (0.56± 0.26LogMAR) improved to 0.05± 0.09LogMARpostoperatiely (p= 0.0001).

Preoperatively, only 18% of eyes had UCDA of 20/20 or better. This improved to 73% postoperatively. For UCNA, 100% were 20/63 or worse preoperatively. Postoperative improvement wasnoted as UCNA was 20/20 or better for 73% of eyes. None of the eyes lost two or more lines of best corrected distance and near acuity.

Conclusions: Implantation of a fully diffractive trifocal toric intraocular lens resulted in excellent distance vision as well as significant presbyopia correction with restoration in near vision. No significant safety issues were observed.

Commercial Relationships: Harvey Uy, None; Richard Nepomuceno, None

324 - P34-8

Evaluation of Incomplete Corneal Incision in Femtosecond-Laser Assisted Cataract Surgery

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Purpose: The benefit of applying femtosecond laser in cataract surgery has been reported. Compared with the laser application to the crystal lens, the application to the corneal tissue has rarely been investigated. We retrospectively reviewed the cases with incomplete corneal incision and evaluated their causes in femtosecond-laser assisted cataract surgery.

Methods: LenSx® (Alcon) with SoftFit™ patient interface was used in all eyes. The configuration of primary incision is three plane with 2.2 or 2.4 mm width and that of secondary incision is single plane with 1.0 mm width. The energy setting for corneal application was 6 μJ with spot and layer separations of 6 μm for the primary incision, and spot and layer separations of 5 μm for the secondary incision. The width and length of laser application was planned in real-time imaging, and the depth and penetrating design was planned in the three-dimensional view of optical coherence tomographer. Caution was paid to avoid the senile ring and vessels around the cornea-scleral limbal area. We defined the incomplete incision to be the one which required the blade resection to create the incision into the anterior chamber after the partial separation of the laser applied area with the spatula. The recorded video of surgical microscope was reviewed to investigate the place and the cause of the incomplete incision.

Results: There were 14 eyes with incomplete incision at either the primary or secondary incision among 216 eyes that received femtosecond-laser assisted cataract surgeries. In the cases of the incomplete incision, 7% were temporal primary incision in the right eye, 47% were secondary incision in the right eye, 13% were temporal primary incision in the left eye, 27% were superior primary incision in the right or left eye, and 7% were secondary incision in cases with superior primary incision. In all cases, the corneal incision was completed with the blade resection without any consequent complications. As for the causes of incomplete incision, 20% were due to limbal invasion of the vessels, 6.7% were senile rings, and 66.7% were a combination of both.

Conclusions: In femtosecond-laser assisted cataract surgery, the corneal incision at the 12 o' clock position has a higher risk of incomplete incision due to the vessels and senile rings. To achieve the complete corneal incision with laser application, these areas should be preoperatively confirmed and avoided.

Commercial Relationships: Shinichi Oki, None; Manabu Hirasawa, None; Michiru Tanaka, None; Keiichiro Minami, None; Hiroko Bissen-Miyajima, None

325 - P35-9

Correlation of Cumulative Dissipated Energy and Intraocular Pressure After Routine Phacoemulsification with Posterior Chamber Intraocular Lens Implantation in Patients with Age-related Cataract

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Purpose: The results of the study will further strengthen the importance of the CDE value as a measure of efficiency in phacoemulsification surgery. Eye surgeons and ultimately, the patients are likely to benefit.

Methods: This prospective cohort study using convenient sampling technique comprised 27 eyes of 27 patients with age-related cataract who underwent routine phacoemulsification with posterior chamber intraocular lens implantation under one surgeon using the same technique at Makati Medical Center. Intraocular pressure (IOP) was measured pre-operatively and on follow-up post-operatively at 24 hours, 7 days and 1 month. The correlation between the CDE value and changes in post-operative IOP was evaluated using correlation coefficients and the paired *t*-test.

Results: The mean baseline preoperative IOP was 12.93 + 2.4 mmHg. The mean CDE value was 6.71 + 4.02. The mean post-operative IOP was 18.30 + 3.01 mmHg (24 hrs), 12.50 + 2.99 mmHg (7 days), and 11.52 + 2.24mmHg (1 month). The correlation between CDE value and the post-operative IOP was not significant at a=5%, with p-values of 0.847 (24 hrs),0.126 (7 days), and 0.190 (1 month).

Conclusions: There is no correlation between CDE and IOP after routine phacoemulsification with posterior chamber intraocular lems implantation in patents with age-related cataract.

Commercial Relationships: Pearl Padilla, None

Figure 1. Postoperative IOP course (mmHg)

Table1. IOP (mmHg) from baseline to 24 hours to 7 and 30 days postoperatively

N	Baseline IOP	24 hours post-op	7 days post-op	30 days post-op	Mean CDE
	Mean IOP	Mean IOP	Mean IOP	Mean IOP	
27	12.93 ± 2. 43	18.29 ± 3.01	12.5 ± 2.99	1 1.52 ± 2.24	6.71 ± 4.03

326 - P36-10

Incidence of Bacterial Contamination of Cataract Day-Packs Used in a Tertiary Hospital

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Purpose:

In this study, the investigator aims to determine the incidence of bacterial contamination of cataract day-packs used in a tertiary hospital.

Methods: Culture sampling was done for six days. Samples from the end of the irrigation and aspiration tubings of the cataract pack were obtained at the start of each case. The cataract packs were kept sterile by coveirng it with sterile drape after each case. Specimens were immediately submitted to Microbiology for 24 and 48 hours incubation and analysis.

Results: The use of cataract day-packs was monitored for six days. One culture medium was assigned per case, which was divided into 3 areas for irrigation, aspiration and fluid samples. A total of 32 culture media were used and 96 samples were obtained from all the cases for six days. Out of the 32 cases, 15 used a new cataract pack, which were all negative for growth after 24 and 48 hours of incubation.

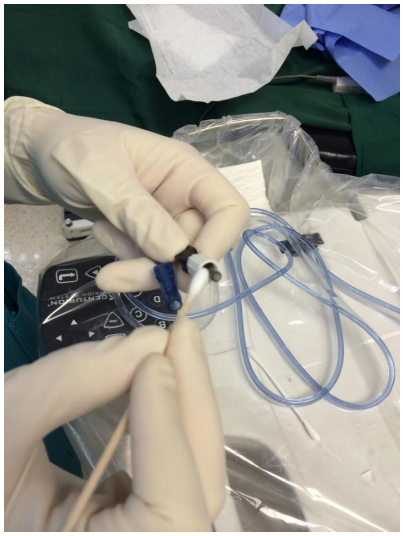
The maximum number of cases that the same cataract day-pack was used was three in both operating rooms. The longest time interval of sampling from the previous case was on the 5th day at 1 hour and 30 minutes in OR 1, and 2 hours and 42 minutes in OR 2; while the shortest time interval of sampling in between cases for both rooms was 6 minutes. From opening of the pack until the last case, the longest time interval was 6 hours and 41 minutes.

Immediately after sampling, the culture medium was sent to Microbiology for incubation for 24 and 48 hours and analysis. All samples submitted from day 1 to 6 from both operating rooms showed negative for bacterial contamination of the packs used. However, media contaminants were identified in some samples from days 2, 3 and 4.

Conclusions: There was no incidence of bacterial contamination of cataract day-packs used in our hospital after three uses and almost seven hours interval from the opening of the cataract pack until the last case. Hence, its use in our hospital remains safe and efficient in delivering the quality of care to patients undergoing cataract surgery.

Commercial Relationships: Aileen Viguilla, None; Sherman Valero, None

1. Sampling of the aspiration tubing



2. Sampling of the irrigation tubing

327 - P37-11

A comparison of keratometry measurements taken with the IOL Master 500 the NIDEK AL-Scan Optical Biometer, and the Oculus Pentacam

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Purpose: Accurate biometry is important for optimising the refractive outcome of cataract surgery. We aimed to compare corneal power measurements taken with three devices, a new non-contact optical biometer (NIDEK AL-Scan Optical Biometer, V1.06.01, Nidek Co Ltd), a new version of the gold standard optical biometer (IOLMaster 500, V. 7.5, Carl Zeiss Meditech), and a Scheimpflug imaging device (Pentacam HR, Oculus, Wetzlar, Germany)

Methods: As part of a wider twin eye study, twin subjects underwent biometry using the three devices. Each subject was examined by the same operator, and scans were performed consecutively in the automated mode. Subjects who had undergone previous corneal or cataract surgery, or who had recently worn contact lenses in the study eye (the right eye), or who had poor quality scans, were excluded. The mean corneal power in dioptres was compared (pairwise comparisons made between instruments using the paired t test)

Results: Scans from 57 subjects were included. Mean (SD) age was 59 (9.9) years. Mean corneal power was 44.17 D and 44.01 D for the IOLMaster 500 and Pentacam respectively. Mean power was 44.29 D and 44.25 D for the AL-Scan reading at 2.4 and 3.3 mm respectively. As expected, pairwise correlations between the instruments were very high (with correlation coefficients of 0.99 or greater), but the differences in corneal power were statistically significant.

Conclusions: All three devices gave broadly similar readings that were highly correlated. However, mean corneal power measures were highest with the AL-Scan and lowest with the Pentacam, with all inter-device differences found to be statistically significant. Whether such differences are clinically significant for cataract surgery would depend on whether the readings altered choice of intraocular lens (IOL) power. IOL powers typically vary by 0.5 D. If choosing an IOL predicted to give an outcome closest to emetropia (SRK/T formula), using IOLMaster 500 or AL-Scan measurements, identical IOLs would be chosen in 52% of our subjects. The IOL chosen with the IOLMaster 500 would be 0.5 D more powerful than that chosen with the AL-Scan in 40%, 1 D more powerful in 2%, and 0.5 D less powerful in 4%

Commercial Relationships: Tariq Ayoub, None; Alex Baneke, None; Mohammed Oomerjee, None; Christopher Hammond, None; Omar Mahroo, None

328 - P38-12

Pattern of Visual Outcome After Nd:YAG Laser Capsulotomy In Patients with PCO and it's relationship to intraocular pressure, A Study of 60 Cases

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Purpose: This study was carried out to evaluate the changes in visual acuity following Nd, YAG laser posterior capsulotomy and to evaluate intraocular pressure before and after performing Nd:YAG laser capsulotomy.

Methods: Study design, Prospective study. Settings, At Islamia Eye Hospital. Sample, A total of 60 patients. Inclusion Criteria, Patients attending at Islamia Eye Hospital with posterior capsule opacification with VA 6/12(no improvement with pin hole) during the period of January 2011 to June 2011,of either sex and aged between 40-85 years.Exclusion criteria:The study will exclude the patients with corneal opacity,glaucoma,posterior segment disease like vitreous opacity,macular disease,optic nerve disease or any other retinopathy causing functional impairmentat of vision.Exposure of interest:Nd:YAG laser. Outcome of interest:Visual acuity for distant and near and its relationship to IOP.

Results: 17 patients(28.33%)had pre-capsulotomy visual acuity 6/36 to 6/60,18 patients(30%)had 6/18 to 6/24,14 patients (23.33%)had 6/60 ,21 patients(35%) with< 6/60 and 4 patients(6.67%)had6/12.After 7 days of capsulotomy 45 patients (75%)gained6/12 or better vision(p-value 0.002 ,which is significant).Visual acuity improved to 6/12 in 58 patients(96.67%) after 30 days with refractive correction(p-value 0.001).Pre –laser mean IOP was 13.133 mm of Hg and post laser mean IOP (within 2 hrs) was 17.006 mm of Hg(p-value significant which is 0.001).All the patients were adviced to use anti-glaucoma medication.Post–laser mean IOP (after 24 hours) was 13.90 mm of Hg(p-value 0.06 which is not significant) and after 7 days the mean IOP was 13 mm of Hg(here p-value is not also significant which is 0.06).

Conclusions: Nd:YAG laser capsulotomy in posterior capsule opacification can substantially improve vision. This improvement can be further augmented with needed refractive correction. And there is significant rise of IOP

after performing YAG LASER capsulotomy.So we should concern about this.Pre-laser and post-laser IOP should be measured.After performing YAG LASER capsulotomy antiglaucoma medication should be given to all patients.
Commercial Relationships: Dr. Farhana Hossain, Green Life Hospital Medical College (E); Prof. Ava Hossain, Green Life Hospital Medical College (E)

329 - P39-13

Optical simulation of water clefts

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Purpose: We have reported that water clefts (WC), which are a type of cataract, caused hyperopia, and increased higher order aberrations. In this study, we investigated the effect of WC on visual function by optical simulation.
Methods: KMU Cataract Classification & Grading System was used for grading of WC, and optical design software LightTools and CODE V (Synopsis) and Navarro model eye to simulate center type WC that occur in the pupillary zone. The physical property of WC was set as water, which was positioned 0.50 mm from the anterior lens surface. WC form was a sphere of radius 0.3mm and 3 cones (height (I), peak (θ)) radially extended from the sphere at intervals of 90 ° , 135 ° , and 135 ° . Spherical power, cylindrical power, total higher order aberration, and spherical aberration were evaluated by altering geometry parameters.
Results: Spherical power, total higher order aberration, and spherical aberration increased with increase in volume of WC, but cylindrical power showed no notable change. Spherical power and total higher order aberration (for 6mm pupil) were 0.15D and 0.31 μ m, 0.45D and 0.97 μ m, 1.16D and 2.59 μ m, respectively under the following conditions; I:0.7mm and θ :10° , I:0.7mm and θ :15° , I:1.0mm and θ :15° .
Conclusions: WC increased spherical power, total higher order aberration, and spherical aberration. It was indicated that WC in central pupillary area caused hyperopia and deterioration in visual function by increase of higher order aberrations. This model is useful for investigating relationships between form of WC and visual function.
Commercial Relationships: Yoriko Takahashi, None; Takushi Kawamorita, None; Yusuke Seki, None; Norihiro Mita, None; Eri Kubo, None; Kimiya Shimizu, None; Hiroshi Sasaki, None

330 - P40-14

Usefulness of absorptive glasses for incipient cataracts

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Purpose: Cataract surgery is mostly performed in the early stage, but some patients prefer to delay surgery as much as possible. We prescribed absorptive glasses to cataract patients with optic symptoms.
Methods: Forty-nine patients (24 men, 25 women) with 0.7 or more corrected visual acuity in both eyes and subjective optic symptoms (glare, dim vision, etc.) were examined. The observation period was between 6 months and 46 months. The glasses were effective when the patients felt improvement of the subjective symptoms. The patients were classified by subjective symptoms and types of cataracts.
Results: Thirty-nine out of 49 patients (80%) reported improvement in symptoms. Regarding classification based on type of symptoms, 21 out of 24 (92%) had "glare," 9 out of 12 (75%) had" dim vision," and 9 out of 13 (70%) had "eyesight deterioration or double vision." The absorptive glasses were effective in 37 out of 47 cases of cortical cataract and in 2 out of 2 cases of nuclear cataract cases. Twenty-four patients preferred the CCP400AC glasses; 14 preferred CCP400TS, and 1 preferred both, all glasses are products of TOKAI OPTICAL CO LTD. Six out of 39 (15%) patients in whom treatment was effective had thought about cataract surgery before this study. One patient underwent the surgery on the 7th months after glasses prescription.
Conclusions: It is important for an ophthalmologist to consider not only visual acuity but also subjective symptoms and state of the everyday life of the patient before recommending cataract surgery. Absorptive glasses weaken the perception of visible light of 400-500 nm, which causes light scattering in eyes, and improve contrast vision. There were no significant relationships between the effectiveness and types of cataract, and subjective symptoms and types of glasses, but 80% patients showed improvement with these glasses. Moreover, 15% patients considered undergoing cataract surgeries. Therefore absorptive glasses are a good option for managing cataract symptoms because unlike surgery, they have no complications such as ocular infection, expulsion hemorrhage, and retinal detachment.Absorptive glasses should be recommended to cataract patients who has more than 0.7 or more corrected visual acuity before surgery.
Commercial Relationships: Michihiro Onishi, None

Retinal Cell Biology - Poster

331 - P41-1

The neoangiogenesis profile of the murine laser-induced CNV mode

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Purpose: We investigated the expression and distribution of neoangiogenic molecules during the development of choroid neovascular (CNV) lesions in a laser-induced mouse model of neovascular age-related macular degeneration (AMD), with particular focus on the choroid of these lesions.

Methods: Lesions were induced on C57Bl6 mice using laser photocoagulation. Animals were sacrificed in a timely maner and eye-cups were dissected from enucleated eyes. Choroids were immunostained for NG2 (pericytes), VEGFR2 (vascular endothelial growth factor receptor 2; sprouting endothelial cells (ECs)), or VEGF with co-staining for CD31 (vascular ECs). lmages of the choroidal membranes were obtained by microscopy. Vascular permeability and cellular recruitment were also determined.

Results: Vascular permeability showed a trend of two events of increased vascular permeability, events concomitant with increased cellular recuitment throughout the time-course. CNV lesions displayed positive staining for the four angiogenic markers studied. The distributions of the angiogenic markers were determined in correlation to the area of neovascularization, as observed by increased CD31 staining. Both NG2 and VEGFR2 displayed a radial progression, while VEGF displayed a uniform distribution across the choroidal membranes of the lesions in the studied times.

Conclusions: Our data shows that the choroidal membranes of CNV lesions recover through a canonical sprouting angiogenesis pathway, in response to a VEGF-mediated signal. ln addition, these membranes display an increasing radial endothelial sprouting with concomitant pericyte recruitment from the laser injury to the exterior of the lesions, in a time-dependent fasion. These results have implications on how to best take advantage of the widely used laser-induced mouse model of CNV, particularly in widows for screening of new antiangiogenic treatments, as well as time frames for evaluation of specific molecular and cellular related results. The results also give insight into how clinical CNV may progress through the various stages of maturation and how drug intervention may affect this process.

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332 - P42-2

The effect of aflibercept on the barrier function of polarized retinal pigment epithelium cells

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Purpose: The purpose of this study was to determine the effect of aflibercept on barrier function of cultured highly polarized human retinal pigment epithelial (HP-hRPE) cells, which can be a mirror of human RPE barrier in vivo.

Methods: HP-hRPE cells were cultured in Boyden chamber by our method (Terasaki et al, PLos One 2013). Twenty four hours after inoculation of aflibercept (400 μg/ml) to the upper chamber, the cytotoxicity on polarized RPE was evaluated by TUNEL stanining and morphological analysis. Barrier function was also measured by transepithelial resistance (TER) of the RPE cells on the Transwells filters. The presence of ZO-1, a tight junction-associated molecule, was determined immunohistochemicallyin RPE cells.

Results: After the treatment of HP-hRPE with aflibercept, the number of TUNEL positive cells was not changed. No apparent morphological cell damage was observed electron- or light microscopically. The distributional change of cell adhesion molecule ZO-1 was not observed, either. In addition, the TER in RPE cells was not significantly changed by exposure to the aflibercept compared to controls.

Conclusions: Aflibercept did not show any toxic change on HP-hRPE in vitro. Considering its great similarity to human RPE in vivo, aflibercept might not have a significant effect on barrier function of RPE in human at least with the clinical dosage.

Commercial Relationships: Naoya Yoshihara, None; Hiroto Terasaki, None; Hiroki Otsuka, None; Shozo Sonoda, None; Taiji Sakamoto, None; Toshio Hisatomi, None; Tatsuro Ishibashi, None

333 - P43-3

The role of Sirt1 for regulating VEGF in human RPE

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Purpose: Age-related macular degeneration (AMD) is a major cause of blindness worldwide. Oxidative stress is known as an important factor for developing AMD through a change of vascular endothelial growth factor (VEGF) expression in retinal pigment epithelium (RPE). Sirtuin1 (Sirt1) works as the protective factor against oxidative stress. However, little is known about the correlation between VEGF and Sirt1 in RPE under

oxidative stress. In the current study, we investigated the role of Sirt1 in the pathogenesis of AMD.
Methods: Cultured fetal RPE cells were incubated in the presence or absence of t-butyl hydroperoxide (tBH). And the VEGF gene expression was examined by real-time RT-PCR. The VEGF protein expression in the supernatant was demonstrated by ELISA. We also treated RPE with tBH following by pretreatment of resveratrol (a Sirt1 activator) or sirtinol (a Sirt1 inhibitor), and examined VEGF gene and protein expression as well.
Results: The VEGF mRNA and protein expression in RPE were significantly increased by tBH comparing with control. The increase of VEGF by tBH was significantly decreased by pretreatment of resveratrol. Conversely, tBH treatment following by pretreatment of sirtinol significantly upregulated the expression of VEGF more than only tBH treatment.
Conclusions: Sirt1 may play an important role in pathogenesis of AMD and Sirt1 may be a new therapeutic approach for treatment of AMD.
Commercial Relationships: Tomoka Ishida, None; Takeshi Yoshida, None; Kosei Shinohara, None; Koutei Ryu, None; Kyoko Ohno-Matsui, None

334 - P44-4

TAK1 inhibition accelerates cellular senescence of retinal pigment epithelial cells, possible new pathway underlies early stages of AMD

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Study Group: Ophthalmology research laboratory. Kaplan Medical Center

Purpose: This study aimed to investigate the role of the transforming growth factor-beta-activated kinase 1 (TAK1) in cellular senescence and apoptosis of the retinal pigment epithelium (RPE) cells, as a model for the development of the dry form of age-related macular degeneration (AMD).
During their life time, RPE cells are subjected to a high level of oxidative stress from several sources, which can contribute to the development AMD. However, the precise mechanism by which oxidative damage involved in AMD is unclear. One possible pathway involves cellular senescence of the RPE cells that share similar feature to cell atrophy. Interestingly, TAK1 was reported to be involved in the response to stress in variety of cells.
Methods: ARPE-19 tissue culture cells were used to study the role of TAK1 in the hemostasis of RPE cells. cells were subjected to FACS analysis, immunofluorescence staining, XTT analysis, western blots analysis. Cells were treated with oxidative stress with or without TAK-1 inhibitor (5Z-7 oxozeaenol). Cells were harvested and subjected to analysis in the different methods.
Results: Inhibition of the TAK1 kinase activity reduced the rate of apoptotic RPE cells and shifted the cells to cellular senescence upon oxidative damage. Moreover, TAK1 inhibition altered the expression of p53, which is the hall-mark of apoptosis, thus affecting the signal-transduction which underlies apoptosis. The aberrant signal transduction led to increase of the senescence phenotype, accompanied by, 1. augmented SA-b-gal expression 2. pigmentary and 3.morphology abnormalities. 4. Augmented secretion of inflammatory chemokine. Finally, the inhibition of TAK1 affected RPE cells similarly to changes that characterize early AMD *in vivo*.
Conclusions: This study demonstrates that TAK1 is involved in the response of RPE cells to oxidative stress. Cells in which TAK1 was inhibited displayed phenotypes similar to those associated with dry AMD. Our results revealed that TAK1 is essential to the maintenance of healthy RPE cells by regulating p53 activity. Lastly, the alternation in the morphology of the senescent RPE cells might affect the structure of the blood retia barrier and induce the development of the later stages of AMD. This data may imply a potential novel approach to maintain the RPE cells, thus may halt the progression of dry AMD
Commercial Relationships: Zeev Dvashi, None; Ayala Pollack, None

335 - P45-5

Characterization of HTRA1 Promoter in Patients with Exudative Age-Related Macular Degeneration

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Purpose: Age-related macular degeneration (AMD) is a leading cause of vision loss and blindness in the elderly. The dry form is more common and accounts for about 85~90% of AMD patients in US, while Japanese AMD patients predominantly progress to wet-form or polypoidal choroidal vasculopathy (PCV). Recent studies have shown HTRA1, a serine protease gene, as major risk factor for wet form AMD. Furthermore, we reported that the Japanese typical wet form AMD patients showed significant association with ARMS2/HTRA1. The purpose of study is to elucidate the function of ARMS2-HTRA1 gene promoter in wet-form AMD patients.
Methods: Human peripheral blood was obtained from patient with control (cataract patient, 228 case) and Wet-AMD (226 case). Genome DNA was extracted from peripheral blood samples using Magtration System 8Lx and DNA sequenced using ABI 3130 Genetic analyzer. ARMS2/HTRA1 promoter activity was measured by luciferase assay. Double strand DNA probe was designed based on the wild-type and mutant sequence and Electrophoresis Mobility Shift Assay (EMSA) was performed. The same probe was used to isolate binding transcription factors and to determine the peptide sequence using liquid chromatography-mass spectrometry (LC-MS/MS). More, We also generated a transgenic (Tg) mouse, which overexpress mouse Htra1 and human ARMS2 in the entire body and observed the pathological change by fundus observation, fluorescein angiography, indocyanine green angiography and optical coherence tomography over 12 month after birth.
Results: The promoter sequence experiment showed that a great number of AMD patients had specific indel mutation in 3.8 Kb upstream of HTRA1 gene. 2~3-fold increase of promoter activity was observed in indel HtrA1

promoter compared to control sequence. Furthermore, we detected indel specific binding factors using EMSA and LC-MS/MS. These results suggest that Htra1 gene expression is influenced by transcription factor specifically binding to this region. And more, using transgenic mice ubiquitously overexpressing mouse HTRA1 using the chicken actin promoter, continuous induction of HTRA1 in vivo was shown to lead to CNV, similar to wet AMD patients.

Conclusions: Human HtrA1 expression is enhanced by AMD specific indel mutation in the promoter region of HtrA1 gene. Specific transcription factor, which likely to be involved in this enhancement was isolated and peptide sequence determined.

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336 - P46-6

Enhanced differentiation of mouse induced pluripotent stem cells towards retinal pigment epithelium cells by using low-level laser irradiation

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Purpose: Currently, the efficiency of pluripotent stem cells differentiated into mature RPE is still low. Low-level laser irradiation (LLLI) has been suggested to promote the proliferation and differentiation of stem cells. The aim of the present study is to evaluate the effect of LLLI (635 nm) on differentiation of mouse iPSCs into mature RPE.

Methods: Mouse iPSCs were seeded in RPE differentiation medium and received LLLI (2J/cm^2) daily. Controls were sham irradiated. After several days of culturing and laser irradiation, the different stage of cells were harvested and prepared for further analysis. Cell differentiation was assessed by immunostaining, quantitative RT-PCR, western blotting, and flow cytometry assay.

Results: When power density is 0.78mw/cm^2, the optimal dose of laser irradiation is 2J/cm^2. After 8 days of differentiation, LLLI significant increased the number of Nestin and β III-tubulin -positive colonies. The mRNA level of neuronal progenitor marker was also up-regulated. At the later stage of differentiation, more polygonal RPE cells were observed in LLLI group than the control group. The mRNA and protein level of mature RPE cells were also enhanced. Phagocytosis assay also confirmed this result.

Conclusions: LLLI significantly increased the efficiency of iPSCs differentiation, promote the RPE progenitors cells maturation and decrease the risk of tumor formation. Such results may be used in vitro as a prerequisite prior to differentiation in order to improve current protocols of cell therapy.

Commercial Relationships: Wentao Wu, None; Chun Zhang, None

337 - P47-7

Suppression of transient receptor potential canonical channel 4 inhibits vascular endothelial growth factor-induced retinal neovascularization

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Purpose: Based on the importance of canonical transient receptor potential (TRP) channels in VEGF signaling, we investigated whether blockade of TRP channels inhibits retinal neovascularization in the mouse model of oxygen induced retinopathy.

Methods: The expressions of TRP channel isoforms on mouse retina and human renal microvascular endothelial cells (HRMECs) were evaluated by RT-PCR. The anti-angiogenic activity of TRP channel inhibitors and calcium ion chelators was evaluated by vascular endothelial growth factor (VEGF)-induced proliferation, migration and in vitro tube formation assay of HRMECs. In the mouse model of oxygen induced retinopathy, calcium ion level was evaluated by TOF-SIMS, and retinal neovascularization was evaluated after intraocular injection of TRP channel inhibitors and calcium ion chelators, whose effect on MAPK signaling pathway was evaluated by Western blot analysis.

Results: All seven TRPC channels were expressed in mouse retina. TRPC4 channels were chosen for further analysis based on their upregulation on hypoxic retina according to the GEO database under the identifier GSE19886. Interestingly, TRPC4 suppression by intravitreal injection of siRNA against mTRPC4 significantly inhibited retinal neovascularization. To further investigate the effect of TRPC4 suppression on neovascularization, human retina microvascular endothelial cells (HRMECs) that are responsible for initiating neovascularization in response to increased VEGF in OIR retina were transfected with siRNA against TRPC4. As we have expected, suppression of TRPC4 effectively inhibited VEGF-induced migration and tube formation as well. Further evaluation on VEGF signaling pathway discovered that VEGF-induced activation of ERK, p38 MAPK and AKT signaling pathways were inhibited by suppression of TRPC4.

Conclusions: These findings suggest that suppression of TRPC4 could be an alternative therapeutic option for VEGF-induced retinal neovascularization.

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338 - P48-8

Different Distributions of M1 and M2 Macrophages in the Mouse Model of Laser-induced Choroidal Neovascularization

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Study Group: Ophthalmology

Purpose: Choroidal neovascularization (CNV) is a serious complication of age-related macular degeneration (AMD). The purpose of the study was to investigate the roles played by M1 and M2 macrophages in the laser-induced CNVs in mice.

Methods: CNVs were induced by laser photocoagulation of the retina in adult mice. The expressions of the mRNA of CD80 and CD86, M1 macrophage markers, CD206, a M2 macrophage marker, and F4/80, a pan macrophage marker, were examined by real-time RT-PCR. Immunohistochemical studies were used to determine the location of the macrophages. Aflibercept was injected into the vitreous to determine the effect of the blockage of VEGF on the recruitment of macrophages.

Results: The expression of the mRNA of M1 macrophage markers increased more than that for the M2 markers in the RPE-choroid complexes after laser treatment, although both M1 and M2 markers increased significantly at Day 3. In contrast, M2 macrophages increased mainly in the retinas and reached a peak at Day 5. Immunohistochemistry showed that the increase of CD206-positive cells existed mainly on the retina, while the CD80-positive cells were located around the site of CNV at the choroid-RPE interface. Intravitreal aflibercept did not significantly affect the mRNA expression of M1 and M2 macrophage markers in the RPE-choroid complexes.

Conclusions: Both M1 and M2 macrophages were activated in response to laser-induced CNV. M1 macrophages were located mainly around the CNVs in the choroid, and M2 macrophages were mainly distributed on the retinas. These findings indicate that M1 macrophages may play a more direct role in inhibiting the laser-induced CNVs in mice.

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339 - P49-9

Alteration of N-glycan Profiles in Diabetic Retinopathy

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Purpose: Glycans are biopolymers bearing biological information and regarded as the third major class of cellular macromolecules, following nucleic acids and proteins. There are two types of glycans that attach to peptide chains; *N*-glycans that bind to nitrogen (N) in the side chain of asparagine residues and *O*-glycans that bind to the hydroxyl group (O) in the serine or threonine residues. So far, using recent-established methods for *N*-glycan analysis structural alterations of *N*-glycans, *e.g.*, increase of sialic acids, in plasma have been reported in patients with systemic diseases such as malignant tumor. However, due to the technical difficulties, little is known about the intraocular *N*-glycan alterations in ocular diseases. In this study, we sought to analyze the alteration of vitreal *N*-glycans in patients with proliferative diabetic retinopathy (PDR).

Methods: Plasma and vitreous samples were collected from 17 patients (10 females and 7 males) with PDR, and 17 patients (8 females and 9 males) with idiopathic macular hole or epiretinal membrane (non-DR). The vitreous samples were collected without dilution at the start of the pars plana vitrectomy. Profiles of *N*-glycans were analyzed by glycoblotting-based high throughput protocol, which we recently developed.

Results: The concentration of *N*-glycans in the vitreous of PDR (495.5 ± 37.4pmol/100 μ g protein) was significantly higher than those of non-DR (142.7 ± 30.8pmol/100 μ g protein, $P<0.001$), whereas there was no difference in plasma *N*-glycans between PDR and non-DR. In addition, the content ratio of *N*-glycans with sialic acids increased in the vitreous of PDR (68.7 ± 1.8%) compared with those in non-DR (61.8 ± 2.7%, $P<0.05$), whereas there was no significant change in plasma.

Conclusions: Our data suggest the structural alterations of *N*-glycans including increase of sialylation in the vitreous of PDR.

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340 - P50-10

Toll-like receptor 4 is implicated in high-fat diet induced activation of macrophage/microglial cells and DNA damage of retina

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Purpose: Toll-like receptor 4 (TLR4) signaling pathway is involved the chronic inflammation, insulin resistance

associated with obesity and diabetes mellitus. We hypothesized that metabolic disorders associated with overnutrition will trigger the inflammatory responses via TLR4 in retina

Methods: C57BL/6 mice of wild type (WT) and TLR4 knockout (TLR4KO) were raised with chow diet (CD) as control or high-fat diet (HFD) for 6 months. The expression of TLR4, macrophage/microglial (CD11b+ and CD45+) cells, and DNA damage with phosphorylated histone H2AX (γ H2AX) expression were assessed by immunofluorescence studies.

Results: HFD induced obesity, glucose intolerance in mice after 6 months. TLR4 expression was found in vascular pericytes at the inner retina. In retinal ganglion cell layer and inner plexiform layer of HFD WT mice, the number of CD11b+, CD45+ and γ H2AX+ cells were higher (203%, p = 0.001; 191%, p = 0.011; 236%, p = 0.009) than those in the CD-fed mice. On the contrary, TLR4KO mice were relatively spared from the effect of HFD. When compared with CD, HFD induced insignificant increases in CD11b+ (120%, p = 0.404), CD45+ (133%, p =1.000), and γ H2AX+ (127%, p = 1.000) cells in retina of TLR4KO mice.

Conclusions: Our results showed that HFD induces macrophage/microglia infiltration in retina. The activation of inflammatory cells and associated damage was reduced in TLR4KO mice. Innate immunity response via TLR4 signaling pathway could be involved in the pathogenesis of diabetic retinopathy.

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341 - P51-11

SP1 expression in AGEs exposed rat retinas and effects of neurotrophic factors

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Purpose: To determine the effect of advanced glycation end-products (AGEs) on neurite regeneration and on the regenerative effects of different neurotrophic factors (NTFs) on rat retinal explants.

Methods: The NTFs studied were; neurotrophin 4 (NT-4), hepatocyte growth factor (HGF), glial cell line-derived neurotrophic factor (GDNF), and tauroursodeoxycholic acid (TUDCA). The isolated retinas of adult SD rats were cultured in three-dimensional collagen gels and incubated in 6 types of media, 1) serum-free control culture media; 2) 100 μ g/ml AGEs-BSA media; 3) AGEs-BSA+100 ng/ml NT-4 media; 4) AGEs-BSA+100 ng/ml HGF media; 5) AGEs-BSA+100 ng/ml GDNF media; or 6) AGEs-BSA+100 μM TUDCA media. After 7 days, the number of regenerating neurites was counted. The explants were immunostained for specificity protein 1 (SP1).

Results: The numbers of neuritis were fewer in retinas incubated with AGEs than in control media. All of the NTFs increased the number of neurites, and the increase was more significant in the NT-4 group. The number of SP1 immunopositive cells was higher in retinas exposed to AGEs than in the control media. All of the NTFs did not significantly affect the SP1 overexpression in AGEs-exposed retinas.

Conclusions: SP1 overexpression may be related to neuronal regeneration in retinas incubated in neurotrophic factors. This indicates atherapeutic potentials of the neurotrophic factors as axoprotectants in AGEs-exposed retinas.

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342 - P52-12

Protective effects of systemic treatment with methylprednisolone in a rodent model of non-arteritic anterior ischemic optic neuropathy (rAION)

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Purpose: This study investigated the protective effects of the systemic administration of steroids on optic nerves (ON) and retinal ganglion cells (RGCs) in a rodent model of non-arteritic anterior ischemic optic neuropathy (rAION).

Methods: We induced rAION using rose bengal and argon laser irradiation in a photodynamic procedure on the optic discs of rats. The treated groups received methylprednisolone (MP) via peritoneal injection for 2 weeks. The control group received intraperitoneal injections of phosphate-buffered saline (PBS) post-rAION. Rats were euthanized at 4 weeks post infarct. Density of retinal ganglion cells (RGCs) was counted using retrograde labeling of Fluoro-gold. Visual function was assessed by flash visual-evoked potentials (FVEP) at 4 weeks. TUNEL assay in the retinal sections and ED1 stain in the optic nerve were investigated.

Results: At the 4th week post-infarct, MP treatments significantly rescued the RGCs (mm^2) in the central retinas (1920 ± 210, p<0.001) and mid-peripheral retinas (950 ± 240, respectively, p=0.018) compared with those of the PBS-treated rats (central, 900 ± 210 and mid-peripheral, 440 ± 180). Functional assessment with photopic flash visual-evoked potentials demonstrated that P_1 latency (ms) was shortened in the MP group compared to the PBS group (108 ± 14 and 147 ± 9, respectively, p<0.001). In addition, the P_1 amplitude (uV) was enhanced in the MP group compared to the PBS group (55 ± 12 and 41 ± 13, respectively, p<0.05). TUNEL assays showed a decrease in the number of apoptotic cells in the RGC layers of MP-treated retinas compared to the PBS-treated group (p<0.05). ED1 positive cells (/HPF) were significantly decreased in the ONs of the MP group compared to the PBS group (p<0.001).

Conclusions: In conclusion, systemic administration of MP had neuroprotective effects on RGC survival and ON function in the rAION animal model.

Commercial Relationships: Tzu Lun Huang, None; Rong Kung Tsai , None

343 - P53-13

In vivo imaging of stress responses in retinal ganglion cells using AAV2-mediated delivery of pathway-specific promoter driven reporters

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Purpose: This study aimed to assess the utility of Adeno-associated virus (AAV)-mediated delivery of various pathway-specific promoter-driven reporters *in vitro* and to noninvasively study the stress responses in retinal ganglion cells (RGCs) using these viruses *in vivo* in a murine model of optic nerve injury.

Methods: Promoters that drive specific element of various stress pathways, i.e., ATF6, NF-kB, hypoxia response element (HRE), p53 promoter and others, were fused with enhanced green fluorescent protein (EGFP) cDNA, and packaged into AAV2. The efficacy of each of these AAV2 containing reporter EGFP was assessed *in vitro* using a specific activator of the related stress pathway. Then the virus was injected intravitreally into the eyes of adult C57BL6 mice. Four weeks later, each animal received an optic nerve crush injury, after which the promoter activation level was monitored *in vivo* through the detection of EGFP fluorescence by *in vivo* confocal ophthalmoscopy.

Results: *In vitro* assessment of the promoter activity showed detectable increases in the activation of all promoters after specific stress induction. In principle, this indicates that all of the reporter constructs delivered by AAV2 respond properly to the specific stress. *In vivo* assessment of the promoter activity in mice after optic nerve crush injury revealed mixed results. The NF-kB and p53 promoters were quiescent before the injury and were least responsive to the stress in RGCs. Meanwhile, the HRE promoter showed some transcriptional activity in RGCs before the injury but the activity changed little after the stress. On the contrary, the ATF6 promoter was activated only after optic nerve crush injury.

Conclusions: AAV-mediated delivery of various stress response reporter followed by *in vivo* imaging is a powerful strategy to characterize molecular pathways involved in retinal diseases. Only the ATF6 promoter that probes the endoplasmic reticulum (ER) stress pathway was shown to be activated by optic nerve crush injury. This results support the results of recent comprehensive gene expression studies demonstrating that the ER stress is the most significantly up-regulated stress pathway in this injury model.

Commercial Relationships: Koji Nishiguchi, None; Kosuke Fujita, None; Satoru Tsuda, None; Yusuke Fujii, None; Kotaro Yamamoto, None; Kota Sato, None; Masayuki Yasuda, None; Toru Nakazawa, None

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344 - P54-14

Effects of Intervention timing with systemic methylprednisolone in a rat model of anterior ischemic optic neuropathy (rAION)

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Purpose: Our studies have demonstrated that systemic methylprednisolone treatment protects retinal ganglion cells (RGCs) from death after rAION. The aim of this study is to investigate whether different intervention timings with systemic methylprednisolone will have different therapeutic effects after rAION.

Methods: We induced rAION in 60 adult Wistar male rats using rose bengal and argon laser irradiation in a photodynamic procedure on the optic discs of rats. Rats were divided into five groups, those are shame, rAION with PBS, rAION with intraperitoneal injections of methylprednisolone starting at 1 day, 1 week and two weeks after induction of rAION. The treated groups received methylprednisolone (MP) via peritoneal injection for 2 weeks. The control group received intraperitoneal injections of phosphate-buffered saline (PBS) post-rAION. At the 4th weeks post-infarct, functional assessment with photopic flash evoked-potentials were measured in rats and the RGC survival rate was measured in the flat-mounted retinas using retrograde labeling of RGCs with Fluoro-gold injections.

Results: In the central retinas,the RGC density was 2360 ± 240/mm^2 in the shame group. At 4 weeks after rAION, the RGC density was 820 ± 190/mm^2 (rAION and PBS-treated rats),2050 ± 160/mm^2 (rAION and MP treated at 1 day; $p<0.05$ comparing with 1 week and 2 weeks) , 1230 ± 590/mm^2 (rAION and MP treated at 1 week)and1230 ± 550/mm^2 (rAION and MP treated at 2 week) respectively. In flash visual-evoked potentials (FVEP) the latency of P1 wave was 74 ± 5ms (sham group), 145 ± 8ms (rAION and PBS-treated rats), 108 ± 14ms (rAION and MP treated at 1 day; $p<0.05$ comparing with 1 week and 2 weeks), 160 ± 20ms (rAION and MP treated at 1 week), 166 ± 23ms (rAION and MP treated at 2 week) respectively.

Conclusions: Systemic pulse therapy with methylprednisolone has neuroprotective effects on RGC mophometry and visual function preservation in a rAION model, as demonstrated by RGC density and FVEP evaluations. And the MP treatment starting at one day has better protection than that starting at one or two weeks after rAION induction. Early treatment with pulse systemic methylprednisolone has bettter therapeutic effects on rAION.

Commercial Relationships: Rong-Kung Tsai, None; Chung-Hsing Chang, None

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345 - P55-15

ATP6AP2/(pro)renin receptor contributes to glucose metabolism via stabilizing the E1 β subunit of pyruvate dehydrogenase complex

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Purpose: ATP6AP2, also called (pro)renin receptor, was originally identified as a component of vacuolar H^+-ATPase (v-ATPase), a multi-subunit proton pump involved in diverse and fundamental cellular physiology, and in recent years has attracted growing attention as an activator of tissue renin-angiotensin system (RAS). In addition to its v-ATPase-mediated and tissue RAS-related functions, we recently reported that Atp6ap2 is required for laminar formation during mouse retinal development. In this study, we aimed to elucidate the biological role of Atp6ap2 in the mature mammalian retina.

Methods: Immunoprecipitation and mass spectrometry (IP/MS) experiments were performed to identify candidate proteins interacting with Atp6ap2. To validate the IP/MS results, we carried out known-bait/known-prey yeast two-hybrid and immunofluorescence analyses. Using siRNAs against *ATP6AP2*, we investigated the biochemical function of ATP6AP2 bound with putative interacting proteins in RPE cells.

Results: Pyruvate dehydrogenase (PDH) complex, a key enzyme in energy metabolism linking glycolysis to the tricarboxylic acid cycle, was identified as Atp6ap2-interacting proteins by IP/MS analysis. Yeast two-hybrid assays demonstrated direct molecular binding between ATP6AP2 and the PDH E1 β subunit (PDHB). Pdhb immunoreactivity was ubiquitously detected and co-localized with Atp6ap2 in mouse retinal layers including the retinal pigment epithelium (RPE). Interestingly, siRNA-based *ATP6AP2* knockdown in RPE cells reduced PDH enzymatic activity as well as acetyl-CoA levels while increasing lactate levels, showing a predilection to anaerobic glycolysis. ATP6AP2 protected PDHB from phosphorylation, thus regulating its protein stability and the resultant enzymatic activity. Downregulated PDH activity due to *ATP6AP2* knockdown suppressed glucose-stimulated oxidative stress generation in RPE cells.

Conclusions: Our present data unraveled the novel function of ATP6AP2 associated with PDH activity as a PDHB stabilizer, contributing to the energy-generating pathway of glucose metabolism.

Commercial Relationships: Atsuhiro Kanda, None; Kousuke Noda, None; Susumu Ishida, None

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346 - P56-16

Lutein's effect on repairing the tight junction of the retinal pigment epithelium induced by light exposure

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Purpose: Oxidative stress that induces pathological condition in the retinal pigment epithelium (RPE) is involved in the development and progression of age-related macular degeneration (AMD). It is well-accepted that the light exposure is one of the AMD risks, and lutein, an anti-oxidative nutrient supplement, can prevent AMD progression. In this study, we evaluate the influence of light exposure and the effect of lutein in the RPE.

Methods: Six-week-old BALB/c mice were exposed to light at 3000 lux for 3 hours, after 12 hours of dark-adaptation. Each animal was given an intraperitoneal injection of lutein (100mg/kg) or a vehicle 12 hours after the light exposure. Flat mount RPE samples were immunostained with anti ZO-1 antibody for evaluating tight junction. The ROS level was measured using DCFH-DA, and SOD activity was by SOD Assay Kit in the RPE-choroid complex sample. ARPE-19 cell line was treated with 25 μM and 50 μM lutein for 3 hours to measure SOD activity.

Results: Light exposure disrupted staining pattern of ZO-1 12 hours after light exposure, which was still observed at 48 hours in the vehicle treated mice. However, in the mice treated with lutein, the disruption was already recovered by 48 hours after light exposure. The ROS level that was increased in the RPE-choroid of the vehicle treated mice was already suppressed by lutein treatment 24 hours after light exposure. We found that lutein treatment upregulated SOD activity both in the RPE-choroid 24 hours after light-exposure, and ARPE-19 cells with 3h of treatment.

Conclusions: The Lutein repaired the light-induced disruption of the tight junction in the RPE, reducing ROS and inducing SOD activity in the RPE-choroid. Lutein may have induced SOD activity as well as scavenged ROS in the light exposed RPE to promote the repair of tight junction.

Commercial Relationships: Mamoru Kamoshita, None; Toshihide Kurihara, None; Norihiro Nagai, None; Hideto Osada, None; Saori Kobayashi, Wakasa Seikatsu Co.Ltd (E); Kazuo Tsubota, None; Yoko Ozawa, Wakasa Seikatsu Co.Ltd (F)

347 - P57-17

C1q increases ganglion cell survival in adult rat retinal explants in vitro - compliments of the complement system

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Purpose: The classical complement cascade, part of the innate immune system, has recently been discovered to become reactivated during disease in mature CNS tissues. In the retina, this has been observed in models of glaucoma, where complement component C1q becomes upregulated in retinal ganglion cells. Speculation as to the specific role of C1q in retinal pathology ranges from maintenance of synaptic plasticity, to promotion of neuronal survival through the removal of apoptotic cells and possibly, when dysregulated, to be a cause of neurodegeneration. The purpose of this study was to explore the effects of C1q in an in vitro retinal injury model, with a specific focus on ganglion cell survival and glial cell activity.

Methods: Full-thickness retinal sheets were isolated from adult rat eyes. 6x6 mm retinal explants were cultured for 12h and 2 days in vitro (DIV) using a previously established protocol, with and without C1q (50nM) added to the medium. Adult rat eyes fixed immediately after enucleation were used as a baseline in vivo control. The explants were analyzed morphologically using hematoxylin and eosin staining (H&E), immunohistochemistry with antibodies directed against neuronal markers, microglial cells (Iba1), several markers of Müller cell gliosis, C1q, and apoptosis (TUNEL labeling).

Results: NeuN-labeling of ganglion cells revealed a significant ($p<0,001$) increase in cell survival in C1q-treated cultures at both time points, although overall cell death as measured by TUNEL labeling was unaffected. GFAP and C1q expression was significantly reduced ($p<0,001$) in C1q-treated explants at both time points compared to control explants. Iba1 labeling revealed no difference in microglial activity. Carbonic anhydrase expression was significantly elevated in 2 DIV control specimens compared to C1q-treated explants, whereas GS expression was found to be significantly elevated in controls at 12h compared to C1q-counterparts.

Conclusions: C1q treatment significantly increases ganglion cell survival and attenuates Müller cell reactivity after insult. These changes do not affect the overall cell death, and are not mediated by microglia. This suggests a modulatory role of the complement system of Müller cell gliosis after retinal injury, and provides insight into the contribution of Müller cell reactivity to ganglion cell death.

Commercial Relationships: Linnea Taylor, None; Karin Arnér, None; Fredrik Ghosh, None

348 - P58-18

Ultrathin silk fibroin membranes as prosthetic Bruch's membrane

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Purpose: To evaluate the potential of membranes fabricated from *Bombyx mori* silk fibroin to act as an appropriate Bruch's membrane substitute. The evaluation of novel biomaterials is an important step in the ongoing development of a viable RPE cell therapy option for degenerative diseases of the ageing retina. Our central hypothesis is that the structural and mechanical properties of silk fibroin can be exploited to mimic Bruch's membrane, and support RPE cell functionality.

Methods: Cultures of human RPE cells (ARPE-19 cells grown in Miller's medium) were established on the apical surface of 3-µm thick membranes fabricated from silk fibroin, or a commercially available polyester membrane (0.4-µm porous Transwell® insert). Fibroin membranes were suspended in custom-designed chambers to allow the establishment of a polarised epithelial monolayer in long-term culture (4 months). Cell attachment was facilitated by pre-coating both membrane types with an ECM-blend derived from human placenta. The phagocytic ability of the RPE cells was tested using vitronectin-coated microspheres (2-µm diameter FluoSpheres), and growth factor production and secretion was measured using commercial ELISA kits for VEGF and PEDF. Electron microscopy was used to visualise the development of functional morphology i.e. apical microvilli, basal infoldings and tight junction establishment, and immunocytochemistry with confocal microscopy was used to localise specific functional proteins within the RPE cell monolayers.

Results: Cultures of RPE cells on both membrane types developed a tight, cobblestoned morphology, and displayed selective phagocytic activity and appropriate growth factor secretion of VEGF and PEDF. Electron microscopy revealed the formation of functional morphology, and appropriate localisation of RPE-specific proteins was visualised by immunofluorescence. The ultrathin silk fibroin membranes maintained membrane integrity for the duration of culture time without issue, as expected.

Conclusions: Ultrathin membranes of silk fibroin can be fabricated to mimic Bruch's membrane, and support RPE cell functionality, comparable to commercial polyester membranes. These results (added to the already established mechanical properties and biocompatibility of silk fibroin) suggest an ultrathin silk fibroin membrane is an attractive biomaterial option for use as a Bruch's membrane substitute.

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349 - P59-19

Neuroprotective effect of Rapamycin in Lipopolysaccharide (LPS) -induced uveitis and retinitis

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Purpose: Rapamycin is an inhibitor of PI3K/Akt/mTOR pathway that is widely used in the medical field today. It has immunosuppressive activity, smooth muscle growth inhibitory activity, and anti-cancer effects. However, pharmacological action of rapamycin in the ophthalmic field is not clear. In this study, we investigated the effect of rapamycin on the visual function in lipopolysaccharide (LPS) -induced uveitis and retinitis model mice.

Methods: LPS was injected into the peritoneal cavity of male C57BL/6 mice at the age of 6 week. Then, rapamycin was administered by intraperitoneal injection 1 hour after LPS injection. Visual function was measured by the electroretinogram (ERG) 24 hours after LPS injection. We also measured the levels of protein and mRNA of rhodopsin using immunoblot and real time-PCR analyese in each group, respectively.

Results: Compared to the control group, the amplitude of the a- and b- waves in ERG were reduced and the level of rhodopsin protein was reduced in the vehicle treated LPS-induced uveitis and retinitis models, but these changes were significantly suppressed in the rapamycin treated LPS-induced uveitis and retinitis group. The mRNA levels of rhodopsin showed no changes in all groups.

Conclusions: Rapamycin treatment inhibited the reduction of rhodopsin protein in a post-transcriptional manner. Further studies may help understand the pathogenesis of retinal inflammation.

Commercial Relationships: Tomohiro Okamoto, None; Mamoru Kamoshita, None; Kazuo Tsubota, None; Yoko Ozawa, None

350 - P60-20

Differentiation of human retinal pigment epithelial cells cultured on thin alginate/ gelatin layer

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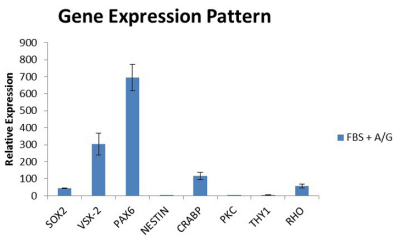
Purpose: Retinal pigment epithelium (RPE) is a pigmented hexagonally packed cuboidal cell located between the photoreceptors the choroid. Neural retinal cells can be damaged in a variety of degenerative eye disorders. A potential treatment could be transplantation of a sheet of functioning hRPE cells, grown on an artificial substrate. In this study a blend of alginate and gelatin were used as a substrate to culture and assess differentiation of hRPE to neural retinal cells.

Methods: hRPE were isolated from neonatal human cadaver eye globes and cultured in DMEM/F12 supplemented with 10% FBS. Alginate and gelatin 2% (w/v) was dissolved in water and A/G blends with 20:80 weight ratios were prepared. 380 µl of prepared blend poured in each well of 12 well micro plates to provide the A/G films with an average thickness of 1mm. hRPE cells at the 4th passages were seeded on substrate. After 7 days cells were harvested, RNA extracted, cDNA synthesis and Real time PCR performed. Relative expressions of genes measured according to the $2^{-\Delta\Delta Ct}$ method using the Bio-Rad software. Cells cultured on 2D polystyrene substrate were used as controls.

Results: Analysis of real time data confirmed that expression of SOX2 (neural stem cell marker), VSX-2 (neural retinal progenitor cells), PAX6 (neural retinal progenitor cells), NESTIN (neural progenitor cell marker), CRABP (cone photoreceptor cell marker), PKC (bipolar neurons) Thy-1 (retinal ganglion cell marker) and RHO (Rhodopsin cell marker) in cells cultured on A/G substrate were increased 44, 300, 695, 1.2, 117, 0.1, 4 and 47 time respectively. It indicated that A/G substrate could potentially induce differentiation of hRPE cells to different kinds of neural or neural progenitor cell.

Conclusions: this study showed that A/G blend as culture substrate can induce differentiation of hRPE cells to different kinds of neural cells and neural progenitor cells.

Commercial Relationships: Hoda Shams Najafabadi, None; zahrasoheila soheili, None; shahram samiei, None; hamid ahmadieh, None



351 - P61-21

Blue light induces inflammatory marker expression in the retinal pigment epithelium-choroid

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Purpose: To evaluate the influence of blue light, we compared the pathogenic effects in the retinal pigment epithelium (RPE)-choroid complexes of mice exposed

to light in a box made of a clear intraocular lens (IOL) material (named 'Clear IOL group') or a yellow IOL material that blocks blue light (named 'Yellow IOL group').

Methods: Seven- to eight-week-old BALB/c male mice were separated into 2 groups, and placed in a cage in one of 2 different light-blocking boxes; one was made of the material used in clear IOLs that blocks ultraviolet (UV) light, and the other was made of the material used for yellow IOLs that blocks blue light and UV light. The cages covered with either of the light-blocking boxes were placed inside a dedicated light-exposure box with stainless-steel mirrors on each wall and the floor, and exposed to a white fluorescence lamp at 3000 lux for 20 minutes after 12h of dark adaptation. We measured the the level of reactive oxygen species (ROS) using 2′,7′-dichlorodihydrofluorescein diacetate, and mRNA level of inflammatory cytokines, that are *mcp-1, il-6, il-1b, mmp-9, tnf-α*, and *tgf-β*1, by real-time PCR, both in the RPE-choroid complex six hours after light exposure. We also analyzed the mRNA level of *f4/80 by real-time PCR* and the protein level of MCP-1 by ELISA in the RPE-choroid complex 24 hours after light exposure.

Results: The ROS level after light exposure was suppressed in the RPE-choroids of light-exposed mice in the yellow IOL group. In parallel, all the inflammatory cytokines that we measured and a macrophage marker were also suppressed in the RPE-choroids of light-exposed mice in the yellow IOL group.

Conclusions: Blue light exacerbated the increase in the ROS level and inflammatory cytokine expression as well as macrophage recruitment in the RPE-choroid after light exposure.

Commercial Relationships: Toshio Narimatsu, None; Kazuno Negishi, None; Manabu Hirasawa, None; Toshihide Kurihara, None; Kazuo Tsubota, None; Yoko Ozawa, None

352 - P62-22

Blue Light Exposure-induced Oxidative Stress and Cell Death in the Retinal Pigment Epithelial Cells are Protected with NF-E2-related Factor 2 Pathway

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Purpose: Blue light (BL) exposure induces mitochondria-derived reactive oxygen species (ROS) in retinal pigmented epithelial cells and causes acute and chronic retinal damage, which indicates the association in the pathogenesis of age-related macular degeneration. NF-E2-related Factor 2 (Nrf2) is anti-oxidative factor to protect cells, although there is no report to elucidate the relationship of BL exposure with Nrf2. Thereofre, we further studied the mechanism of BL exposure and cell damage of retinal pigment epithelium cell.

Methods: Human retinal pigment epitrhelium cells (ARPE-19) were cultured under the exposure of blue light-emitting diodes (blue LED, middle wave length 450 nm). Lactase dehydrogenase (LDH), a marker of cell death, and ROS were measured. To detect apoptosis, TUNEL-staining was performed. *Nrf2* messenger RNA (*Nrf2* mRNA) was detected by real-time polymerase chain reaction (RT-PCR). To detect Nrf2 proteins increasing in nuclear or cytoplasm, western blotting was performed.

Results: BL increased LDH and ROS in ARPE-19 cells time-dependently whereas no increasing in control non-exposure cells. TUNEL-staining showed apoptotic cells in exposure cells, but not in control non-exposure cells. *Nrf2* mRNA was increased in BL exposure cells time-dependently, but not in control non-exposure cells. Nrf2 proteins were increased both in nuclear and cytoplasm in all conditions, however more increased in exposure cells rather than in non-exposure cells. Nrf2 proteins was significantly increased in nuclear than in cytoplasm in BL exposure cells.

Conclusions: BL exposure increased cell death via oxidative stress in ARPE-19 cells causing apoptotic changes. Nrf2 was increased both in nuclear and cytoplasm in BL exposure cells. It would protect ARPE-19 cells from BL exposure.

Commercial Relationships: Kei Takayama, None; Hiroki Kaneko, None; Fuxiang Ye, None; Ryo Ijima, None; Shu Kachi, None; Akiko Higuchi, None; Yosuke Nagasaka, None; Hiroko Terasaki, None

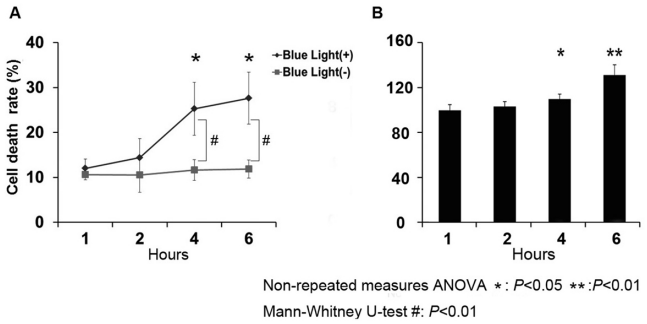

Fig.1 Cell death and reactive oxygen species
BL increased LDH and ROS in ARPE-19 cells time-dependently whereas no increasing in control non-exposure cells.

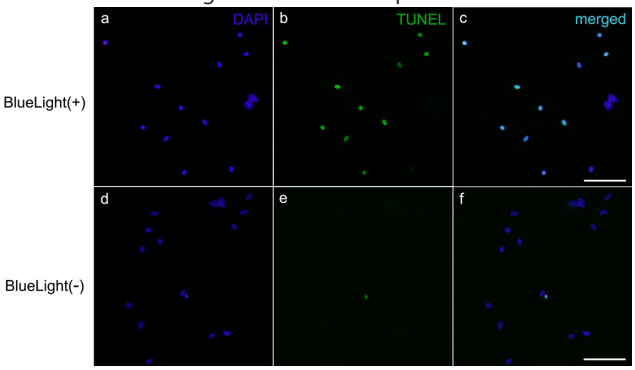

Fig.2 Apoptotoc cell death with BL exposure
Under BL exposure, cell death was increased (a) and TUNEL positive cells(b), which were almost same(c). Without light exposure, TUNEL positive cells were less than with exposure.

353 - P63-23

Neuro-protective effect of bilberry extract on the light-induced retinal degeneration in vivo

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Purpose: Bilberry (*Vaccinium myrtillus* L.) extract is a widely spread micronutrient supplement. It contains high amounts of anthocyanins, well known anti-oxidants, and thought to be beneficial for eyes. However, its biological mechanism for retinal protection remains unknown. In this study, we investigated the effect of the bilberry extract on the light-induced retinal degeneration in mice.

Methods: Eight-week-old BALB/c male mice were dark-adapted for 16 hours. 12 hours and 30 minutes prior to the light exposure, mice were treated with bilberry extract or its vehicle, PBS, by gavage administration. Then, mice were exposed to 3000 lux of a white fluorescence lamp for 1 hour. After the light exposure, the mice were returned to the cages and maintained under dim cyclic light. Eyes were enucleated for histological and biochemical assays. Apoptotic cells were detected by TUNEL assay. The mRNA levels were quantified by realtime PCR. Electroretinogram (ERG) was recorded for investigating visual function.

Results: Administration of bilberry extract to mice led to significant suppression of the number of light-induced cell death in photoreceptor cell layer, following suppression of the photoreceptor cell death. [o1] [o2] The mRNAs of ER stress markers, apoptosis markers, and mitocondrial biogenesis markers were increased in the light exposed retina, while the expressions were suppressed in the bilberry extract treated retina. The ERG showed the reduction of the a-wave amplitude caused by light exposure was attenuated by bilberry extract administration.

Conclusions: Bilberry extract prevented the light-induced retinal degeneration, in terms not only of retinal morphology, but also visual function. Further analysis is needed to reveal the details of the underlying mechanisms of the protective effect in the bilberry extract.

Commercial Relationships: Hideto Osada, None; Tomohiro Okamoto, None; Seiji Miyake, None; Saori Kobayashi, Wakasa Seikatsu Co.Ltd (E); Kazuo Tsubota, None; Yoko Ozawa, Wakasa Seikatsu Co.Ltd (F)

354 - P64-24

Effects of intense visible light on synaptic layers in neonatal chick retina, a morphological and biochemical investigation

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Purpose: The aim of the present study was to investigate the effect of intense visible light on synaptic layers changes and associated molecules in neonatal chick retina.

Methods: Post–hatch day 1 chicks (n=18) were reared in 12 hour light (L), 12 hour dark (D) photoperiod for 7 days at 500 lux. After acclimatization, they were divided into three groups, 12L, 12D, 18L, 6D, and 24L, 0D (continuous light; CL), and reared in light of intensity 2000 lux (at the base of cage) for 23 days. After completion of experiments, retinas were isolated and fixed for immunohistochemistry and electron microscopy, and proteins were extracted for Western blotting. Qualitative immunoexpression of synaptophysin and post synaptic density (PSD-95) protein was evaluated by immunohistochemistry and quantitative expression was evaluated by western blot.

Results: Comparative results from chicks reared in 12L, 12D revealed that animals exposed to CL phase showed significant retinal damage at synaptic level, as observed by the presence of disorganized and shorten synaptic vesicles, vacuolating space in outer plexiform layer (OPL). In inner plexiform layer (IPL), many axonal dead profile was seen along with vacuolating space in CL group compared to 12L, 12D group. In 18L, 6D regime, retinal changes were minimal in OPL and IPL relative to CL regime. Further, there was decreased immunoreactivity in synaptophysin in OPL and enhanced in sublamina of IPL in CL group, while moderate expression was observed in 12L, 12D and 18L, 6D groups. Minimal PSD-95 expression was seen in OPL, but faintly in IPL in CL group as compared to expressions in 12L, 12D and 18L, 6D groups. Moreover, Western blot analysis showed decreased expressions of synaptophysin ($P<0.001$)

Conclusions: From the above results, it can be concluded that continuous intense visible light causes damage to the retina at synaptic level in neonatal chicks. Ultrastructural changes indicated significant damage to OPL and IPL upon intense light exposure. Decreased expression of synaptophysin indicated damage at presynaptic site in OPL and IPL. Similarly, PSD-95 level altered upon intense light exposure, which indicated that oxidative stress caused by light exposure ultimately altered the synaptic architecture in cone dominated neonatal chick retina.

Commercial Relationships: Kumar Jha, None; Tapas Nag, None; Shashi Wadhwa, None; T Roy, None; Pankaj Kumar, None

355 - P65-25

The result of an analysis of fundus photos taken from 1,443 monkeys at Tsukuba Primate Research Center during 2011-2013

Yusuke Fujii [1] Koji M. Nishiguchi [2] Toshinori Furukawa [3] Fumiko Ono [4] Nobuhiro Shimozawa [5] Mutsumi Togo [6] Michihiro Suzuki [6] Toru Nakazawa [1, 2]

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Purpose: To cross-sectionally review a large collection of the fundus photos from monkeys and to identify monkeys with fundus abnormalities.
Methods: We initially carried out a cross-sectional review of 7,109 fundus photos taken from 1,443 monkeys at Tsukuba Primate Research Center, National Institute of Biomedical Innovation as a part of Non-human Primate Reagent and Resource Program. These photos were taken as a part of annual health screening during 2011 – 2013. The monkeys studied included *Macaca mulatta*, *Macaca fascicularis*, and *Cercopithecus aethiops*. For the selected eyes with fundus abnormalities, we additionally reviewed fundus photos from earlier time points to follow the lesion longitudinally.
Results: Initial screen of fundus photo from the monkeys revealed various fundus abnormalities ranging from retinal hemorrhages, drusen, macular degeneration, presumed myopic changes, retinal degeneration, and glaucoma. All of these abnormalities in monkeys were similar to the lesions observed in humans. Reviewing the fundus photo longitudinally from earlier time points provided insight into the development and the natural course of the abnormalities for the eyes with fundus abnormalities.
Conclusions: The screen of the fundus photos of the monkey eyes revealed various pathologies that may serve as models of human disease. Longitudinal data on the fundus appearance enhanced the understanding of the pathologies. A large cohort of monkeys with longitudinal fundus data is potentially a valuable resource for eye research.
Commercial Relationships: Yusuke Fujii, None; Koji Nishiguchi, None; Toshinori Furukawa, None; Fumiko Ono, None; Nobuhiro Shimozawa, None; Mutsumi Togo, None; Michihiro Suzuki, None; Toru Nakazawa, None
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356 - P66-26

Retinal Neuro-degeneration and Oxidative Stress, Vascular Endothelial Growth Factor-B (VEGF-B) in action?!

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Purpose: Retinal and macular degeneration is the leading cause of irreversible central vision loss in Americans 50 years and older. Retinal degeneration results in neurodegeneration especially of the retinal ganglion cells and photoreceptors leading to gradual visual impairment. Till date there is no effective treatment with demonstrated mechanisms of neuro-protection and recovery of RGCs, photoreceptor or retinal pigment epithelium. Oxidative stress has been proposed as a link to retinal degeneration. The molecular mechanism of VEGF-B overexpression in mice retina is still unknown. We hypothesize that the potent antioxidant-defensive and anti-apoptotic effect of VEGF-B can be applicable as potential therapeutics for diverse degenerative diseases.
Methods: Retinal thickness was measured by H&E staining in VEGFB-KO, VEGFB-tg and VEGF-B protein treated mice. Microarray and mouse oxidative stress/ Antioxidant defense PCR-array were performed in VEGFB-treated mice and confirmed by qPCR. Glutathione peroxidase1 (Gpx1) expression was examined by qPCR, IF, western in VEGF-B-injected, VEGFB-tg and intercrossed $Gpx1^{-/-}$ mice compared with their littermate wild type mice retinae.
Results: Retinal thickness was reduced in VEGFB-KO mice whereas increased and protected from apoptosis in VEGF-B treated and overexpressed mice compared with age-matched wild type. VEGF-B inhibits expression of the protein and oxidative/mtDNA damage, apoptotic/cell death-related genes whereas activates and regulates the expression of numerous antioxidant defense-related genes via Gpx1 in VEGFB-Tg compared with the wild type and intercrossed $Gpx1^{-/-}$ mice.
Conclusions: We reveal a novel function of VEGF-B as a potent survival and rescue factor. Our finding shows that VEGF-B overexpression up-regulate Gpx1, which activates the mitochondrial glutathione defense system and protect photoreceptor cells from oxidative stress or DNA-damage, thus rescues retinal degeneration. VEGF-B may potentially offer a new therapeutic intervention for treating retinal degenerative diseases including AMD, Retinitis pigmentosa, Alzheimer' s etc.
Commercial Relationships: Pachiappan Arjunan, None; Zhongshu Tang, None; Lijin Dong, None; Zhijian Wu, None; Pamela Martin, None; Christopher Cutler, None; Xuri Li, None

357 - P67-27

Promotion of retinal progenitor-like cells from adult human retinal pigment epithelial cells using AAV-2 viruses harboring hSox2

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Purpose: Retinal pigment epithelium (RPE) is a monolayer of cells underlying and supporting the neural retina. Developmentally, RPE and neural retina originate from the same structure, the optic vesicle. Sox_2 an HMG box transcription factor, plays an essential role in the maintenance of retinal progenitor cells undifferentiated state.

The aim of this study was evaluation of recombinant AAV-2 containing sox_2 to reprogram adult human RPE cells to retinal progenitor cells.
Methods: Coding region of sox_2 gene was cloned into pAAV-MCS vector. Validity of cloning was approved by colony PCR, digestion and sequencing. To detect gene expression, a 1.3kb IRES-EGFP segment as a reporter gene was ligated into the expression vector.. Transformed bacteria by ligation products were confirmed by quick check extraction. Digestion, PCR and sequencing methods were done to verify the final expression vector. pAAV-lacZ was used as a reporter vector to optimize calcium phosphate method for transfection. HEK293T cells were co-transfected with recombinant vector containing SOX_2-IRES-EGFP, pAAV-RC and pHelper. Recombinant viruses were harvested after 72 hours. To determine virus titration, flow cytometry and absolute real-time polymerase chain reaction (PCR) were used. Cultured RPE cells obtained from adult human cadaver globes were infected by viruses. Real-time PCR and immuno cytochemical(ICC) analysis were utilized to evaluate the expression of Sox_2 and retinal cells' markers.
Results: Titration of viral particles revealed a concentration of 1.8×10^6 infectious virus/mL of primary preparations. Suspance infection method was selected as the best way of RPE infection. Real time PCR showed Nestin overexpression followed by *sox2* overexpression. ICC represented Nestin protein expression as a neural progenitor marker in cells that were infected by AAV-SOX2 viruses.
Conclusions: The presented data implied that *sox2* overexpression induced adult human RPE cells retro differentiation toward neural progenitor-like cells. This would promis for a convenient source for cell replacement therapy in retinal degenerative diseases.
Commercial Relationships: Azade Etemadzade, None; Zahrasoheila Soheili, None; Shahram Samiei, None; Razie Ezati, None; Ehsan Ranaei Pimardan, None; Bagher Yakhchali, None; Hamid Ahmadieh, None

358 - P68-28

Intravitreal human neural stem cells are neuroprotective in a rat model of optic nerve transection in part by upregulation and secretion of gremlin-1

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Purpose: Progressive degeneration of retinal ganglion cells (RGCs) is a major pathologic feature of various optic neuropathies and retinal diseases. We investigated the neuroprotective effect of intravitreal human neural stem cells (hNSCs) on the survival of RGCs in the rat retina with optic nerve transection (ONT).
Methods: On the day of intraorbital ONT, intravitreal injections of 100,000 hNSCs, which were obtained from primary cultures of adult human temporal lobe tissues, 100,000 apoptotic hNSCs after freeze and thaw, and the same volume of vehicle were done. Survival of hNSCs was analyzed by real time RT-PCR assay for mRNA for human GAPDH. Retrograde labeling of RGCs with fluorescence tracer was done to evaluate the survival of RGCs. RNA was isolated from eyes and assayed on human microarrays for transcriptional profiling of intravitreal hNSCs. The upregulated transcripts were subjectively examined for candidate genes of interest, and real-time RT-PCR assays were used to confirm the data. Neuroprotective effects of candidate protein were analyzed using SH-SY5Y cell line and rat model of ONT.
Results: The real time RT-PCR assay for mRNA for human GAPDH to follow the fate of the intravitreally administered hNSCs indicated that more than 20% of the intravitreally administered hNSCs were recovered after 1 and 5 days. Intravitreal hNSCs increased the number of RGCs in the retina at days 7 and 14 after ONT. However, intravitreal hNSCs did not integrate into host retina and intravitreal apoptotic hNSCs did not show neuroprotective effect. These results suggest the possible paracrine effects of intravitreal hNSCs on RGCs. Transcriptome analysis showed that intravitreal hNSCs upregulated multiple genes including 22 secretory protein-encoding genes. The upregulated 22 human transcripts including gremlin-1 were further investigated. Recombinant human gremlin-1 significantly increased survival of RGCs in a rat model of ONT and increased survival of SH-SY5Y cell in vitro.
Conclusions: The results suggest that intravitreal hNSCs increase survival of RGCs in part by upregulation and secretion of gremlin-1. Intravitreal hNSCs or secretory factors from hNSCs might be a useful therapy for retinal or optic nerve diseases in which RGCs undergo apoptosis.
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359 - P69-29

Extra Ocular Muscles and Stem Cells

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Purpose: To isolate stem and progenitor cells from extra ocular muscle tissue and compare it with bone marrow derived stem cells.
Methods: The Extraocular muscle tissue was obtained from more than 100 patients undergoing strabismus surgery with an average age of 21 years. The tissue was mechanically dissociated and stem cells were isolated by adherence selection. Differentiation was analyzed by addition of specific induction factors and gene expression was analyzed by real time PCR. The statistical significance was determined using SPSS soft ware.
Results: The cells were adherent in nature and spindle shaped, could be passaged upto several generations and had a doubling time of 34 hours. The cells were

positive for cell surface expression of CD13,CD44,CD49b ,CD49e,CD73,CD90,CD105, HLA class I and negative for CD34,CD45,CD104,CD146,CD200 and HLA class II. The EOM cells could be differentiated into adipocytes and osteocytes. The cells also expressed embryonic markers such as SSEA4, and transcription factors OCT4, NANOG and SOX2. The cells had a high expression of NESTIN and differentiated readily into neuronal cells identified by the expression of GFAP,MAP2B and TUBBIIIB.

Conclusions: Our study shows for the first time that multipotent progenitor cells are present in the human extra-ocular muscle tissues and could be successfully isolated. The presence of multipotential stem cells in the ocular muscle tissue has significant therapeutic implications as they can be differentiated into retinal progenitor cells to treat retinal degeneration.

Their superior neuronal differentiation potential and multipotent nature make these cells highly desirable for cell therapy.

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360 - P70-30

The effect of low level laser irradiation on the proliferation of bmscs

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Purpose: Glaucoma is one of the chronic neuro-degenerative diseases .Progressive loss of retinal ganglion cells (RGCs) and visual field defects are the most common manifestations of glaucoma.MSCs can probably inhibit apoptosis of retinal ganglion cells so that they may relieve optic nerve damage and reduce the loss of visual field. LLLI may enhance stem cells differentiation, including MSCs. The objective of this study is to discuss the effects of mesenchymal stem cells proliferation caused by low level laser irradiation experiments at different doses and to investigate the optima laser level.

Methods: Bone mesenchymal stem cells were cultivated by primary culture method and those cells during logarithmic growth phase were chosen to plant them into plate. Then 635nm laser of different doses（0J、0.5J、4J、24J）were used to irradiate bone mesenchymal stem cells and cell vitality and relative cell amounts of every culture dish were measured by cell counting kit-8(CCK-8) methods. Afterwards the most typical group was selected to carry out Brdu immunofluorescence staining to photo those bone mesenchymal stem cells under proliferative phase. Finally, statistical analysis of experimental results was performed.

Results: Obviously promoting effects on the proliferation of BMSCs could be noticed from 0.5J, 4J and 24J group and the highest effects could be seen in 0.5J group .

Conclusions: This study demonstrated that low level laser irradiation(LLLI) could promote the proliferation of mesenchymal stem cells and provide us a new approach to amplify MSCs in vitro. A great many advantages of LLLI could be concluded as follows：firstly, LLLI was belonging to physical stimulation and it could prevent us from the issue of protein contamination caused by small molecular protein amplification; secondly, LLLI had strong stability for its stable wave length and constant output power and even amplification effects; thirdly, all the irradiation work could be performed in the incubator in order to avoid extra work and save cost of labor and reduce possibilities of germ contamination；finally, LLLI was relatively cheap. It only took us 50 thousands RMB to buy an 635nm laser machine with low irradiation cost per time. Therefore, low level laser irradiation(LLLI) could promote the proliferation of MSCs and act as a new approach to amplify MSCs. In a word, this study provided us a new method for MSCs amplification and had certain applicable values.

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Biochemistry/Molecular Biology - Poster

470 - P01-1

Polyphenolic Compounds Fisetin and Luteolin decrease Inflammation and Oxidative Stress-induced Cytotoxicity in ARPE-19 Cells

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Purpose: Age-related macular degeneration (AMD) is not only the leading cause of blindness in the western world but also a significant burden on the healthcare system. Despite the severity of the disease and its high prevalence in the aged population, much research remains to be done to understand the pathways of disease formation and progression and to find viable treatment options. In this study, we evaluate the anti-inflammatory potential of naturally occurring polyphenols fisetin and luteolin, and investigate the pathways by which inflammation is controlled in human retinal pigment epithelial cells.
Methods: ARPE-19 cells were treated with the lipid peroxidation end product 4-hydroxynonenal (HNE) to simulate high oxidative stress. One hour after the initial stimulation, fisetin or luteolin were added to the culture medium. In order to assess the importance of the MAPK pathway, experiments were performed with or without pretreatment with specific MAPKinase inhibitors. Cytotoxicity of treatments was assessed using the MTT and the lactate dehydrogenase assay. Inflammatory cytokines IL-6 and IL-8, as well as phosphorylation of transcription factor CREB and MAPKinases p38, ERK1/2, and JNK were measured using ELISA. Moreover, DNA-binding activity of the NF-κB subunit p65 was analyzed with a DNA-binding transcription factor assay.
Results: Both fisetin and luteolin protected ARPE-19 cells from cell death induced by the HNE stimulation. Additionally, both polyphenols significantly reduced the inflammatory response in ARPE-19 cells. HNE stimulation led to increased activation of CREB and the MAPKinases p38 and JNK. Fisetin and luteolin reversed the increased phosphorylation of these factors and additionally reduced the activity of ERK1/2, but had no effect on p65 DNA-binding. The inhibition of JNK by a specific chemical inhibitor also lead to a decrease in the production of IL-8.
Conclusions: Fisetin and luteolin protect retinal pigment epithelial cells from oxidative stress-induced cell death even when added one hour after the initial insult, suggesting not just preventive but therapeutic potential. Furthermore, both compounds exhibit potent NF-κB-independent anti-inflammatory properties.
Commercial Relationships: Maria Hytti, None; Niina Piippo, None; Eveliina Korhonen, None; Kai Kaarniranta, None; Anu Kauppinen, None
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471 - P02-2

Suppression of high fat diet-induced inflammation in the RPE by angiotensin II type 1 receptor blockade

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Purpose: High fat diet is one of the risk factors of age-related macular degeneration (AMD), in which the oxidative stress in the retinal pigment epithelium (RPE) and choroid contribute to the pathogenesis. However, its underlying mechanisms were still obscure. In the retina, we have reported the interaction between oxidative stress and angiotensin II type 1 receptor (AT1-R) signaling. In this study, we analyzed the impact of high fat diet and the involvement of AT1-R signaling in the pathological changes of the RPE and choroid.
Methods: Balb/c mice were fed with a normal or a high fat diet for 4 weeks. These mice were treated daily with an AT1-R antagonist, valsartan (5 or 20 mg/kg body weight) or vehicle for the last 1 week. Macrophage infiltration to RPE-choroid was analyzed by quantitative realtime PCR (qPCR) for F4/80. RPE-choroidal expression of interleukin-1b was also analyzed by qPCR.
Results: AT1-R blockade with valsartan at the dose of 20mg/kg body weight significantly suppressed RPE-choroidal mRNA expression of F4/80 and interleukin-1b induced by high-fat diet.
Conclusions: AT1-R signaling blockade inhibited macrophage infiltration to RPE-choroid and RPE-choroidal expression inflammatory molecule in the high-fat diet model. These results suggest the potential use of an AT1-R antagonist as a therapeutic agent to reduce RPE-choroidal inflammation induced by high-fat diet.
Commercial Relationships: Norihiro Nagai, None; Kazuo Tsubota, None; Yoko Ozawa, None

472 - P03-3

Preliminary study on expression of uncoupling protein 2 in human retinal pigment epithelial cells

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Purpose: To analyze the expression of UCP2 in RPE cells at different human age, further explore the possible new target of RPE cells protection.
Methods: Cultivate ARPE19 cells and RGC cells and detect the expression of UCP2 in ARPE19 cells and in primary RPE cells at different ages by RT-PCR, Western Blot, laser scanning confocal microscopy.
Results: Results of RT-PCR, Western Blot, confocal microscopy detection all showed that UCP2 was highly

expressed in ARPE19 cells and in primary cultured human RPE cells at the age of 9 and 52, but lowly expressed in primary cultured human RPE cells at the age of 62 and 76.
Conclusions: As a mitochondrial membrane protein, UCP2 can prevent apoptosis of mitochondrial pathway and inhibit oxidative stress. Expression of UCP2 in RPE cells increased at the lower age groups, but decreased obviously at the older age groups. The results showed that the antioxidant capacity of RPE cells at older age groups weakened, while oxidative stress and apoptosis increased significantly. Further prompt that if the expression level of UCP2 was increased, the ability of anti-oxidation damage in RPE cells might enhance and the cell apoptosis and oxidative stress might reduce, which might be a new target of RPE cells protection.
Commercial Relationships: Zhang Chuntao, None

473 - P04-4

Studying GRP78 over-expression in cultured adult human RPE cells and its effects on the expression of the inflammatory factor

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Purpose: Age-related macular degeneration (AMD) is caused by death of photoreceptor cells and their supporter tissue, retinal pigmented cells (RPEs) and consequent macula breakdown. Progression of AMD is linked to byproducts accumulation of cellular stresses such as oxidative stress, proteotoxic stress, inflammation and hypoxia. These conditions can trigger stress in endoplasmic reticulum (ER), which controls protein quality in cells via unfolded protein response (UPR). A large number of studies suggest that induction of 78 kDa glucose-regulated protein (GRP78), is a marker for ER stress due to its role in controlling the activation of transmembrane ER stress sensors (IRE1, PERK, and ATF6). In the present study the linkage between ER stress and inflamatory responses would be explored through over-expression of GRP78 in adult RPE cells by means of adeno-associated virus (AAV) helper free system as a prominent gene delivery system.
Methods: Validated GRP78 coding sequence was cloned into the expressive pAAV-MCS plasmid vector. Afterwards the recombinant expression plasmid was co-transfected into the HEK239T cells conjointly with pHelper plasmid (carrying adenovirus-derived genes) and pAAV-RC plasmid (carrying AAV-2 replication and capsid genes). pAAV-LacZ plasmid was used as control in a parallel co-transfection. Recombinant AAV-2 viral particles were prepared from infected HEK293T cells and then were used to infect adult RPE cells according to spin infection method. At the final step the infected RPE cells would be examined for over-expression of GRP78 and changes in expression of CRP, CFH, C3, C5, MCP1, DAF and Caspase4 inflammatory factors by Real-Time PCR.
Results: Restriction digestion and sequencing of constructs confirmed the accuracy of cloning for GRP78 coding sequence into pAAV-MCS plasmid vector. Recombinant AAV-2 viral particles production and their infection into RPE cells were monitored by beta-galactosidase expression. Real-Time PCR verified the overexpression of GRP78 and significant changes in expression of C3 and CRP factors in transduced RPE cells.
Conclusions: The Results verify that ER stress could help the pathogenesis of AMD by complement system activation. So AMD could be controlled by decline of complement system extension through controlling ER stress and its main marker, GRP78.
Commercial Relationships: Amirhossein Mansoori, None

474 - P05-5

Aqueous level of angiopoietin-like 4 correlates with phenotypes of diabetic retinopathy, Nonperfusion area and macular volume

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Purpose: To investigate the aqueous level of angiopoietin-like 4 (ANGPTL4) from patients with diabetic retinopathy (DR) and ascertain their association with phenotypes of DR.
Methods: This study is prospective nonrandomized, comparative case series. The DR group enrolled 104 eyes of 104 patients who had intravitreal anti-VEGF injections including 51 eyes of severe nonproliferative diabetic retinopathy (NPDR) and 53 eyes of proliferative diabetic retinopathy (PDR). Control group enrolled 54 eyes of 54 non-diabetic controls who had cataract surgery.
The aqueous humor samples were obtained from subjects before intravitreal injections or cataract surgery. The aqueous level of ANGPTL4 was measured with enzyme-linked immunosorbent assay. Capillary nonperfusion area (NPA) was calculated from encircled angiography using 7 standard field (7SF) images described in the Early Treatment Diabetic Retinopathy Study protocol. Total macular volume (TMV) was measured by spectral domain optical coherence tomography.
Results: The mean aqueous ANGPTL4 level was 352.2 ± 665.3 pg/ml in the control, 5,639.1 ± 35.1 pg/ml in the severe NPDR, and 17,174.9 ± 6,410.0 pg/ml in the PDR groups, and they were significantly different among the 3 groups ($P<0.001$).
The aqueous ANGPTL4 level of both the severe NPDR and PDR groups were higher than that of the control group ($P<0.001$, respectively). The aqueous ANGPTL4 level of all patients from the severe NPDR and PDR groups significantly correlated with the extents of NPA ($r = 0.820$, $P<0.01$). Furthermore, the aqueous ANGPTL4 level correlated positively with the TMV ($r = 0.763$, $P<0.01$).
Conclusions: The ANGPTL4 could be induced by ischemia retina and promote vasopermeability in patients with diabetic macular edema. Thus, the ANGPTL4 can be an alternative target for the treatment of diabetic retinopathy.
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Clinical Trail: NCT02026843

475 - P06-6

Does endothelin 2 have a role in diabetic retinopathy?

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Purpose: The endothelins are a family of three highly conserved and homologous vasoactive peptides that are expressed across all organ systems. Endothelin dysregulation has been implicated in a number of pathophysiologies, including diabetes and diabetes-related complications. The purpose of the study was to examine endothelin 2 (Edn2) and endothelin receptor B (Endrb) expression in mouse models of retinal neovascularisation and diabetes.
Methods: Edn2 and Ednrb mRNA and Edn2 protein expression were assessed in young (8-week old) and mature (24-week old) C57Bl/6 (control), Kimba (a vascular endothelial growth factor-driven -driven mouse model of retinal neovascularisation), Akita (a Type 1 diabetes mouse model) and Akimba mice (a retinal neovascularisation model on a diabetic background from crossing Kimba with Akita mice) by qRT-PCR and immunohistochemistry. Edn2 protein concentration in serum was measured using ELISA.
Results: Fold-changes in Edn2 and Ednrb mRNA were seen only in young Kimba (Edn2, 5.3; Ednrb, 6.0) and young Akimba (Edn2, 7.9, Ednrb, 8.8) and in mature Kimba (Edn2:9.2, Ednrb:11.2) and mature Akimba (Edn2:14.0, Ednrb:17.5) mice. Co-localisation of Edn2 with Müller-cell-specific glutamine synthetase demonstrated Müller cells and photoreceptors as the major cell types for Edn2 expression in all animal models. Edn2 serum concentrations in young Kimba, Akita and Akimba mice were not elevated compared to control mice. However, in mature mice, Edn2 serum concentration was increased in Akimba (6.9pg/mg total serum protein) compared to control, Kimba and Akita mice (3.9, 4.6, and 3.8pg/mg total serum protein, respectively; $p<0.05$).
Conclusions: These results demonstrated that long-term hyperglycaemia in conjunction with a vascular endothelial growth factor-driven-driven retinal neovascularisation increased Edn2 serum concentration suggesting Edn2 might be a sensitive, quantifiable biomarker for vascular changes in diabetic retinopathy.
Commercial Relationships: Chooi-May Lai, None; Nicolette Binz, None; Ireni Ali Rahman, None; Elizabeth Rakoczy, None

476 - P07-7

Increased vitreous concentrations of MCP-1 and IL-6 after vitrectomy in patients with PDR, Possible relation to postoperative macular edema

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Purpose: To determine whether the concentrations of MCP-1, IL-6, and IL-8 in the vitreous are altered after vitrectomy in patients with proliferative diabetic retinopathy (PDR) and to investigate whether the altered levels of these cytokines are associated with postoperative macular edema after vitrectomy.
Methods: Vitreous samples were collected from 36 eyes of 33 patients with PDR before pars plana vitrectomy without IOL implantation, and also from the same 36 eyes during IOL implantation surgery. The interval between the vitrectomy and implantation surgery ranged from 93 to 722 (mean 219.5) days. The levels of MCP-1, IL-6, IL-8, and VEGF were measured by flow cytometry using cytometric bead array (CBA) technology.
Results: The mean vitreous levels of MCP-1, IL-6, and IL-8 in the samples collected before vitrectomy were significantly higher in patients with PDR than in the control patients ($P<0.0001$). The levels of MCP-1 and IL-6 in the samples collected at the time of IOL implantation were significantly higher than those collected before vitrectomy ($P<0.05$). In contrast, the level of IL-8 was significantly lower after vitrectomy ($P<0.05$). The levels of IL-6 and IL-8, but not MCP-1, in the vitreous from eyes with PDR were inversely correlated with the interval between the initial vitrectomy and the time of the implantation surgery. Among the vitrectomized patients, the mean vitreous level of MCP-1 in eyes with diabetic macular edema (DME) was significantly higher than in those without DME ($P=0.028$).
Conclusions: The elevated levels of MCP-1 and IL-6 may indicate prolonged inflammation even after successful vitrectomy which can cause postoperative DME.
Commercial Relationships: Yoshiyuki Kobayashi, None; Shigeo Yoshida, None; Yedi Zhou, None; Takahito Nakama, None; Keijiro Ishikawa, None; Shintaro Nakao, None; Yukio Sassa, None; Yuji Oshima, None; Toshihiro Kono, None; Tatsuro Ishibashi, None
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477 - P08-8

Functional analysis of semaphorin 3F in rat retinal ganglion cells after optic nerve crush

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Purpose: Semaphorin are a family of glycoproteins that play an important role in repulsive axon guidance during embryogenesis, also, it has been reported to the functional involvement in neuronal apoptosis. We have now investigated that the expression of class 3 semaphorins, and the role of semaphorin3F in rat retinal ganglion cells after optic nerve crush.
Methods: Optic nerve crush injury was induced in Long avans rats. Eyes and retinas were collected at intervals of 3, 7 and 14 days after procedure. Using RT-PCR, immunoblot and immunohistochemical analysis, the expression levels of class 3 semaphorins were studied. Also, the expression and role of semaphorins in retinal ganglion cells after optic nerve crush was examined by Laser Microdissection and retinal organ culture system.
Results: We found a decrease in semaphorin 3F levels in the retina at 7 and 14 days after optic nerve crush, but no change in others semaphorin 3 class (sema 3A, 3B or 3C). Particularly, these decreasing expression was seen in the retinal ganglion cell layers of retina. Furthermore, mRNA expression also was decreased in retinal ganglion cell layers with Laser Microdissection system.
Conclusions: These results suggest that neural guidance gene semaphorin 3F may play an important role in the regulation of retinal ganglion cells functions and provide more information about RGC survival. It is indicated a useful factor from the viewpoint of neuroprotection.
Commercial Relationships: Ji-Ae Ko, None; Chihiro Ohki, None; Akira Minamoto, None; Yousuke Sugimoto, None; Junko Hirata, None; Yoshiaki Kiuchi, None

478 - P09-9

Genetic analysis of hereditary retinal dystrophies in consanguineous Pakistani families

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Purpose: Identifying genetic defects associated with retinal dystrophies common in Pakistani population.
Methods: Patients of unrelated families (A-C) with documented consanguineous marriages were recruited from the rural Pakistani populations. Blood samples were collected from available members of the families and a proband from each family was subjected to ophthalmic assessments. Families were assessed for involvement of retinal disease genes/loci reported in the Pakistani population and un-linked families were subjected to genome-wide genotyping applying the 250K NspI genotyping array (Affymetrix, USA). Homozygosity Mapper was applied to identify homozygous regions shared by affected family members. Candidate genes were further analyzed by Sanger sequencing, deletion mapping & minigene functional assay.
Results: Genome-wide SNP analysis revealed large homozygous intervals 14Mb, 79Mb & 4Mb on chromosomes 2, 8 & 15 in families A, B & C respectively. The identified homozygous regions were analyzed to find known RD genes and Sanger sequencing of these genes led to the identification of a novel and a recurrent mutation. Affected members of family A with autosomal recessive retinitis pigmentosa (RP) shared a homozygous region on 2p24.3-p23.1 that contains ZNF513, C2ORF71 & VSNL1 genes. Yet sequencing all coding exons of these genes revealed no pathogenic mutation. Similarly, we identified a 79Mb region on chromosome 8p21.3-q22.3 in family B, which harbors the candidate gene, C8ORF37. Mutation screening revealed a novel splice acceptor site variation, which was further verified by the minigene splice assay. The splice variant results in the activation of a cryptic splice site resulting in the partial deletion of the adjacent exon 3 in transcripts and eventually leads to a translational frameshift followed by premature protein truncation. Sanger sequencing also confirmed the presence of a recurrent homozygous missense mutation in TRPM1 gene in the third family (C), which was mapped on chromosome 15q12-q13.3. The identified variants segregate in the respective families and were absent in 60 healthy controls from Pakistan.
Conclusions: Our research highlights the influence of consanguineous marriages and presence of genetic heterogeneity in arRDs in the Pakistani population. It is anticipated that these findings will broaden our perception of the disease pathogenesis.
Commercial Relationships: Zeinab Ravesh, None, Higher Education Commission of Pakistan (F); Nicole Weisschuh, None; Bernd Wissinger, None; Muhammad Ansar, higher education commission pakistan (F)

479 - P10-10

Autosomal recessive bestrophinopathy, a novel mutation in a Korean family

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Purpose: To describe the clinical and genetic characteristic of patients with autosomal recessive bestrophinopathy from a Korean faimly
Methods: Clinical investigations in patients included best-corrected visual acuity, slit-lamp examination, indirect opthalmoscopy, fundus photography, fundus autofluorecence imaging, spectral-domain optical coherence tomography (OCT), fluorescein angiography and idocyanine green angiography, full-field electro-retinography (ERG), and electro-oculaography (EOG). Blood samples from patients and their family members were obtained to analyze the *BEST1* gene for biallelic mutations that confirmed the diagnosis of ARB.
Results: We identified a previously described mutation (A195V) and a novel mutation (L40P) in the *BEST1* gene

in two patients from a same family. One patient was a 66 year-old man who reported central visual loss at the age of 25. The other patient was a 52-year-old younger sister who noted central visual loss at the age of 20. She presented with concurrent macular edema associated with branched retinal vein occlusion and macular edema in the left eye, which was successfully treated with two monthly intravireal bevacizumab. Both patients showed absent light rise on the EOG, but full-field ERG results were relatively unaffected.

Conclusions: Autosomal recessive bestrophinopathy is a recently described macular dystrophy, characterized by recognizable phenotype and autosomal recessively inhertized mutation in the *BEST1* gene. A new mutation in *BEST1* was identified in a Korean family with autosomal recessive bestrophinopathy. Autosomal recessive nature and a relatively broad therapeutic window may make this disease a suitable candidate for regeneration medicine including gene therapy.

Commercial Relationships: Christopher Lee, None; Ikhyun Jun, None; Ji Lee, None; Hye Kim, None; Sung Lee, None; Eung Kim, None

480 - P11-11

Modulation of Glutathione S-Transferase Pi Isoform (GSTP1) Expression by Lethal and Non-Lethal Oxidative Stress in Human Retinal Pigment Epithelial Cells

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Purpose: Glutathione S-transferase pi isoform (GSTP1) is an intracellular detoxification enzyme that catalyzes reduction of chemically reactive electrophiles and is a zeaxanthin-binding protein in the human macula. We have previously demonstrated that GSTP1 levels are decreased in human age-related macular degeneration (AMD) retina compared to normal controls. We also showed that GSTP1 levels parallel survival of human retinal pigment epithelial (RPE) cells exposed to UV light, and GSTP1 over-expression protects them against UV light damage. In the present study, we determined the dose-response effect of oxidative stress on GSTP 1 expression in the survival of human RPE cell exposed to hydrogen peroxide (H_2O_2) and cigarette smoke component hydroquinone (HQ).

Methods: Human RPE cell culture (ARPE-19) was maintained using established protocols. ARPE-19 cells were plated at sub-confluent density in six-well plates and grown to confluence. Cells were treated with H_2O_2 (50, 100, 200, 300 μM) or HQ (50, 100, 200, 300 μM) for 8-12 hours. Cell survival was measured using trypan blue exclusion assay. GSTP1 expression was assessed using Western blot analysis.

Results: ARPE-19 cell viability was not negatively affected when exposed to increasing concentrations of H_2O_2 compared to untreated control. In contrast, ARPE-19 cell survival decreased when exposed to increasing concentrations of HQ. GSTP1 expression decreased in response to both H_2O_2 and HQ.

Conclusions: Oxidative stress parallels survival of human RPE cells and reduces GSTP1 levels in ARPE-19 cells exposed to HQ. H_2O_2 down-regulates GSTP1 expression in ARPE-19 cells but does not lead to decreased cell viability. GSTP1 levels may be modulated by lethal and non-lethal oxidative injury.

Commercial Relationships: Wen-Hsiang Lee, None; Matthew Donovan, None; Ali Saeed, None

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Cornea - Poster

481 - P12-1

Severe Intraepithelial Neoplasia Association with Conjunctival Squamous Papilloma

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1. Ophthalmology, Tokyo Dental College, Ichiawa, Japan. 2. Pathology and Laboratory Medicine, Tokyo Dental College, Ichikawa, Japan.

Purpose: To investigate the association between conjunctival squamous papillomas and dysplastic histopathological findings in patients undergoing surgical resection

Methods: We retrospectively reviewed the ophthalmic pathology reports of all patients between 2004 and 2014 at the Pathology Department of Ichikawa General Hospital. Specimens with a diagnosis of "conjunctival papilloma" were reevaluated and additional hematoxylin-eosin and immunohistochemistry stainings were conducted for p53, Ki 67, and human papilloma viruses. The relation between clinical patient characteristics and the histopathological characteristics, especially with dysplastic features was investigated. Informed consent was obtained from all subjects for the study procedures.

Results: Out of the total 73,183 specimens, 1,195 specimens (%1.6) during 2004-2014 were from the Department of Ophthalmology. Of the 1195 ophthalmic pathology reports scanned, 7 patients (6 males, 1 female; age range, 21~89 years, mean age, 42.7 years) had a diagnosis of conjunctival papilloma which constituted to 0.58% of the diagnosis for the ophthalmology specimens. Papillomatous growths originated from the lower lid margin (2 cases), conjunctival fornix (1 case), bulbar conjunctiva (2 cases) and a rare caruncular location in 2 cases. All specimens displayed multiple fronds of conjunctival epithelium enclosing cores of vascularized connective tissue. In the two caruncular and one lower eyelid margin lesion, the epithelium comprising the papillomatous growth was replaced totally by cells displaying densely stained large nuclei, increased mitotic figures, dyskeratosis, koilocytosis and loss of cellular polarity constituting to Grade 3 conjunctival intraepithelial neoplasia. Epithelial histopathology showing intranuclear inclusion bodies suggested a viral ethiology. All patients underwent simple resection and/or amniotic membrane transplantation. Two patients with caruncular or pericaruncular lesions and intraepithelial neoplasia developed recurrence after resection.

Conclusions: Conjunctival papillomas may rarely arise from a caruncular location and develop into papillary squamous cell carcinoma in situ. Clinical differentiation from squamous cell carcinomas with papillary growths and papilloma with dysplastic features is important. Close follow up for recurrence and/or progression into invasive carcinoma with appropriate histopathology stainings is essential.

Commercial Relationships: Murat Dogru, None; Jun Miyauchi, None; Yoichi Tanaka, None; Takefumi Yamaguchi, None; Seika Den Shimazaki, None; Yoshiyuki Satake, None; Jun Shimazaki, None

482 - P13-2

Laser Therapy for Ocular Lesions of Nevus of Ota in a child

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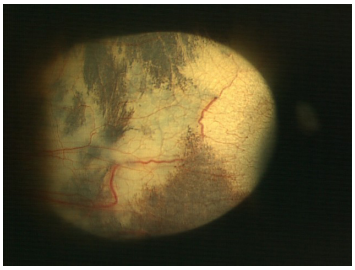
Purpose: To confirm the efficacy and safety of Q-switched Nd, YAG laser therapy for ocular lesions of nevus of Ota in children.

Methods: A 16-year-old Japanese girl fulfilled the criteria for the treatment. Namely, the patient was cooperative enough to fix the face and the eye and had thin capsule of Tenon. Corrected visual acuity, intraocular pressure and fundus findings showed no abnormality. There were no complications such as glaucoma. The cutaneous lesion of nevus of Ota was already treated successfully when she was 8 y/o. Oxybuprocaine ophthalmic solution 0.4% was used for surface anesthesia. She was treated by Tango (Ellex) at SLT mode (wave length, 532 nm, diameter, 400 μ m, laser irradiation time, 3 nsec). After an informed consent was obtained, the patient was treated six times and followed-up five times. Irradiation output was 1.3 mJ and a treatment was with irradiation of 326 to 641 shots.

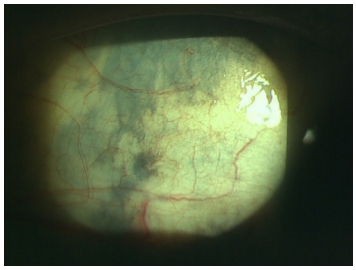
Results: There were no risk of ectoic irradiation. An improvement was confirmed on slit-lamp examination after one treatment. Subjective improvement by the patient were noted after three treatments. The scleral lesion responded well in the upper layer, which contributed to objective and subjective improvements. Although the deeper lesion remained unresponded, this was recognized as a merit because it means there was no intraocular damage.

Conclusions: The Q-switched Nd, YAG laser therapy for ocular lesions of nevus of Ota was safe and effective, well tolerated in a 16-year-old girl and could be a treatment option.

Commercial Relationships: Sachi Amaki, None; Masahiro Kobayashi, None; Masaru Tanaka, None



Befor the treatment



After 5 treatments

483 - P14-3

The Effect of Rebamipide to the Human Conjunctival Epithelium

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Purpose: We revealed acceleration of cultivated human conjunctival epithelial sheet stratification due to the increased cell proliferation by rebamipide (Mucosta® Otsuka Pharmaceutical Co., Ltd, Tokyo), which enhanced the barrier function of the conjunctival epithelium (ARVO 2014). However, the mechanism of action of rebamipide in this phenomenon are still unclear. In this study, we investigate the effect of rebamipide on cell proliferation of human conjunctival epithelial cells.
Methods: Small pieces (1mm x 1mm) of human limbal conjunctival tissues from donor cornea were cultured on the transwell insert until confluent in culture medium containing epidermal growth factor (EGF). After harvesting cultivated human conjunctival epithelial cells, the single cell suspension (1 x 10^4 cells/well) were transferred into 6 well-plate and cultured in the presence of rebamipide, but in the absence of EGF for 2 weeks. Then, cultivated human conjunctival epithelial cells were stained with rhodamine B to examine the ability of cell proliferation.
Results: In the presence of rebamipide, proliferation of human conjunctival epithelial cells were enhanced obviously.
Conclusions: This study revealed that rebamipide enhanced the ability of proliferation of human conjunctival epithelial cells. This phenomenon may induce the acceleration of stratification of cultivated human conjunctival epithelial sheet as well as the enhancement of barrier function.
Commercial Relationships: Yoshiyuki Satake, Otsuka Pharmaceutical Co., Ltd (F); Kazunari Higa, Otsuka Pharmaceutical Co., Ltd (F); Jun Shimazaki, Otsuka Pharmaceutical Co., Ltd (F)

484 - P15-4

NEW TECHNIQUE FOR PRIMARY PTERIGIUM EXCISION-WITHOUT CONJUNCTIVAL GRAFT

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Purpose: To present a new surgical concept for the excision of pterigium, a disorder that consists physiopathologically in sick conjunctiva advancing towards the limbal area , and finally invading the peripheral cornea . The concept of the technique is to reverse the tissues to its original anatomical position, based on the known elasticity of the conjunctiva.
Methods: The procedure is performed only with instillation of lydocaine eye drops in order to keep the anatomical boundaries of the surgical area. After placing a blepharostat, a traction suture is placed at the contra lateral limbus to facilitate the manipulation of the eye globe all the time . Using a cardiovascular Prolene 8-0 with round not cutting spatula needle . The lateral body of the pterigium is pulled expanding its surface first superiorly about 6 to 8 mm and fixing it transconjucntivally to the epiesclera , a holding to knot is made a this point, an oblique fold is created , and this redundant conjunctiva should be excised. Bleeding is controlled leaving a small piece of Weck cell during 40 seconds and removing it after profuse wetting . A similar step is done at the inferior zone. At this moment the body of the pterigium has moved towards the limbal area . With a Vannas scissor the body is incised and separated from stretched normal conjunctiva , some bleeding will appear and again using Weck cells , no cauterization is needed. The final step is the dissection of the head of the lesion starting in the upper part , and separating the whole pterigium ,leaving a small free limbal zone and an uniform conjunctival surface. A mega-22mm- soft contact lens (Surgilens) is placed to cover the free ends of the suture knots . Sutures and contact lens are removed one week after the procedure.
Results: We have performed 20 surgeries with this technique with only 2 cases that have to be submitted for a second small retouch.
Conclusions: This novel technique for the management of pterigium and similar conjunctival lesions is easy to perform, less invasive and has a short postoperative evolution with minimal complications. This procedure is advised only for primary pterigiums.
Commercial Relationships: Alexandra Mieth, None; Eduardo Arenas, None

485 - P16-5

Comparison of the Coefficient of Friction of New and Worn Daily Disposable Silicone Hydrogel Contact Lenses to Human Corneal Tissue

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Study Group: Johnson and Johnson Vision Care, Inc

Purpose: Compare the coefficient of friction (CoF) of narafilcon A contact lenses removed from packaging and after up to 14 hours of wear to fresh human corneal tissue.
Methods: CoF was measured on 13 narafilcon A contact

lenses (CL) after removal from manufacturer packaging. Ex vivo narafilcon A CL CoF was measured for 10 eyes post 12 (± 2) hours of wear. The CoF was measured on 10 fresh human corneas within 9 hours of donor death (range 3.30-8.48 hrs). All CoF testing was conducted using the same methodology utilizing the Basalt Must Micro-Tribometer in Tear Like Fluid (TLF-PBS) solution. The applied normal force varied between 0.25 and 4.0 mN with a measured stroke length of 1.0mm at normal speed of 0.1mm/s. A mucin coated hydrophobized glass counter surface was used for all measurements. Contact lens CoF values were recorded for 0, 50, and 100-cycles and at 0-cycles for the human corneas. CL CoF was analysed using a linear mixed model to calculate least square (LS) mean differences for within cycles and time points, respectively. The statistical equivalence margin was set at 0.05. Pairwise comparisons between CL' s at 100-cycle and the cornea were conducted using t-tests on LS Means. All statistical analyses were carried out using SAS 9.4 (Cary, NC, 2014).

Results: CoF for lenses removed from packaging resulted in mean values of 0.010 (SD 0.005) at 0-cycles, 0.009 (SD 0.003) for 50-cycles, and 0.008 (SD 0.004) for 100-cycles. CoF for ex vivo lenses resulted in mean values of 0.025 (SD 0.009) for 0-cycles, 0.017 (SD 0.004) for 50-cycles, and 0.017 (SD 0.003) for 100-cycles. The LSMean Differences between packaging and ex vivo CL wear were
-0.0147 (0.0039SD) for 0-cycles p=0.0077, -0.0080 (0.0039) for 50-cycles p=0.4626, and -0.0085 (0.0039SD) for 100-cycles (p=0.3846). CoF values of worn lenses demonstrated statistical equivalency to lenses measured out of the package at all cycles (0, 50, and 100-cycles). The CoF average for human cornea was 0.015 (0.009 SD) and is not statistically different from narafilcon A out of packaging (p=0.051) or from ex vivo lenses (p=0.513).

Conclusions: This pilot investigation successfully demonstrated narafilcon A contact lens lubricity value (CoF) measured out of the package is maintained throughout the wear period and is comparable to that of the human cornea. Results should be verified in a larger, appropriately- powered study.

Commercial Relationships: Tawnya Wilson, Johnson and Johnson Vision Care, Inc (E); Kristy Canavan, Johnson and Johnson Vision Care, Inc (E); Rudolf Aeschlimann, SuSoS (C); Samuele Tosatti, SuSoS (C); Ryan Butterfield, MaxisIT (E); Kathrine Osborn-Lorenz, Johnson and Johnson Vision Care, Inc (E)

486 - P17-6

Cosmetic and mechanical lens fit acceptance of 1-DAY ACUVUE® DEFINE™ Brand Contact Lenses with LACREON® Technology

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1. Johnson & Johnson Vision Care, Inc., Jacksonville, FL, United States.

Purpose: The purpose of this study was to evaluate the mechanical lens fit acceptance, the cosmetic fit acceptance, and the absence of the hula hoop effect of 1-DAY ACUVUE® DEFINE™ Brand Contact Lenses with LACREON® Technology.

Methods: This study was a non-masked, brand de-identified, binocular, multi-site, non-dispensing clinical trial. Asian females between 18-40 years of age were stratified into three contact lens powers (+1.00D, -4.00D, -9.00D) based on their optimal sphero-cylindrical refraction. Three primary outcomes were tested in this clinical evaluation, (1) mechanical lens fit acceptance (full corneal coverage and acceptable movement); (2) cosmetic fit acceptance (no sclera exposure inside the limbal ring, and no iris exposure outside the limbal ring) in primary gaze at conversational distance; and (3) absence of the hula hoop effect (sclera visible inside limbal ring when the subject blinks, becoming more or less visible as lens settles) in primary gaze at conversational distance.

Results: One hundred and seventy-seven (177) subjects successfully completed the study per protocol. The mechanical fit was found to be acceptable in 345 of the 354 (97.5%) of the eyes wearing 1-DAY ACUVUE® DEFINE™ Brand Contact Lenses with LACREON® Technology. An acceptable cosmetic lens fit in primary gaze at conversation distance was found in 349 of the 354 eyes (98.6%) and 99.4% of the wearers did not exhibit the hula hoop effect.

Conclusions: 1-DAY ACUVUE® DEFINE™ Brand Contact Lenses with LACREON® Technology provide very high overall mechanical lens fit and cosmetic fit acceptance. Hula hoop was not present in over 99% of all wearers fit with the lens.

Commercial Relationships: Meredith Jansen, Johnson & Johnson Vision Care, Inc (E); Charis Lau, Johnson & Johnson Vision Care, Inc (E); Michael Mayers, Johnson & Johnson Vision Care, Inc (E); Danielle Boree, Johnson & Johnson Vision Care, Inc (E); Lenora Copper, Johnson & Johnson Vision Care, Inc (E)

Clinical Trail: 2036320

487 - P18-7

Effect of Mini Scleral Lenses on Higher Order Aberration in Irregular Cornea

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Purpose: To evaluate whether fitting of mini scleral contact lenses can reduce higher-order aberrations in irregular corneas such as keratoconus eyes with and without corneal intacs.

Methods: A prospective clinical trial was planned which included 29 established keratoconus eyes of 18 patients with or without corneal Intacs. Best corrected visual acuity, topographic refractive analysis, spherical, and cylindrical refraction were determined prior to contact lens fitting. Total error and higher order aberration was measured by iTrace system (Tracey Technologies Corp., Houston, TX) before and after fitting of mini scleral contact lens. Paired sample *t*-test and descriptive statistics were used to evaluate the differences in study parameters before and after wearing the scleral lens.

Results: Both the keratoconus groups with and without intacts, showed significant reduction ($p<0.01$) in resultant higher-order aberration and total error after fitting mini scleral lenses. There was significant improvement ($p<0.01$) in best corrected visual acuity after scleral contact lens fitting.

Conclusions: The mini-scleral lens was useful in reducing total error and higher-order aberrations in patients with irregular astigmatism such as keratoconus eyes with and without intacts. Mini scleral contact lens is an effective solution for patients with highly irregular cornea.
Commercial Relationships: Mukesh Kumar, None; Dr Rohit Shetty, None

488 - P19-8

Comparative Evaluation of Microbial Adhesion on Cosmetic and Clear Hydrogel Lenses

Carol Lakkis [1] Scott Piper [2] Danielle Boree [1]

1. Research & Development, Johnson & Johnson Vision Care, Jacksonville, FL, United States. 2. Kelly Services, Inc, Troy, MI, United States.

Purpose: To determine the levels of primary (reversible) and irreversible microbial adhesion to two limbal ring cosmetic hydrogel contact lenses (CLs) 1-DAY ACUVUE® DEFINE™ NATURAL SHINE™ (etafilcon A) and 1-DAY ACUVUE® DEFINE™ NATURAL SHIMMER™ (etafilcon A with polyvinylpyrrolidone (PVP)) compared to corresponding clear hydrogel CL material controls 1-DAY ACUVUE® (etafilcon A) and 1-DAY ACUVUE® MOIST® (etafilcon A with PVP).
Methods: Viable microbial adhesion was determined at 2 h (primary adhesion) and 20 h (irreversible adhesion) following inoculation using 3 bacterial clinical isolates, *P. aeruginosa* GSU#3, *S. marcescens* K675, and *S. aureus* CKM-S1, at an initial inoculum of approximately $1x10^6$ colony forming units (CFU)/mL. All experiments were conducted in triplicate. Adherent bacteria were recovered from the CLs using a modified vortex device and the resulting bacterial supernatant was enumerated for viable microorganisms by pour plate technique. CFU counts were determined using an automated colony counter and logarithmic (base 10) transformed to allow statistical comparisons to be made between the test and control CLs.
Results: There were no statistically significant differences in microbial adhesion between the cosmetic and clear control CLs for each microorganisms at both 2 & 20 h ($p>0.05$). Non-inferiority was met for all comparisons across all microorganisms at both time points. In addition, statistical equivalence (within ± 0.5 $\log_{10}$ CFU/mL) of the cosmetic and clear control CLs was met for all comparisons with the exception of the etafilcon A with PVP CLs with *S. marcescens* K675 at 20 h, where average microbial adhesion was 0.3 log lower on the cosmetic lens compared to the clear lens. The *P. aeruginosa* clinical isolate showed greater adhesion to all CLs compared to the *S. marcescens* and *S. aureus* isolates.
Conclusions: The presence of the enclosed limbal ring pigment in the cosmetic CLs did not appear to impact primary or irreversible microbial adhesion of clinical isolates of *P. aeruginosa*, *S. marcescens*, and *S. aureus* compared to clear CLs of the same material.
Commercial Relationships: Carol Lakkis, Johnson & Johnson Vision Care (F), Johnson & Johnson Vision Care (E); Scott Piper, Johnson & Johnson Vision Care (F); Danielle Boree, Johnson & Johnson Vision Care (E)
Support: This study was supported by Johnson & Johnson Vision Care.

489 - P20-9

Analysis of first 100 refractive surgeries with a 500-Hz scanning spot excimer laser

Rupali Jain [2, 1]

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Study Group: Jankikund

Purpose: To evaluate visual and refractive outcomes of first 100 refractive surgeries with 500-Hz scanning spot excimer laser
Methods: Retrospective analysis of 100 eyes of 53 patients underwent refractive surgeries(95 LASIK,5 TransPRK)
Outcome measures:Uncorrected(UCVA) and Best corrected(BCVA) visual acuity,refraction and complications
Results: Mean followup was 74.64 ± 70.29days. Mean spherical equivalent was reduced from -3.70 ± 3.23D(range-13.5to+7.5D) to -0.05 ± 0.36D(range-2to+1D) [P<.001,Wilcoxon sign rank test] at last followup.92% had spherical equivalent within ± 0.50D and 82% had UCVA≥20/20 at last followup.86% achieved UCVA equal or better than preoperative BCVA.Statistically significant correlation was found among achieved and intended correction(r=0.99,P<.001).None of the eyes lost their BCVA,2 eyes were overcorrected by ≥1.00D,3 eyes undercorrected by ≥1.00D and 1 eye required flap reposition
Conclusions: Refractive surgeries with 500-Hz scanning spot laser were safe,effective and predictable
Commercial Relationships: Rupali Jain, None

490 - P21-10

Wavefront-optimised Vs. topography guided ablation, a contralateral eye study

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1. Department Of Ophthalmology, Advanced Eye Centre, Chandigarh, India.

Purpose: To compare the outcomes of wavefront-optimized and topography guided treatment in fellow eyes of patients having laser in situ keratomileusis (LASIK) for myopia.
Methods: This prospective comparative study comprised 20 patients who had wavefront-optimised(WO) LASIK in 1 eye and topography- guided(TG) LASIK in the fellow eye. The IntraLase iFS(Abbott Medical Optics) was used to create a superior-hinged flap and the MEL80 Excimer Laser (Carl Zeiss MeditecAG), for photoablation. The WASCA analyzer(Carl Zeiss Meditec AG) was used to measure ocular wavefront aberrations and the Functional Acuity Contrast Test(FACT) chart, to measure contrast sensitivity before and 1week,1month and 3 month after LASIK. The refractive and visual outcomes and the changes in aberrations and contrast sensitivity were compared between the 2 treatment modalities.
Results: Preoperative mean spherical equivalent refraction was -4.22 ± 1.22D and -4.381 ± 1.30D in wavefront – optimised and topographthy- guided groups, respectively. At three months , 95%of eyes in the wavefront-optimised group and 100% in the topography-guided group had

uncorrected visual acuity of 20/20 or better; there was no statistical difference in the number of patients who had postoperative spherical equivalent refraction of ± 0.5D . Higher order aberrations(RMS) increased from 0.238 ± 0.06 μm (range, 0.17 to 0.44 μm) and 0.224 ± 0.06 μm (range, 0.13 to 0.40 μm) to 0.385 ± 0.14 μm (range, 0.18 to 0.73 μm) and 0.324 ± 0.11 μm (range, 0.20 to 0.64 μm) in the wavefront-optimized and topography guided groups, respectively. Contrast sensitivity did not decrease in either group and no statistically significant differences between groups were noted.
Conclusions: Although both wavefront-optimized and topography-guided lasik gave excellent refractive correction results, the later induced fewer higher-order aberrations and tissue conserving for same amount of refractive correction
Commercial Relationships: Anand Pasari, None; Arun Jain, None; Chintan Singh, None; Partha Chakma, None

491 - P22-11

Corneal biomechanical measurement of patients with congenital glaucoma using Scheimpflug noncontact tonometry

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Purpose: To investigate the corneal biomechanical properties in congenital glaucoma (CG).
Methods: We studied eight eyes of patients with CG. Scheimpflug noncontact tonometry (SNT) was used to evaluate the corneal biomechanical parameters including central corneal thickness (CCT), intraocular pressure (IOP), deformation amplitude (DA), radius (R), peak time (PT), and peak distance (PD) at the highest concavity, and applanation time (TAin, TAout), applanation velocity (VAin, VAout), and applanation length (LAin, LAout) at the first and second applanation points. Anterior-segment optical coherence tomography (AS-OCT) was used to measure the AS morphologic parameters including CCT (CCT) and corneal diameter (CA). Another standard noncontact tonometer and a Goldmann applanation tonometer (GAT) were used to evaluate the IOP. JMP9.0 software (SAS Institute Inc., Cary, NC) was used for data analysis.
Results: SNT showed the mean CCTs was 478.17 ± 40.78 μm, the mean intraocular pressure (IOPs) was 13.13 ± 1.36 mmHg. DA was1.10 ± 0.11 mm, R was 6.92 ± 0.95 mm, PT was 16.23 ± 1.07 msec and PD was 4.58 ± 1.34 mm, TAin was 7.43 ± 0.22 msec, TAout was 22.2 ± 0.43 msec, VAin was 0.12 ± 0.02 m/ sec,VAout was -0.27 ± 0.22 m/sec, LAin was 1.65 ± 0.26 mm, LAout was 1.37 ± 0.48 mm. Anterior segment OCT showed CCTa was 512.3 ± 23.2 μm, and CA was 13.2 ± 0.69 mm. The other non contact tonometer showed the mean IOPn was 13.6 ± 2.8 mmHg, and Goldmann applanation tonometer showed the mean IOPa was 13.9 ± 2.8 mmHg. There was no significant difference between CCTs and CCTa（P=0.22, Student's paired t-test）. And no significant difference was found in the mean IOPs between different tonometers (IOPs vs IOPn; P=0.43, IOPs vs IOPa; P=0.50, Student's paired t-test).
Conclusions: Our results showed that patients with CG had a larger corneal diameter, thinner CCT, elongated applanation length, and peak distance at the highest concavity compared with normal reported data (Maeda et al., Jpn J Ophthalmol 2014). We concluded that decreased CCT and increased corneal diameter may affect the corneal biomechanical profile in patients with CG, although the SNT recorded the same IOP value as any other type of tonometers.
Commercial Relationships: Takanori Matsuoka, None; Kenji Matsushita, None; Rumi Kawashima, None; Kohji Nishida, None

492 - P23-12

Rabbit model of corneal ectasia with excimer laser

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Purpose: To apply excimer laser stromal photoablation to the development of a rabbit model of corneal ectasia that simulates the human condition.
Methods: Ten eyes of 10 New Zealand White rabbits had femtosecond laser flap creation with 120 μm thickness and 7.9mm diameter (VisuMax, Carl Zeiss Meditec) followed by LASIK (Technolas; Bausch & Lomb). Three eyes had -9 dioptres(D) correction, 3 had -7D correction and 4 had -5D correction, all with 6.5mm optical zone. All eyes had Visante Omni (Carl Zeiss Meditec) topography before intervention and weekly subsequently. At each scan, 4.5mm zone mean keratometry (K-4.5mm), 4.5mm astigmatism (Astigm-4.5mm) and maximum posterior elevation measurements were recorded. The treatment group that simulated human ectasia better was followed up for 2 months; on sacrifice the corneas were examined with transmission electron microscopy(TEM). Mean ± standard deviation topography measurements at each time interval were compared to baseline (paired t-test).
Results: The -9D LASIK rabbits developed corneal micro-perforation in 2 of the 3 eyes immediately after LASIK. The 3 eyes treated with -7D LASIK developed topographic ectasia rapidly within 2 weeks of intervention. The -5D LASIK eyes developed qualitative (vertical keratometric asymmetry, increase in posterior elevation) and quantitative topographic ectasia changes gradually over 4 weeks, simulating most closely the progressive human ectasia. K-4.5mm decreased from 43.3 ± 0.6 dioptres(D) at baseline to 41.8 ± 0.7D at 4 weeks (p=0.007) and 40.6 ± 1.3D at 8 weeks (p=0.017). Astigm-4.5mm increased from 0.8 ± 0.3D to 2.3 ± 0.6D at 4 weeks (p=0.022) and 1.9 ± 1.8D at 8 weeks (p=0.242). Maximum posterior elevation increased from 7.7 ± 2.1 μm to 21.8 ± 10.0 μm at 4 weeks (p=0.073) and 22.1 ± 10.3 μm at 8 weeks (p=0.069). On TEM, mean collagen fibril diameter in the -5D eyes was smaller than in the fellow control eyes (34.0 ± 2.2 vs. 44.6 ± 9.1nm; p<0.005), as was interfibril distance (47.1 ± 7.8 vs. 82.4 ± 27.6nm; p<0.005).
Conclusions: Excimer laser stromal photoablation and serial Visante Omni imaging enabled the development of

a rabbit model of ectasia that simulated human ectasia in topography and microarchitecture. The -5D treatment produced the most appropriate model, with the gradual development of ectasia simulating the slowly progressive human condition. This model could allow the evaluation of treatment modalities that aim to halt or slow ectasia progression.
Commercial Relationships: Aristides Konstantopoulos, None; Yua Chi Liu, None; Andri Riau, None; Jinesh Shah, None; Nyein Chan Lwin, None; Ericia Pei Wen Teo, None; Gary Hin Fai Yam, None; Jodhbir Mehta, None

493 - P24-13

Association of Sex Hormone Levels and Corneal Hysteresis During Menstrual Cycle

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Purpose: To determine corneal ocular hysteresis change during phases of the menstrual cycle and to correlate with sex hormone levels.
Methods: 31 eyes of 31 healthy young women (18-27 years old) were recruited. Hormones including follicle stimulating hormone (FSH; mU/ml), luteinizing hormone (LH; mU/ml), progesterone (ng/ml) and 17-β estradiol (pg/ml) were measured in serum three times in a cycle during the follicular (day 3 to day 9), luteal phases (day 21 to day 28) and ovulation (day 13 to day15). At every time point, corneal hysteresis (CH) were measured with the Ocular Response Analyzer.

Results: The mean corneal hysteresis values at follicular, ovulatory, and luteal phases were 12.8mm Hg, 11.7mm Hg, and 12.4 mmHg ($p < 0.05$), There was a reverse correlation between serum estradiol and CH (Pearson correlation=-0.28, P=0.039)
Conclusions: The corneal hysteresis is a bimechanical property of the cornea that changes during a menstrual cycle that might be due to serum estradiol levels.
Commercial Relationships: Azam Bakhtiari, None; Mohammadreza Peyman, None

494 - P25-14

Niche function of amniotic membrane stromal cells in corneal epithelial stem cell expansion

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Purpose: Amniotic membrane (AM) has been broadly used as carrier for ex vivo expansion of limbal epithelial stem cells (LESCs), whereby AM works as a surrogate niche for stem cells. Previous studies used cryopreserved AM which contains devitalized stromal and/or epithelial cells. In this study, we intend to evaluate the efficacy of the fresh denuded AM with live stromal cells (LAM) for ex vivo expansion of LESCs.
Methods: Fresh amniotic membrane was harvestded from human placenta, corneal limbal epithelium were harvested from New Zealand white rabbit. Epithelium of fresh AM was removed after brief dispase II digestion. AM stromal cells remained alive, or devitalized through repeated freeze-thaw. Limbal epithelial cell sheets isolated from rabbit limbal explants by dispase II digestion were cultured on the basement membrane side of the live AM (LAM) or dead AM (DAM) in SHEM medium for up to 7 days. Some tissues were labeled with BrdU before culturing and then tracing for 7days. While some epithelial cells were BrdU labeled after 3 days culture. The outgrowth of the epithelial sheets, epithelial colony-forming-efficiency (CFE) on 3T3 feeder layers, and BrdU labeling with or without tracing were performed. The whole-mount and section immunofluorescence staining for cytokeratin K12 and K14, p63, Ki67, type IV collagen and BrdU were conducted. The expression of K12, K14, p63, Ki67 was also determined by Western blot analysis.
Results: Limbal epithelial cells on LAM showed more homogeneous, compact, and smaller in cell size. The CFE of cells expanded on LAM was significantly higher than that on denuded AM with devitalized stromal cells, i.e, DAM. There were more BrdU label retaining cells on LAM. K14, p63, and Ki67 expression was higher in epithelial cells expanded on LAM. K12 was negative in the basal epithelium on LAM, while was full-thickness positive in the epithelium on DAM. Furthermore, the epithelial cells on the LAM could be easily separated as an intact cell sheet, while cells on DAM could not.
Conclusions: AM with live stromal cells facilitates ex vivo expansion and stemness preservation of limbal epithelial stem cells. AM stromal cells may act as niche cells in ex vivo corneal epithelial tissue engineering.
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495 - P26-15

N-cadherin expression in hypoxic spheroidal cultivation of human limbal epithelial cells

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Study Group: CCB

Purpose: Stem cells have a specialized microenvironment for maintaining self-renewal and multipotent capacities. It is believed that a cornea epithelial stem cell niche exists in the limbus. We previously reported that the limbal phenotype can be maintained in spheroids form the human limbus for up to 1 month in medium with KGF+Y27632 using matrigel (2014 ARVO). To further improve cultivation method of limbal epithelial stem cells niche in vitro, we performed spheroidal cultivation from human limbus under hypoxia.
Methods: N-cad+ cells and mesenchymal niche-like cells were prepared from donor corneal sclera used after

cornea transplantation. After treatment with collagenase, limbal epithelial cells and surrounding cells were scraped from limbal tissue, and spread on Matrigel. Cells sere cultivated using medium with KGF and the Rho kinase inhibitor, Y27632 in normoxia (20% O2) or hypoxia (2% O2) conditions. After 1 month, the spheroidal forming ratio and size were estimated, and frozen sections of spheroids were observed by histochemical analysis.
Results: Spheroids were uniformly small and round under hypoxia compared to normoxia,, but spheroidal forming ratio was similar between hypoxia and normoxia. Keratin (K) 15 and p63 were expressed in the edge of spheroids after 1-month cultivation under hypoxia. In addition, N-cadherin was expressed in epithelial spheroid under hypoxia.
Conclusions: The limbal niche can be maintained in spheroids for up to 1 month in hypoxia culture with a N-cadherin positive limbal phenotype.
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Galectin Expression Signature in Murine Corneal Allograft

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Purpose: Galectins constitute a family of widely distributed carbohydrate-binding proteins characterized by their affinity for b-galactoside-containing glycans found on many cell surface and extracellular matrix glycoprotein. In mammals, there are 15 members of the galectin family. There is currently intense interest in characterizing the function of galectins because of their role in the modulation of immune response. Galectins are a novel class of modulators of innate and adaptive immune functions, and potential therapeutic agents for promoting allograft survival. The goal of this study was to determine the galectin expression signature in murine corneal allograft.
Methods: The Balb/c mice (8 to 10 weeks old) were used as graft recipient and C57BL/6 mice (6 weeks old) were used as donor grafts. One week after the transplantation, the sutures were removed and corneal grafts were scored for their graft opacity and neovascularization by slit-lamp microscopy. Combined corneas were harvested four weeks after transplantation, and galectin expression in accepted and rejected grafts were analyzed by Western blot and immunohistochemistry staining. Specificity of antibodies were verified by Western blot analyses using various recombinant galectins.
Results: In normal mouse cornea Gal-1,-3,-7,-8 and -9 were expressed as determined by Western blot. In both, accepted and rejected grafted corneas, protein expression levels of Gal-1,-3,-7,-8 and -9 were upregulated compared to normal corneas. Comparing the both grafts, Gal-1, -3 and -7 were no difference, but Gal-8 expression was higher in rejected grafts, Gal-9 expression was higher in accepted grafts ($p<0.05$, Student's t-test). In IHC staining, Gal-8 expression in rejected grafts was higher than accepted grafts in stroma. Immunoreactivity of Gal-9 was detected at the interface of host-graft stroma and was stronger in accepted grafts compared to rejected grafts.
Conclusions: Our data show differential expression pattern of Galectins-8 and -9 in rejected corneal allografts. These results lay foundation for assessing the role of these two galectins in modulating the outcome of corneal allografts.
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497 - P28-17

Derivation of mesenchymal stromal cells from pluripotent stem cells through a neural crest lineage using small molecule compounds with defined media

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Purpose: The neural crest cell (NCC) is an embryonic migratory cell population that possesses an ability to differentiate into a wide variety of cell types contributing to craniofacial skeleton, cornea, peripheral nervous system, and skin pigmentation, suggesting the promising role of NCCs as a source of cell-based therapy. Although several methods have been published to induce NCCs from human pluripotent stem cells (PSCs) further modifications are required to improve robustness, efficacy and simplicity, and also to use chemically-defined culture condition for clinical application.
Methods: Chemically defined medium (CDM) was used as a basal medium both in the induction and maintenance steps. By optimizing the culture condition, we found that the combination of GSK3 β inhibitors and TGF β inhibitor with a minimum growth factor (insulin) was enough to induce NCCs from PSCs with a high efficiency. The induced NCCs expressed cephalic NCC-related genes and stably proliferated in CDM supplemented with EGF and FGF2 at least up to 10 passages without changing the gene expression profiles.
Results: Differentiation property for peripheral neurons, glia, melanocytes, and corneal endothelial cells were confirmed. In addition, cells compatible with the

differentiation property as multipotent mesenchymal stromal cells (MSCs) were induced from NCC using CDM specific for MSC.
Conclusions: Our simple, robust, and chemically defined induction protocol enables to generate NCCs as an intermediate material producing terminally differentiate cells for cell-based innovative medicine.
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498 - P29-18

Genetic association of TCF4 intronic polymorphisms, CTG18.1 and rs17089887 with Fuchs' endothelial corneal dystrophy in Indian population

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Purpose: To assess the genetic association of *Transcription factor 4* (*TCF4*) intronic polymorphisms and CTG18.1 allele in Fuchs' Endothelial Corneal Dystrophy (FECD) individuals from Indian population.
Methods: Forty four FECD patients and 108 unrelated age-matched controls were recruited with informed consent for this study. Three single nucleotide polymorphisms (SNPs) spanning the third intronic region of *TCF4* (rs613872, rs17089887 & rs17089925) and an unstable trinucleotide repeat CTG18.1 allele were genotyped by direct sequencing using Sanger's method. Association of polymorphisms was analyzed using Chi squared test and logistic regression.
Results: SNP rs17089887 (P = 0.013) and CTG18.1 (P = $2x10^{-4}$) alleles were found to be significantly associated with FECD in Indian population. However, the other two SNPs, rs613872 and rs17089925, were not found to be associated. Thirty four percent of FECD subjects and five percent of control individuals harbor more than 50 trinucleotide repeats which was considered as disease threshold.
Conclusions: *TCF4* poses a major contributor to FECD manifestation globally, with significant association of rs17089887 and CTG18.1 allele in Indian population.
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499 - P30-19

A COL4A3 locus (rs7606754) has an additive effect on the corneal curvature of 20-year olds

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Purpose: Keratoconus is a complex disease typically affecting younger individuals. Previously, genome-wide association studies (GWAS) have identified associations between keratoconus and multiple single polymorphisms (SNPs). We investigated the effect of six risk alleles for keratoconus in young adults with Northern European ancestry.
Methods: At the 20-year follow-up participants of the Western Australian Pregnancy Cohort (Raine) Study underwent a comprehensive eye examination including anterior segment tomography assessment. Individuals with subclinical keratoconus were identified according to modified Rabinowitz/McDonnell methods. Genotype data and consents for genetic studies were available from the previous assessments. For the current study, genotype and phenotype data were available for 814 individuals. Quantitative trait analyses of six loci (QTL) (rs7606754, rs4894535, rs1324183, rs1536482, rs7044529, rs9938149) with corneal curvature (CC) were performed using linear regression with additive genetic inheritance model. The relationships between genotype groups were assessed with a trend test. Individuals with subclinical keratoconus were excluded from QTL analysis.
Results: A total of 12 (1.5%) individuals were identified with subclinical keratoconus. No distinctive allele frequency pattern was present in the six loci of these individuals. rs7606754 was found to be associated with CC in the remaining 802 individuals (beta=0.154, p=0.035). However no trend for corneal steeping was present in individuals with homozygous A/A genotype compared to individuals with heterozygous A/T and homozygous T/T genotypes (p=0.085).
Conclusions: Of the six SNPs investigated, only rs7606754 had an additive effect on CC of young adults. However, a clinical feature of keratoconus, steepening of CC was not dependent on the rs7606754 genotype in our cohort. Better-powered future studies in this age group and older ages are required to assess the effects of associated SNPs on CC variations.
Commercial Relationships: Seyhan Yazar, None; Alex Hewitt, None; Jenny Mountain, None; David Mackey, None

500 - P31-20

Association of ZEB1 variations and TCF4 SNP rs613872 with late onset Fuchs endothelial corneal dystrophy in Indian Patients

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Purpose: Fuchs endothelial corneal dystrophy (FECD) results in loss of vision associated with progressive corneal edema and loss of corneal transparency. Candidate genes implicated are *COL8A2*, *SLC4A11* and *ZEB1* but identified changes show mixed evidence as causal variants. Genome wide association analysis implicated *TCF4* rs613872 SNP with increased risk of FECD validated by several studies. To evaluate genetic changes in the candidate genes in FECD patients.

Methods: 82 patients diagnosed with FECD from the outpatient department of Rajendra Prasad Centre for Ophthalmic sciences (RPC), AIIMS and 100 age and sex matched controls from the general population were recruited for the study. Patients were examined under slit-lamp and clinically evaluated using specular microscopy, confocal scan, ultrasound pachymetry and applanation tonometry. PCR amplification of genomic DNA from peripheral blood was followed by direct sequencing to screen for presence of genetic changes in *ZEB1, COL8A2, SLC4A11* and *TCF4* genes.

Results: The patients showed mean age of onset of symptoms at 59.14 ± 17.14 years, male to female ratio of 1:1.5, mean duration of disease 4.372 ± 0.718 years, mean specular count (endothelial cell density) 1629 ± 93.62 cells/mm2 and mean central corneal thickness 617.30 ± 15.73 µm. No changes were identified in *COL8A2* and *SLC4A11* gene except for a reported *COL8A2* SNP. *ZEB1* gene showed presence of a novel SNP in about 15% and reported SNPs rs149166539 and rs77516068 in 4% of the FECD patients and in none of the controls.

Other changes identified were a novel nonsense mutation in two patients, two novel missense mutations in one patient each and a reported mutation p.840Q>P in one patient while these changes were absent in the controls. Genotype frequency of ZEB1 rs220060 and TCF4 rs613872 were significantly different between cases and controls with ZEB1 rs613872 heterozygous genotype "CA" being significantly higher in the patients.

Conclusions: This is the first report of genetic variations in ZEB1 and TCF4 in FECD from India suggesting its role in the pathogenesis of the diseases.

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501 - P32-21

Attenuation of Lysyl Oxidase and Collagen Gene Expression in Keratoconus patient corneal epithelium corresponds to disease severity

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Purpose:
To assess level of Lysyl Oxidase (LOX) and Collagen Gene Expression (COL1A1 ad COL4A1) in Keratoconus (KC) patient corneal epithelium and its relation to disease severity.

Methods: Gene expression levels of key proteins LOX, Collagens (COL1A1 and COL4A1), MMP9 and IL6 in debrided corneal epithelium from a large cohort of KC patients (n=80) and compared them to non-ectactic controls (n=48).

Results: There was significant reduction of LOX transcript levels in epithelium and LOX activity in the tears. In the same samples, MMP9 transcript levels were upregulated and correlated strongly with disease severity. IL6 transcript levels were moderately increased in KC patients. Collagen transcripts (COL1A1 ad COL4A1) were reduced in KC patients but demonstrated differential reduction profiles across different grades of KC. There was marked reduction in protein expression levels of LOX in the epithelium

Conclusions: There is deregulation of gene expression of important factors of corneal homeostasis in corneal epithelium of KC patients. LOX and MMP9 expression levels appear to correlate with disease severity and collagen expression.

Commercial Relationships: Tejal SJ, Narayana nethralaya, Bangalore, India (E); Rohit Shetty, Narayana nethralaya, Bangalore, India (C); Vishal Arora , Narayana nethralaya, Bangalore, India (C); Rudy A. Nuijts, Narayana nethralaya, Bangalore, India (S); Arkasubhra Ghosh, Narayana nethralaya, Bangalore, India (C); Abhijit Roy, Narayana nethralaya, Bangalore, India (C); Natasha Pahuja, Narayana nethralaya, Bangalore, India (E)

502 - P33-22

Safety of Intracameral Moxifloxacin/ Dexamethasone Fixed-Dose Formulation on the Corneal Endothelium in a Rabbit Model

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Purpose: To determine the safety of intracamerally injected preservative-free 0.5% moxifloxacin/ 0.1% dexamethasone fixed-dose combination on the corneal

endothelium in a rabbit model and compare it to intracamerally injected preservative-free 0.5% moxifloxacin as control.

Methods: This experimental study included twenty eyes from ten Philippine Albino rabbits. The eyes were assessed for baseline corneal clarity and anterior chamber (AC) inflammation using slit-lamp biomicroscopy. A specular microscope (SP-9000, Konan Medical Inc., Hyogo, Japan) measured the corneal endothelial cell density (ECC) and corneal thickness (CT).

Intracameral injections of 0.1mL 0.5% moxifloxacin ophthalmic solution were administered to the 10 right eyes (IPFM group) and 0.1mL of 0.5% moxifloxacin/ 0.1% dexamethasone fixed-dose preparation were administered to the 10 left eyes (IPFMDex group).

In both groups, ECC, CT, corneal clarity, and AC inflammation at Day 0 (baseline) were compared with Day 1 (one day post-injection) and Day 7 (seven days post-injection). The IPFMDex group was also compared with the IPFM group at Days 0, 1, and 7. The endothelial cells of harvested corneas at Day 1 and 7 were exposed to vital stains composed of trypan blue and alizarin red, and analyzed using a previously published quantitative method of assessing collective endothelial cell damage (ECD) using Adobe Photoshop software and the two groups were compared. Data was analyzed using paired and independent samples *t*-test with a significance value of $P < 0.05$.

Results: In both the IPFM and IPFMDex groups, ECC and CT values on baseline (Day 0) were not statistically different from Day 1 (IPFM, ECC $P = 0.07$, CT $P = 0.76$; IPFMDex, ECC $P = 0.41$, CT $P = 0.94$) and Day 7 (IPFM, ECC $P = 0.95$, CT $P = 0.28$; IPFMDex, ECC $P = 0.29$, CT $P = 0.34$).

No significant difference in ECC, CT, and ECD were found between the IPFM and IPFMDex group at Day 1 (ECC $P = 0.82$, CT $P = 0.36$, ECD $P = 0.96$) and Day 7 (ECC $P = 0.95$, CT $P = 0.22$, ECD $P = 0.61$).

Throughout the study, the cornea in both groups were clear and showed no signs of AC inflammation.

Conclusions: Intracameral injection of preservative-free moxifloxacin/dexamethasone fixed-dose formulation was safe on the rabbit corneal endothelium and was no different from preservative-free moxifloxacin.

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503 - P34-23

Expression of Eph receptors in corneal endothelium

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Purpose: The posterior surface of the cornea is lined with an endothelium cell layer that functions to maintain corneal clarity. Adult corneal endothelial cells lack the ability to proliferate and this can result in endothelial dysfunction and vision loss if cell density falls below a critical threshold. The only treatment for this condition is transplant surgery. We aim to develop strategies to increase endothelium cell density in patients with endothelial dysfunction, circumventing the requirement for surgery. Previous studies indicate that some Eph receptor tyrosine kinases are expressed in corneal tissues. Since Eph receptors are known to regulate cell migration and proliferation, we have initiated a study to examine their expression and role in corneal endothelial cells. Our studies are focused on the ligand ephrin-A1, and on two of its receptors, EPHA1 and EPHA2. We hypothesise that they will be expressed in adult corneal endothelial cells and that their normal role will be to suppress migration and proliferation.

Methods: Expression characteristics of ephrin-A1, EPHA1 and EPHA2 were analysed in fresh samples of human corneal endothelium, and in cultures of primary human corneal endothelial cells, using immunofluorescence techniques. Expression studies were also performed with transformed human corneal endothelial cells (B4G12s) using RT-PCR.

Results: Our results showed that ephrin-A1, EPHA1 and EPHA2 proteins were present in the nuclei, and more variably at cell borders, of human corneal endothelial cells *in vivo*. Immunoreactivity for all three proteins was also demonstrated in both subconfluent and confluent primary corneal endothelial cell cultures. In addition, the results of our RT-PCR studies indicated that ephrin-A1, EPHA1 and EPHA2 were also expressed in both subconfluent and confluent B4G12 cultures.

Conclusions: Our data indicates that ephrin-A1, EPHA1 and EPHA2 are normally expressed in human corneal endothelial cells, both *in vivo* and *in vitro*. This data is supportive of a potential role for these factors in regulating the proliferation and migration behaviour of endothelial cells in the adult cornea. We aim to test this hypothesis further by performing functional assays with both transformed and primary corneal endothelial cells *in vitro*.

Commercial Relationships: Jennifer Young, None; Neil Richardson, None; Damien Harkin, None

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504 - P35-24

The influence of dextran on the in-vitro development of corneal endothelium in corneorscleral rims prior to transplantation

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Purpose: Dextran is added to corneal culture medium 5 days prior to transplantation to ensure that the cornea is osmotically dehydrated. It is presumed that dextran has a certain toxic effect on corneal endothelium but the degree of severity of its effect has no so far been determined. We believe that the quantification of the toxicity of dextran on corneal endothelium could have an impact on the time frame and logistics of corneal preparation in eye banking.

Methods: In a retrospective data analysis we studied the development of corneal endothelium of 1334 corneal explants cultured in dextran medium. The loss of

endothelium per day in dextran medium was compared to the loss of endothelium in culture medium not containing dextran. In a separate analysis we studied if age, sex and cause of death of the donor also play a role in the development of the endothelial cell count in dextran medium.
Results: Corneas cultured in dextran-free medium showed a mean endothelium cell count decrease of 0,7% per day. After dextran was added the endothelium cell loss rose to 2,01% per day- representing an increase by a factor of 2,9. Most of this effect is seen on the first day after dextran was added. Age, sex and cause of death of the donor did not play a significant role in the rate of endothelial cell loss.
Conclusions: Dextran has a significant toxic effect on corneal endothelium in vitro. This data analysis could help design an algorithm to calculate the probable endothelium cell count in vitro in a medium containing dextran and possibly influence the time management and planning of corneal transplantations.
Commercial Relationships: Filip Filev, None

505 - P36-25

A case of a corneal bee sting monitored by anterior segment optical coherence tomography

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Purpose: Corneal honey bee sting is an uncommon environmental eye injury. However, if undiagnosed and not properly diagnosed, they can lead to corneal scar or chronic inflammation.
Anterior segment optical coherence tomography, a technology typically used to examine the anterior segment, may be useful in monitoring the therapeutic interventions for conditions involving corneal edema by the consequences with corneal honey bee sting.
Methods: A 63-year-old man presented with a left eye pain and decreased vision after honey bee injury while driving a bicycle. Visual acuity was 20/40 in left eye, 40/40 in right eye at the day2 after injury. Slit lamp examination revealed marked edema in paracentral area of the injured eye. The patient was successfully treated with topical steroids and levofloxacin. This case was followed to resolution using anterior segment OCT(optical coherence tomography) using an imaging analyzing software to quantify decrease in edema throughout treatment. The thickest area of the cornea was located and measured across time using anterior segment OCT until resolution was achieved. Anterior segment OCT allowed precise localization of stromal as well as the objective measurement of therapeutic interventions resulting in reduced edema and thickness.
Results: The maximum thickness part was located in paracentral area and its thickness was 969 uM at day 2 after injury and it resolved to 629 um at day7 after injury. Visual acuity recovered 40/40 at 7 days after injury.
Conclusions: The use of anterior segment OCT allows objective measurements of corneal thickness and presents an additional method for following stromal edema due to corneal bee stings with greater accuracy than can be appreciated using slit lamp examination alone.
Commercial Relationships: Emi Inagaki, None; Shigeto Shimmura, None; Yutaka Hara, None; Shin Hatou, None; Kazuo Tsubota, None

506 - P37-26

Tear expression profiling of cytokine, chemokine and soluble receptor in keratoconus patients

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Purpose: To determine whether the expression levels of cytokines, chemokines and soluble receptor in tear of keratoconus patients contribute to the pathogenesis of keratoconus
Methods: The patients with keratoconus were diagnosed based on the following criteria, (1) symptoms of keratoconus (Munson sign, protrusion, Vogt' s striae, corneal thickness, scarring, Fleishcher ring, photokeratoscopy signs, video keratography signs, and refractive errors) and (2) medical histories (age, gender, contact lense wearing, eye rubbing, systemic disease, atopy, and connective tissue disease). Tears were collected from 28 keratoconus patients (56 eyes) and 30 healthy subjects (60 eyes) by a polyurethane minisponges. Control subjects with no history of ocular disease were also enrolled. The concentrations of cytokines/chemokines were analyzed by Luminex 200 using Human cytokine/chemokine (42 molecules), Human cytokine/chemokine Panel II (23 molecules) and Human soluble cytokine receptor (14 molecules). The Median Fluorescent Intensity (MFI) was used to obtain the calculating cytokines, chemokines and soluble receptor concentrations in tears
Results: Of the 79 cytokines, chemokines and soluble receptors, we detected 16 molecules that demonstrated a significant differences in tears from Keratoconus patients. In cytokines, G-CSF ($p<0.001$), IL1-ra ($p<0.05$), VEGF ($p<0.05$), IL-16 ($p<0.05$) and IL-20 ($p<0.05$) were significantly increased in Keratoconus patients compared to control subjects. MDC ($p<0.05$) and GRO ($p<0.05$) of chemokines and sIL-1RI ($p<0.05$) and sIL4R ($p<0.001$) of soluble receptors were increased in keratoconus patients. Whereas IL4 and IFN gamma (all $p<0.001$) and FGF2 ($p<0.05$) of cytokines, MIP1a and MIP1b (all $p<0.001$) of chemokines, sCD30 and sIL6R (all $p<0.05$) were significantly decreased in keratoconus patients
Conclusions: In tears of keratoconus patients, nine molecules were elevated keratoconus patients, whereas 7 molecules were decreased. It is suggested that different levels of inflammatory regulated cytokines/chemokines/soluble receptors may all play an important role in the pathogenesis of keratoconus
Commercial Relationships: Jeewon Mok, None; Kyung-Sun Na, None; Choun-Ki Joo, None
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507 - P38-27

The use of corneal densitometry in assessing the treatment outcome after corneal transplantation

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Purpose: To compare the corneal densitometry between Penetrating Keratoplasty (PK) and Deep Anterior Lamellar Keratoplasty (DALK) in patients with keratoconus, and between PK and Descement' s Stripping Endothelial Keratoplasty (DALK) in patients with Fuchs' Endothelium Dystrophy (FED) using Oculus Pentacam.
Methods: Two retrospective comparative studies were carried out at Manchester Royal Eye Hospital, UK. Post-operative BCVA, corneal densitometry, Central Corneal Thickness (CCT) and other relevant clinical details were extracted from clinical notes and Pentacam database after one year of the corneal transplantation for each group.
Results: Analysis form the keratoconus group (n=22) found there was a significantly higher corneal densitometry measurement after DALK than PK in the posterior layer at the central 2mm of the cornea (p=0.01) and at the surrounding 2mm-6mm annulus zone of the cornea (p=0.04). Analysis from the FED group (n=23) found higher corneal densitometry after PK than DSEK but was not significant. There was no strong correlation between post-operative BCVA and corneal densitometry in keratoconus group. There was also no strong correlation between CCT and corneal densitometry in FED group. Combined analysis showed there was significantly higher corneal densitometry in FED group than keratoconus group after PK in the middle layer (p=0.01) and in the posterior layer (p=0.00). All in which needs further investigations.
Conclusions: There was different in corneal densitometry outcomes after different type of corneal transplantation in the different group of disease. Oculus Pentacam provides an objective evaluation to monitor the cornea status after the surgery. Further investigation with prospective design, longer study period and larger sample size is recommended to increase its clinical validity in the future.
Commercial Relationships: Syarifah Faiza Syed Mohd Dardin, None

508 - P39-28

Inhibition of lymphangiogenesis and hemangiogenesis in corneal inflammation by subconjunctival Prox1 siRNA injection in rats

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Purpose: Prospero homeobox 1 (Prox1) siRNA is a small interfering RNA that is designed to specifically bind Prox1 mRNA. We determined whether Prox1 siRNA inhibits lymphangiogenesis and hemangiogenesis after acute corneal inflammation.
Methods: Three Prox1 siRNAs were synthesized and tested for their effect on Prox1 mRNA expression and tube formation in human dermal lymphatic endothelial cells (HDLECs) *in vitro*. The *in vivo* effects of Prox1 siRNA were assessed in alkali burn-induced inflammatory corneal neovascularization of rats. Prox1 siRNA was administered via subconjunctival injection. Corneal flat mounts were stained with lymphatic vessel endothelial hyaluronan receptor (LYVE)-1 to reveal lymphatic vessels. Lymphangiogenesis and hemangiogenesis were analyzed morphometrically using Image J software. Corneal inflammatory cell infiltration was evaluated by immunostaining for F4/80 and CD45. Western blot assay was used to analyze the protein levels of LYVE-1, podoplanin, vascular endothelial growth factor receptor 2 (VEGFR2), and VEGFR3.
Results: Prox1 siRNA treatment decreased Prox1 mRNA expression and tube formation in cultured HDLECs. Subconjunctival injection of Prox1 siRNA significantly inhibited alkali burn-induced lymphangiogenesis and hemangiogenesis in the cornea compared to those of scrambled siRNA (negative control). This inhibition was comparable to that induced by bevacizumab (positive control). Prox1 knockdown by Prox1 siRNA also inhibited macrophage and leukocyte infiltration into the cornea. All four protein expression were downregulated by Prox1 siRNA.
Conclusions: Prox1 siRNA is a strong inhibitor of inflammatory corneal lymphangiogenesis and hemangiogenesis *in vivo*. Prox1 siRNA may be useful in preventing immune rejection after penetrating keratoplasty by suppressing lymphangiogenesis.
Commercial Relationships: Chang Rae Rho, None; Jun-Sub Choi, None; Choun-Ki Joo, None

509 - P40-29

Suppression of corneal inflammation by inhibition of lymphangiogenesis in rat alkali burn model

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Purpose: Corneal inflammation is induced by various stimuli including infection, chemical burn, dry eye and graft rejection. Also, lymphangiogenesis is induced during inflammatory response. This study was investigated to

suppress inflammation by inhibition of lymphangiogenesis by Prox1 siRNA.
Methods: SD rats (Male, 8 weeks) were used for this study. Inflammation and lymphatic vessels are induced by alkali burning in cornea. 2 mm disc with 1N NaOH was applied in center of cornea rat for 10 sec. Prox1 siRNA was designed from human Prox 1 gene (NM_001270616.1). LYVE-1 antibody for lymphatic vessels, F4/80 and LSP-1 antibody for inflammatory cells were used in this study. To inhibit corneal lymphangiogenesis, Prox1 siRNA liposome was treated 2 times per day after burning, topically.
Results: The lymphatic vessels were observed in injured cornea by LYVE-1 immunostaing. And the F4/80 and LSP-1 positive cells were observed in this cornea. However, the lymphagiogenesis by alkali burn was inhibited by prox1 siRNA. And also, F4/80 and LSP1 positive cells were decreased in lymphangiogenesis inhibited cornea with prox1 siRNA treatment.
Conclusions: In this study, we observed that inhibition of lymphangiogenesis suppressed corneal inflammation in chemical burn model. These results suggested that prox1 is a regulatory protein for corneal lymphangiogenesis and this prox1 siRNA should be used for inhibition of corneal inflammation.
Commercial Relationships: Jun-Sub Choi, None; Chang Rae Rho, None; Choun-Ki Joo, None
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510 - P41-30

Decellularized human corneal limbus for limbal reconstruction

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Purpose: The limbus presents a unique microevironment for the maintenance of limbal epithelial stem cells (LESC) as it provides a specific three-dimensional ultrastructure with a particular basement membrane composition and vascularization. We created a decellularized human limbal tissue construct, which could mimic the distinct three-dimensional structure of the native limbus and might serve as surrogate niche matrix for expansion and transplantation of LESC.
Methods: Research consented human corneas were obtained after approval of the local Ethics Committee. The corneas were processed for decellularization with sodium desoxychelate in ultra-pure water and DNase treatment. Absence of DNA was verified by Feulgen reaction and DNA quantification. The native and decellularized limbus was analyzed histologically by hematoxylin and eosin staining and transmission electron microscopy. The maintenance of main basement membrane proteins was verified by immunohistochemistry.
Results: The DNA content was reduced from 1.5 ± 0.3 µg/mg to 0.15 ± 0.01 µg/mg. The lamellar arrangement of the collagen fibers and the radial vessels were preserved. Collagen IV staining was well preserved at the limbal basement membrane and vascular structures of the limbus. Laminin-staining showed a linear expression at the limbal and corneal epithelial basement membrane and along Descemet´s membrane before and after decellularization. Fibronectin additionally revealed a distinct homogenous stromal staining pattern.
Conclusions: Decellularization of human limbal tissue revealed a histologically intact, three-dimensional, cell-free scaffold. Main basement membrane components such as collagen IV, laminin, and fibronectin and the vascular structures were well preserved. Structure and composition of basement membrane components are organ-specific and its integrity is a prerequisite for cellular adhesion, proliferation, migration, and stem cell maintenance. Thus, decellularized human limbal tissue with preserved basement membrane components might provide a "limbus-specific" microenvironment and serve as a three-dimensional scaffold for LESC transplantation.
Commercial Relationships: Kristina Spaniol, None; Mathias Roth, None; Gerd Geerling, None; Stefan Schrader, None

511 - P42-31

P2Y2 agonist promotes corneal epithelial wound healing

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Purpose: The aim of this study was to evaluate the effect of P2Y2 agonist on corneal epithelium, especially wound healing process
Methods: BrdU cell proliferation assay and scratch-cell migration assay were performed on SV40-human corneal epithelial cells in vitro to determine whether the P2Y2 agonist, diquafosol tetrasodium, may affect epithelial cells. Additionally, we made ethanol-assisted 3 mm round epithelial wound on rat cornea and measure the wound closure over time with topically administration of P2Y2 agonist, to assess the effect of P2Y2 agonist on epithelial wound healing process in vivo. P2Y2 receptor expression in cornea epithelium was evaluated by immunostaining and western blot.
Results: Diquafosol 1.0mM promoted significantly SV40-HCEC (human corneal epithelial cell) proliferation, compared to control ($p<0.001$). P2Y2 antagonist, suramin, inhibited cell proliferation in medium both with and without diquafosol. Adding diquafosol made no impact on migration of immortalized HCEC. Suramin also showed the inhibitory effect on cell migration, in vitro scratch migration assay. Diquas treated eyes showed significantly wound closure than the control eyes after wound. P2Y2R was strongly stained near the margin of wounded cornea epithelium was in wound margin, while there was no positive stain in normal unwounded epithelium.
Conclusions: Topical application of P2Y2 agonist, diquafosol tetrasodium, accelerated corneal epithelial wound healing in vitro and in vivo animal study. This stimulatory effect of P2Y2 agonist may affect the cell proliferation or rather than cell migration of wound healing process.
Commercial Relationships: Yong Soo Byun, None; Woong-

Joo Whang, None; Choun-Ki Joo, None

512 - P43-32

Upstream Signal Transduction Pathway of HSP27 Phosphorylation Can Control Corneal Epithelial Cell Migration and Apoptosis during Wound Healing

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Purpose: To investigate the signal transduction pathway of heat shock protein 27 (HSP27) phosphorylation in the corneal epithelial wound healing and feasibility to control epithelial migration using its upstream signal transduction pathway.

Methods: Telomerase-immortalized human corneal epithelial cells (hTCEpi) were cultured. After scratch-induced directional wounding was created, Western blotting was performed using antibodies against phsphor-HSP27, phosphor-p38 mitogen-activated protein kinase (MAPK), phosphor-Erk, and phosphor-Akt. After the inhibitors of p38 MAPK and Akt and Erk kinase was administered in the wounded epithelia, epithelial migration and expression of phsphor-HSP27, phosphor-p38 MAPK, phosphor-Erk, and phosphor-Akt were observed.

Results: Western blot assay showed that phosphor-p38 MAPK increased immediately after scratch-induced directional wounding, phosphor-HSP27 increased just after p38 MAPK phosphorylation, and phosphor-Akt increased at about two hours. The inhibitor of p38 MAPK decreased phosphor-HSP27 and migration, but that of Akt increased phosphor-HSP27 and migration.

Conclusions: P38/MAPK and Akt pathways were involved in HSP27 phosphorylation in corneal epithelial wound healing. Modulation of p38/MAPK and Akt pathways can control the corneal epithelial migration during the wound healing.

Commercial Relationships: Jae Yong Kim, None; Soon-Suk Kang, None; Eun-Soon Kim, None; Hungwon Tchah, None

513 - P44-33

High prevalence of sleep and mood disorders in dry eye patients-The survey of 1000 eye clinic visitors-

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Purpose: To explore the prevalence of sleep and mood disorders in eye clinic visitors.

Methods: Outpatients were invited to complete a questionnaire containing the Pittsburgh Sleep Quality Index (PSQI) and Hospital Anxiety and Depression Scale (HADS). All outpatients were examined by certified orthoptists and board-certified ophthalmologists. A final diagnosis was made after comprehensive evaluation on the same day and patients were classified into six diagnostic groups.

Results: In total, 1,000 outpatients (mean age, 54.7 ± 21.7 years; range, 5-96 years; 635 females) participated in the study; they completed questionnaires and underwent ophthalmic examinations. The mean PSQI and HADS scores across all patients were 5.1 ± 3.0 and 9.5 ± 5.9, respectively. For the diagnostic groups, the mean PSQI and HADS scores, respectively, were 5.7 ± 3.3 and 10.2 ± 6.0 for dry eye (n = 254); 5.4 ± 3.2 and 9.2 ± 5.7 for bilateral cataracts (n = 159); 5.5 ± 3.4 and 8.1 ± 5.2 for bilateral pseudophakia; and, 5.2 ± 3.1 and 9.6 ± 6.4 for glaucoma (n = 117). Overall, 35.4% of patients were poor sleepers (PSQI ≥ 6) and 39.4% had possible mood disorders (HADS ≥ 10). Stepwise regression analysis revealed that the PSQI and HADS scores were significantly correlated with both age ($P < 0.05$) and the presence of dry eye ($P < 0.05$). In addition, 43% of patients with bilateral pseudophakia also had dry eye.

Conclusions: The prevalence of sleep and mood disorders was significantly higher in patients with dry eye. Patients with bilateral cataracts or bilateral pseudophakia had the second highest PSQI, adjusted for age and sex. Further evaluation is necessary to uncover the relationship between these psychiatric and ophthalmic diseases.

Commercial Relationships: Masahiko Ayaki, None; Motoko Kawashima, None; Kazuno Negishi, None; Taishiro Kishimoto, None; Masaru Mimura, None; Kazuo Tsubota, None

514 - P45-34

Sjögren's syndrome-like exocrinopathy and dry eye of TRAF6 deficient mice

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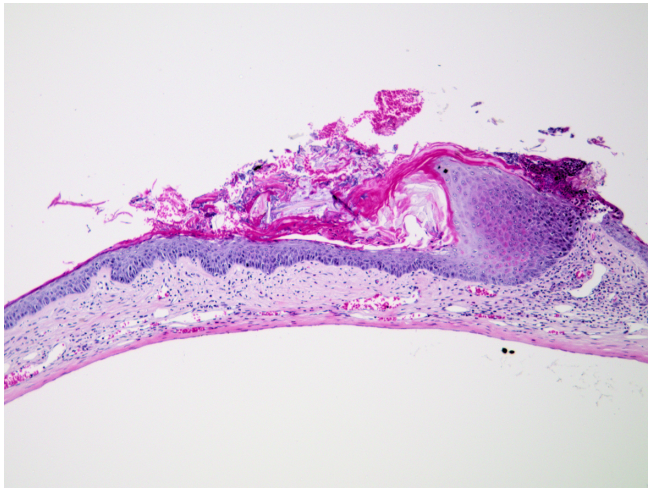
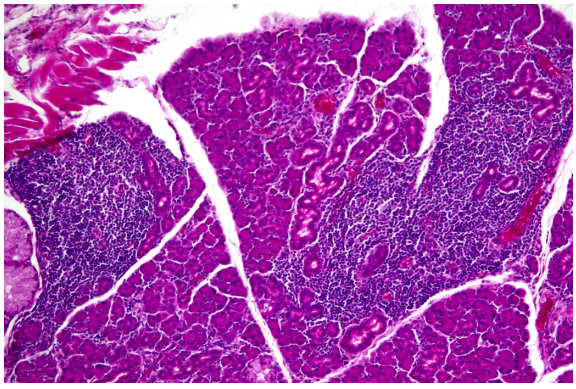
Purpose: Sjögren's syndrome (SjS) is an autoimmune disease characterized by exocrine gland dysfunction that leads to the development of xerostomia and keratoconjunctivitis sicca. Although the inflammatory process is considered to play an important role in progression of SjS, its pathogenesis is not obvious due to a few SjS model animals. TRAF6 was known to play an essential role in activating NF-kB pathways and in the development of T regulatory (Treg) cells. Treg cells are reported to markedly diminish in salivary glands of patients with SjS, and dysfunction of Treg is associated with human autoimmune diseases. We evaluated the SjS-like severe exocrinopathy and dry eye in TRAF6 deficient mice.

Methods: Corneal fluorescein staining of TRAF6 deficient mice and wild type mice was performed and photographed with a slit lamp biomicroscope. Phenol red threads tests were used to measure tear volume. Hematoxylin and eosin staining were employed for morphological observation of cornea lacrymal gland. Gene expression microarray analysis on the cornea was performed (6-7 months old, n=3). Real-time quantitative RT-PCR and

immunohistochemistry were used to examine mRNA and protein detected by microarray analysis.
Results: Increases corneal fluorescein staining and decreases in phenol red threads wetting were observed in TRAF6 deficient mice. Corneal erosion, squamous cell hyperplasia, inflammatory cells infiltration, and angiogenesis were observed in the histological specimen of old TRAF6 deficient mice cornea. Many inflammatory cells infiltrated in lacrimal glands. Gene expression microarray analysis revealed 1,296 transcription alterations. *SPRR1B* (small proline-rich protein 1B, 18,537-fold), *S100A8* (S100 calcium binding protein A8, 8,908-fold), *KRTDAP* (keratinocyte differentiation associated protein) were frequently increased in dry eye mice and patients with like SjS in many previous reports. These expressions were verified by real-time quantitative RT-PCR and immunohistochemistry.
Conclusions: TRAF6 deficient mice had severe exocrinopathy and keratoconjunctivitis sicca resembling SjS. This new mouse model of SjS might be useful to investigate a mechanism between Sjogren's syndrome and inflammation.
Commercial Relationships: Satoko Nakano, None; Kaori Noguchi, None; Takashi Kobayashi, None; Toshiaki Kubota, None



1. Corneal erosion, squamous cell hyperplasia, inflammatory cells infiltration, and angiogenesis were observed in the cornea of TRAF6 deficient mice.



2. Many inflammatory cells infiltrated in the lacrimal glands of TRAF6 deficient mice.

515 - P46-35

Compensatory increase in tear volume and mucins in Meibomian gland dysfunction caused by stearoyl-CoA desaturase-1 deficiency

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Purpose: The stearoyl-CoA desaturase (SCD) family of enzymes catalyzes monounsaturated fatty acid synthesis by inserting a cis double bond at the delta9 position of saturated fatty acids. The major products of SCD activity are oleate (18:1n-9) and palmitoleate (16:1n-7). Disruption of these enzymes has been reported to induce a severe dry skin phenotype. Lipid abnormalities in the meibomian glands have been associated with dry eye. Therefore, we analyzed the eye tissues in SCD-1 deficient mice, including the lacrimal glands and conjunctiva, with a specific focus on lacrimal function.
Methods: C57BL/6J (wild-type) and SCD-1 deficient mice were used in all experiments. Tear secretion was measured once a month using a phenol red thread (Zone-Quick, Menicon, Nagoya, Japan) placed on the temporal side of the lower eyelid margin for 30 seconds. Then, the length from the edge to the moistened area of the thread was measured. The mice were sacrificed and lacrimal glands were removed. Total RNA was extracted from lacrimal gland and conjunctiva of mice and we evaluated by real time-PCR. Pathological analyses were performed to analyze tissue sections using an optical microscopy.
Results: The tear volume of the wild-type and the SCD-1 deficient mice was examined using the cotton thread test. The results showed a significantly higher basal tear volume in SCD-1 deficient mice compared to that in wild-type mice at 10–40 weeks of age. The weight of the lacrimal glands was significantly greater in SCD-1 deficient mice when compared to that in wild-type mice. Histopathological analysis revealed atrophy and loss of meibomian glands, concurrent with increased goblet cell and MUC5AC expression in the conjunctiva.
Conclusions: These phenomena suggest that the weight of the lacrimal glands, the tear secretion volume, and the amount of mucin secreted are enhanced in the absence of lipid secretion as a compensatory mechanism. Thus, human meibomian gland dysfunction patients may have some sort of compensatory mechanism that prevents the tear volume from declining.
Commercial Relationships: Takaaki Inaba, None; Yasuhisa Tanaka, None; Shusaku Tamaki, None; Tomotaka Ito, None; Ntambi James, None; Kazuo Tsubota, None

516 - P47-36

Effectiveness of rebamipide prodrug in a mouse model induced dry eye

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Purpose: Rebampide known as an oral drug for treatment of gastric ulcer, has an effect of increasing mucin secretion. Comparint to it, Rebamipide prodrug improve absoption into the body. Based on the mechanism of increasing mucin secretion, we aim to evaluate the therapeutic effects of rebamipide prodrug in a mouse model induced dry eye.
Methods: Dry eyes were induced in 8-week old female C57BL/6 mice by intramuscular injection of scopolamine and exposure to an air draft for 5 days. The mice were divided into 7 groups according to the type of treatment. Tear volume, corneal smoothness and fluorescein staining were measured on 7 and 14 days after treatment. Periodic acid-Schiff staining, immunohistochemistry and flow cytometry were performed 14 days after treatment.
Results: Mice treated with 2% rebamipide eyedrops and rebamipide prodrug(20mg/kg, 40mg/kg, 80mg/kg) for 7days showed significant differences in tear volume and fluorescein staining compared with controls. The density of conjunctival goblet cells, number of CD4+CXCR3+ T cells and TNF-a stain in 2% rebamipide eyedrops and rebamipide prodrug groups were not significantly different compared with the control group. There were no significant differences in all the parameters between 2% rebamipide eyedrops and rebamipide prodrug(20mg/kg, 40mg/kg, 80mg/kg) groups.
Conclusions: Oral intake of rebamipide prodrug can improve tear production and decrease ocular surface damage in an induced dry eye mouse model. These results suggest that rebamipide prodrug may be useful for the treatment of dry eye syndrome.
Commercial Relationships: Woon hyung Ghim, None; Kyong Jin Cho, None; Moo Hwan Chang, None

517 - P48-37

Expression of the ocular surface mucins in dry eye patients

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Purpose: To evaluate the expression of mucin 1(MUC1), mucin 5 (MUC5AC) and mucin 16(MUC16) in ocular surface of Sjögren Syndrome dry eye (SSDE) patients, Non-Sjögren syndrome dry eye (NSSDE) patients and healthy controls.
Methods: Twenty SSDE patients with mean age 53.9 ± 9.2 years, 20 NSSDE patients with mean age 48.2 ± 11.7 years and 20 gender and age matched healthy controls were recruited in this study. Schirmer I test, tear film break-up time (TBUT), cornea fluorescein staining (FS) were examined. Conjunctival impression cytology specimens were obtained and real-time PCR was performed to evaluate the expression of MUC1, MUC5AC and MUC16 mRNA.
Results: The Schirmer I value, TBUT and FS scores were obviously worse in NSSDE and SSDE patients comparison with the healthy controls, specially in the SSDE group. The expression of MUC5AC mRNA significantly decreased in SSDE and NSSDE groups compared with the healthy controls ($P < 0.05$). However the expressions of MUC1and MUC16 mRNA obviously increased in SSDE and NSSDE groups compared with the healthy controls ($P < 0.05$).
Conclusions: Decreased MUC5AC mRNA expression and increased MUC1 and MUC16 mRNA expressions were found in dry eye patients. This suggested the alteration of ocular surface mucin played important role in the pathogenesis of dry eye and the function of different mucin need further study.
Commercial Relationships: Yan Wang, None; Liangliang Niu, None; Jihong Wu, None; Jianjiang Xu, None
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518 - P49-38

Lagosleep a New Eyelid Syndrome

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Study Group: Dry eye

Purpose: To demonstrate the existence of a not identified syndrome in the ophthalmic literature, responsible for different complaints such as morning eye irritation, chronic pruritus , lid irritation and eye redness ; with a prior diagnose of dry eye, allergies, chronic keratitis and others , some times for many years . A typical staining of the inferior lid border associated with incomplete eyelid closure when the patient is asked to close the eyes , conform a syndrome that we named Lagosleep, which with an appropriate treatment start to improve immediately.
Methods: A total of 1985 consecutive patients were checked between July 2013 to March 2014 founding 98 cases with a complete clinical picture, in which a typical inner lid margin staining was evident with the use of lissamine green .In more severe cases also stains the bottom of the conjunctiva and inferior cornea. A specific questionnaire and a score sheet was designed to classify the intensity of the syndrome. Therapeutic management consists in the use of gel tears before sleeping time , some cases required drape lid closure.
Results: From the 98 patients with this diagnostic 76% where women ,age average was 67, with a maximum age of 82 and minimum of 15. The more frequent symptoms were , dry eye sensation when getting up in the mornings ??? by 34%, followed by 23% red eye, morning secretion 22%, 22% and eyes glued difficulty eyelid opening by 16%.
Conclusions: During the study we found that Lagosleep is a complete syndrome, which classic signs and symptoms mistakenly treated with different medications for a long time without improvement. Our preliminary results, let us to conclude that this is a very common syndrome that requires to be searched as a routine in patients with

chronic irritation with the aid of lisamine green. Further conclusions about the final type of treatment will be published later.
Commercial Relationships: Eduardo Arenas, None; Ingrid Ulloa, None

519 - P50-39

The Relationship between Blinking and Ocular Surface Abnormalities

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Purpose: In the pathophysiology of dry eye, it remains unclear as to what influence blinks have on the ocular surface. The purpose of this present study is to investigate the relationship between blinking and ocular surface abnormalities.
Methods: This study involved 45 eyes of 45 female dry-eye patients (mean age, 58.6 years). In all eyes, tear meniscus radius (TMR, mm), spread grade of the tear-film lipid layer (SG 1-5, 1 being the best), fluorescein breakup time (FBUT, seconds), ocular surface epithelial damage score (OSED, cornea, 15 points maximum, conjunctiva, 6 points maximum), lid-wiper epitheliopathy grade (LG, 6 points maximum) were measured. In addition, measurements of the palpebral aperture height (PAH, mm), the descending distance (DD, mm) and ascending distance (AD, mm) of the upper eyelid movement by use of a blink analyzer, and the completeness of the eyelid descending movement (DC=DD/PAH) and ascending movement (AC=AD/PAH) were calculated. Finally, the Schirmer 1 test (ST1, mm) was measured and the correlation between DC, AC, and the other factors were then investigated.
Results: DC was found to be significantly correlated with TMR (R=-0.36, p=.013), SG (R=0.40, p=.007), FBUT (R=-0.33, p=.025), OSED (cornea) (R=0.41, p=.005), OSED (conjunctiva) (R=0.47, p=.001), and LG (R=0.53, p<.001), yet not significantly correlated with ST1 (R=-0.15, p=.342). AC was found to be significantly correlated with SG (R=0.45, p=.002), FBUT (R=-0.33, p=.025), OSED (cornea) (R=0.47, p=.001), OSED (conjunctiva) (R=0.52, p<.001), and LG (R=0.57, p<.001), yet not significantly correlated with TMR (R=-0.27, p=.078) or ST1 (R=-0.14, p=.371).
Conclusions: The findings of this study show that a greater degree of blinks resulted in reduced tear volume and subsequently more severe ocular surface epithelial damage, thus indicating the possibility that blinks have a strong influence on the ocular surface.
Commercial Relationships: Hiroaki Kato, None; Norihiko Yokoi, None; Rieko Sakai, None; Mengxi Niu, None; Akihide Watanabe, None; Shigeru Kinoshita, None

520 - P51-40

Lactoferrin Alleviates Desiccating and Restraint Stress Induced Dry Eye Disease by Reducing Lacrimal Gland and Corneal Inflammation

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Purpose: Dry eye disease (DED) is highly common multifactorial disease of aging that accumulates as tear hyposecretion due to a loss of function in the lacrimal functional unit. DED causes pain, severely affecting the quality of life of sufferers. Aging populations combined with the fact that only supportive treatments currently exist indicates that the problem of DED will exacerbate with time, and that a curative treatment is greatly needed. Lactoferrin (Lf) is a protein found in high concentrations in exocrine secretions including tears and has been linked to a decline in the function of the lacrimal gland (LG). Lf also posses a plethora of properties including anti-oxidation and anti-inflammation, factors critical to the pathogenesis of DED. We therefore propose that Lf could provide a possible treatment to DED.
Methods: Experiments lasted 7 days (-1 to 5). 20, 11-week old female C57BL/6 mice were randomly divided into 4 groups of 5. Each were given daily administrations of 500 μ l of Lf in concentrations of 100mg/kg(HLf), 50mg/kg(MLf) or 20mg/kg(LLf). Whereas Control mice (C) received PBS. From day 0 all mice were exposed to a daily 4hr restraint and desiccating stress event to induce DED. Tear secretion was then measured via phenol red thread test (PRTT). In a separate experiment PRTT was replaced with corneal fluorescein staining (CFS). Mice were sacrificed on day 5. qPRC and immunofluorescent staining (IFS) was used to analyze tissues.
Results: C displayed a significant decline in PRTT scores immediately after the initial stress event, whereas Lf mice remained close to pre-stress levels. Pre-stress (Ave. day -1-0), C 2.63(± 0.70), HLf 2.50(± 0.73), MLf 2.88(± 0.51), LLf 2.53(± 0.70). Post stress (Ave. day 1-5), C 1.12(± 0.52), HLf 2.52(± 0.62), MLf 2.32(± 0.68), LLf 2.12(± 0.74). CSF indicated higher tissue injury in C, although Lf mice also displayed some increase. We observed reduced expression of inflammatory mediators in the LG of Lf mice. Furthermore we found positive 8-OHdG IFS in both the cornea and LG of C, but very low levels in Lf mice.
Conclusions: Our data indicates that Lf maintains tear secretion in a dose dependent manner but also reduces inflammation and oxidative stress in the LG, protecting tissue function. This was also observed in the cornea, however it is not clear weather this is directly due to Lf or a consequence of increased tear secretion.
Commercial Relationships: Samuel Connell, None; Motoko Kawashima, None; Shigeru Nakamura, None; Toshihiro Imada, None; Kokoro Sano, None; Akiko Ito, None; Kai Jin, None; Ryuji Hisamura, None; Kazuo Tsubota, None

521 - P52-41

Effects of a warm compress with menthol on the tear film conditions

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Study Group: LIME working group

Purpose: It is known that menthol binds to Transient receptor potential cation channel subfamily M member 8, stimulating lacrimation via activation of corneal cool primary afferent neurons.To investigate the effects of a warm compress using a disposable eyelid-warming steamer with menthol on the tear meniscus and the tear film stability.

Methods: Eighteen eyes of 18 healthy volunteers (mean age ± SD, 34.6 ± 6.6 years; range, 22-44, 9 males and 9 females) without dry eye symptoms evaluated by the Dry Eye-related Quality-of-life Score (DEQS < 10) were enrolled. Two types of disposable eyelid-warming steamers that were menthol-containing type (M+) and menthol-free type (M-) were applied to subjects with single warming (once for 10 minutes). Repeated M+ warming (10minutes, twice a day for 2 weeks) was performed for the evaluation of continuous warming effect. We evaluated tear break up time (BUT), strip meniscometry (SM) value which was reflected tear meniscus volume, surface temperature of the central cornea/ upper tarsal conjunctiva/ lower tarsal conjunctiva by thermography, meibum-score and meibo-score by non-contact meibography. Evaluation of repeated warming was performed after at least 8 hours from latest warming.

Results: Single warming with both M+ and M- steamers increased the surface temperature of central cornea, upper and lower tarsal conjunctiva. Single warming with M+ significantly increased SM value (from 4.5 ± 1.7 to 7.1 ± 2.1 mm, $p < 0.001$) and BUT (from 4.2 ± 1.6 to 6.2 ± 2.5 s, $p < 0.05$). Single warming with M- did not affects SM value nor BUT. Repeated warming with M+ significantly increased SM value (from 4.5 ± 1.7 to 6.8 ± 3.2 mm, $p < 0.01$) and BUT (from 4.2 ± 1.6 to 6.2 ± 3.3 s, $p < 0.05$). Repeated warming did not change meibum score, meibo-score nor surface temperature of the tarsal conjunctiva and cornea.

Conclusions: Single warming by the steamer with menthol increased tear meniscus volume and tear film stability. Repeated warming by the steamer with menthol maintained the increased tear meniscus volume and tear film stability. These findings suggested that the warm compress with menthol might have potential to contribute to dry eye disease.

Commercial Relationships: Reiko Arita, TOPCON (P), Kao corporation (F); Naoyuki Morishige, None; Ichiro Sakamoto, Kao corporation (E); Natsuko Imai, Kao corporation (E); Michihito Igaki, Kao corporation (E); Atsushi Suzuki, Kao corporation (E); Kazuo Tsubota, Kao corporation (C)

522 - P53-42

Proteolytic Degradation of Galectin-3 in Dry Eye

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Purpose: Galectin-3 is a multimeric carbohydrate-binding protein known to be upregulated under several pathological conditions, such as inflammation and cancer. Proteolytic cleavage of galectin-3 by matrix metalloproteinases (MMPs) has been associated with abrogation of the biological properties of the lectin and with the progression of the disease. Here, we investigated the secretion and cleavage of galectin-3 in tears of dry eye patients.

Methods: Tear fluid and conjunctival impression cytology specimens were collected from 11 normal subjects (11 eyes) and 16 patients with dry eye disease (20 eyes). Galectin-3 content in tears was analyzed by quantitative Western blot, using recombinant galectin-3 protein as the calibration standard. The relative expression of MMP9 mRNA in conjunctival impression cytology specimens was evaluated using quantitative PCR (qPCR). Galectin-3 proteolytic cleavage was studied using recombinant and endogenous protein from human tears.

Results: The level of galectin-3 was significantly higher in tears of dry eye patients (Ave. 0.38 ng/μg total protein, range 0.04-1.36) compared to controls (Ave. 0.12 ng/μg total protein, range 0.00-0.41)($p<0.01$). By Western blot, all tear samples from normal subjects were characterized by the presence of a single band corresponding to full-length galectin-3, whereas 50% of dry eye patients contained both full-length and cleaved galectin-3. Analyses of conjunctival epithelium by qPCR revealed increased MMP9 mRNA expression in dry eye patients. Importantly, we demonstrated that active MMP9 can cleave full-length galectin-3 from recombinant origin and from tear fluid. These effects were abrogated by use of the pan-specific MMP inhibitor GM6001.

Conclusions: Tear fluid of patients with dry eye contains increased levels of galectin-3. Cleavage of galectin-3 as a result of increased protease activity in dry eye disease may lead to ocular surface barrier dysfunction.

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523 - P54-43

Increase in tear secretion by abdominal breathing in healthy women

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Purpose: We hypothesized the relationship between abdominal breathing and tear meniscus volume in healthy women. We investigated the change in tear meniscus volume in 2 groups, normal breathing and abdominal breathing to determine this hypothesis.
Methods: We used a crossover experimental model and examined 20 healthy women aged 20-54 years (mean ± SD, 32.7 ± 11.1 years). The participants were randomly assigned to 2 groups. During the first visit, the normal breathing group was subjected to normal breathing for 3 min, whereas the abdominal breathing group was subjected to abdominal breathing (4-second inhalation and 6-second exhalation) for 3 min. During the second visit, the protocols were swapped between the 2 groups. We estimated the R wave to R wave (R-R) interval, tear meniscus volume, salivary amylase activity, pulse, and blood pressure before, immediately, 15 min after, and 30 min after breathing initiation.
Results: In abdominal breathing, compared to that before breathing, the tear meniscus volume increased significantly 15 min after breathing (P<.01). Furthermore, systolic blood pressure showed a significant decrease immediately after breathing (P<0.05). No significant difference was found in the test parameters in the normal breathing group.
Conclusions: Our study shows that abdominal breathing for 3 minutes increases the tear meniscus volume in healthy women. Consequently, abdominal breathing may be considered in the treatment of dry eye disease.
Commercial Relationships: Kokoro Sano, None; Motoko Kawashima, None; Kazuhiro Ikeura, None; Reiko Arita, None; Kazuo Tsubota, None

524 - P55-44

An association between SNPs in the TNFA promoter region and Korean Dry Eye Disease patients

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Purpose: To determine whether variations altering the function or expression of TNFA, contribute to the pathogenesis of dry eye disease.
Methods: Genomic DNA was extracted from blood samples of unrelated dry eye disease patients (non-Sjogren's syndrome patients (n=200) and Sjogren's syndrome patients (n=100). Polymerase chain reaction and direct sequencing were used to screen variations in promoter region of TNFA gene. One hundred fifty control individuals without corneal disease were selected from the general population.
Results: We investigated 6 SNPs of TNFA; -1196 C>T, -1031 T>C, -863 C>A, -857C>T, -308 G>A (rs1800629) and -238 A>G (rs361525) in promoter. Among them, -1196 C>T, -857 C>T, rs1800629 and rs361525 were different between patient groups and control groups. The *A allele frequency of rs361525 in dry eye patients (2.6%) and Sjogrens' patients (2.5%) were decreased compared with control subjects (5.1%). The *A allele frequency of rs1800629 was lower in Sjogrens' patients (2.5%) than in the controls (4.8%). In -1196C>T variation, *T allele of both patient groups was decreased compared with control subjects. Whereas, the * T Allele frequency of -857 C>T was higher in the Sjogrens' patients (30.0%) than in the controls (16.8%). The genotype distributions of all polymorphisms of TNFA among the control subjects and the affected individuals were in Hardy-Weinberg equilibrium.
Conclusions: Our results suggested that the genetic variations of TNFA gene seem to be associated with dry eye predisposition in a Korean.
Commercial Relationships: Sung A Lim, None; Jeewon Mok, None; Kyung-Sun Na, None; Hee-Jung Ju, None; Choun-Ki Joo, None

525 - P56-45

Topical rebamipide improves subjective symptoms of dry eye by modulating pain sensation in cornea

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Purpose: Rebamipide was originally developed and approved for treatment of gastric ulcers and gastritis. Today, its ophthalmic suspension is widely used for the patients with dry eye. Subjective symptoms are often improved prior to objective ones when treated with topical rebamipide. In the present study, we examined the corneal sensitivity and pain sensation in patients with dry eye during topical rebamipide therapy.
Methods: Twenty-one patients with dry eye (14 females and 7 males; range, 25-87; mean age, 62.5) were enrolled. Corneal sensitivity and pain sensation were quantified with Cochet-Bonnet corneal aesthesiometer at 0 and 2 weeks after the initiation of rebamipide treatment. Superficial punctate keratitis (SPK) score and breaking up time (BUT) of tear film were evaluated by slit lamp examination. Lacrimal secretion was measured with Schirmer test. Examinees also filled out the questionnaire sheet about the symptoms (7 items and 4 points each entry). Subjects in this survey were categorized as short BUT-type dry eye.
Results: Corneal sensitivity was not significantly changed before and after rebamipide treatment (54.3 ± 14.9mm and 57.1 ± 6.2mm, respectively, P=0.22). However, corneal

pain sensation was significantly decreased after the administration of rebamipide (21.7 ± 19.3mm) compared with pretreatment level (38.1 ± 19.2mm, P<0.01). Questionnaire revealed that subjective symptoms were also significantly improved after rebamipide therapy (9.14 ± 1.07) compared with the baseline value (13.4 ± 0.85, P<0.01). SPK score, BUT, and lacrimal secretion persisted despite the treatment.
Conclusions: Topical rebamipide therapy reduced the corneal hypersensitivity to pain of dry eye patients. It is possible that it elevates the threshold of the nerves that detect pain in ocular surface, and ameliorates the subjective complaints prior to the improvement of objective opinions.
Commercial Relationships: Yoshiaki Tagawa, None; Nobuyoshi Kitaichi, None; Takeshi Ohguchi, None; Erdal Tan Ishizuka, None; Saori Inafuku, None; Satoshi Kinoshita, None; Atsuhiro Kanda, None; Kousuke Noda, None; Susumu Ishida, None

526 - P57-46

Effects of punctal occlusion on dry eye clinical signs and symptoms, and tear cytokine levels using direct measurement and generalized linear modeling

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Purpose: Punctal occlusion is a common treatment option for dry eye but there is limited evidence that occlusion favorably alters tear composition. This longitudinal study evaluated changes in clinical signs and symptoms, and tear cytokine levels, after insertion of non-adsorbable punctal plugs in patients with dry eye.
Methods: Patients with moderate dry eye and persistent symptoms (2 or more symptoms ≥ grade 3) were enrolled. Parasol type silicone plugs were inserted bilaterally in the lower punctum at baseline. Assessments included fluorescein corneal staining, Schirmer's test, and global symptom score in the more severe eye for up to 3 weeks after occlusion. Tears were tested for 15 cytokines and matrix metalloproteinase-9 (MMP-9) by indirect immunofluorescence; concentrations were normalized to amount of wetting of Schirmer's test. General linear model (repeat measures) was performed.
Results: 29 patients (mean age 49.8 years) were evaluated. Compared to baseline, fluorescein staining was reduced in all zones (P<0.01) except inferior zone and global irritation score decreased (P<0.001) after 3 weeks punctal occlusion. At baseline, low Schirmer's scores (≤8 mm) were associated with higher levels of several cytokines, notably TNF- α (P<0.001), IL-2 (P<0.03) and MIP-1 α (P<0.002). No significant changes in raw cytokine levels were observed following punctal occlusion. Multivariate analysis models indicated that Schirmer's score was the most important factor for elevated cytokines; the majority of cytokines elevated in patients with low Schirmer's score at baseline remained high post-occlusion, including Th1-type cytokines TNF- α, IL-1 β and IFN- γ. Using cytokine or MMP-9 as a dependent variable, baseline characteristics and signs, and time from baseline as other covariates, 5 different models were identified that best classified changes after punctal occlusion, Model 1 for IL-13; Model 2 for IL-2 and IL-6; Model 3 for MMP-9, IL-17a, IP-10, and RANTES; Model 4 for IFN- γ, IL-10, IL-12, and TNF- α; and Model 5 for IL-8.
Conclusions: Punctal occlusion reduced corneal staining in all zones except inferior zone and improved irritative symptoms. Insertion of punctal plugs did not significantly change tear cytokine or metalloproteinase levels suggesting a need for earlier treatment with anti-inflammatory agents for the management of dry eye disease.
Commercial Relationships: Louis Tong, None; Roger Beuerman, Allergan (C); Susan Simonyi, Allergan (E); David Hollander, Allergan (E); Michael Stern, Allergan (E)
Clinical Trail: NCT01684436

527 - P58-47

Influence of warm compress and eyelid massage on the tear lipid layer thickness in office workers

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Purpose: To evaluate the tear lipid layer thickness and blink rate after warm compress with or without eyelid massage.
Methods: Sixteen office workers (mean 29.7 ± 8.7 years, 3 male and 13 female) were enrolled in the study. The tear lipid layer thickness and partial blink rate were measured using LipiView Ocular Surface Interferometer (TearScience, Inc., Morrisville, North Carolina) before warm compress, after warm compress with or without eyelid massage. Three measurements were obtained and average value was applied for analyses. Warm compress was performed using Hot Eye Mask (Kao, Tokyo, Japan) for 15 minutes, and subsequent eyelid massage was performed using glass rod.
Results: The mean lipid layer thickness through an Interference Color Unit score (ICU) before warm compress and after warm compress with or without eyelid massage showed 58.9 ± 15.2 μ m, 72.0 ± 19.0 μ m and 78.8 ± 14.9 μ m, respectively. There were statistically significant differences between each 3 conditions (ANOVA, p < 0.0001). The mean partial blink rate before warm compress and after warm compress with or without eyelid massage showed 75.6 ± 28.1%, 73.4 ± 34.8 % and 71.5 ± 28.4 %, respectively. There were no significant differences in blink rate between 3 conditions.
Conclusions: To improve the lipid layer thickness, eyelid massage with warm compress is necessary. The LipiView Ocular Surface Interferometer is promising tool to evaluate the effects of warm compress in office workers.
Commercial Relationships: Takashi Kojima, None; Osama Ibrahim, None; Murat Dogru, None; Kazuo Tsubota, None

528 - P59-48 Withdrawn

Clinical predictors of early dry eye, objective vs subjective comparison

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Purpose: Many clinical techniques are available to measure dry eye, however, at early stages of dry eye are not easily diagnosed. The aim of the study was to compare different objective clinical techniques like tear break up time, Schirmer' s test and tear osmolarity to subjective symptoms of early dry eye and identify the technique that identify dry eye at the earliest.

Methods: 33 subjects participated in the study that included 18 early dry subjects and 15 normal subjects. Early dry eye was defined as subjects who complained of the presence of symptoms like dryness and irritation, however, were not under any medication for the same. Tear break up time, Schirmer' s test and tear osmolarity test were performed by the same investigator on all the subjects. In addition, presence of early dry eye was assessed using standard questionnaire that was rated on a scale of 1 to 5. All the measurements were obtained within a single visit.

Results: Mean (SD) of tear break up time, Shirmer' s test, tear osmolarity in normal and early dry eye subjects was 7.23 (2.82) sec, 17.16 (8.71) mm, 311.83 (14.56) mOsms/L and 5.36 (1.67) sec, 18.83 (11.51) mm, 307.56 (11.83) mOsms/L, respectively. One-way ANOVA revealed no significant difference (all p >0.05) for clinical techniques like Schirmer' s test and tear osmolarity between early dry eye and normal subjects, however, there was a significant difference for tear break up time (p=0.02), and subjective questionnaire scores (p=0.01).

Conclusions: Early dry eye can be screened and identified by the presence of subjective questionnaire and a clinical objective test like TBUT, that other techniques like tear osmolarity and Schirmer' s test takes longer to diagnose

Commercial Relationships: Balamurali Vasudevan, None; Emily Washek, None

529 - P60-49

Study of various lubricants in the eye using modern assessment tools

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Purpose: To compare optical quality and tear film analysis of normal and dry eye individuals after instilling various lubricating eye drops.

Methods: 50 eyes of 25 normal individuals and 20 eyes of 10 dry patients were studied using advanced imaging tools like LipiView Interferometer (TearScience Inc, Morrisville, North Carolina, USA) ,Noninvasive TBUT ,Modulation transfer function and Mean OSI using OQAS(HD Analyser). These patients were subjected to randamised use of A(Polyethylene Glycol 400 0.4% , Propylene Glycol 0.3%) ,B(Sodium Carboxymethyl-cellulose 5mg, Stabilized Oxychloro complex 0.1mg, C (Sodium carboxymethyl-cellulose -5mg), eye drops and the optical quality and tear film at 5 mins and 40mins after applying the drops were assessed.

Results: After 5 and 40 minutes all the indices suggested that the average ICU was -78.23 72.33, 71.2, and with drops A, B, C respectively which suggested that the drop A had a beneficial effect on the lipid layer. The optical scatter index, modulation transfer function and the mean OSI also showed a improvement after the similar drops in both normal and dry eye individuals.

Conclusions: Significant improvement in the optical quality and tear film parameters were seen with the eye drops A.

Commercial Relationships: Dhawal Haria, None; Tejal Sj, None; Dr Rohit Shetty, None

530 - P61-50

Immunohistochemical localization of D- β -aspartic acid-containing proteins, a molecular marker of aging, in pterygium

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Purpose: Biologically uncommon D- β -aspartic acid-containing proteins have been reported to accumulate in organs affected by age-related disorders. In addition, D- β -aspartic acid-containing proteins are known to accelerate some aspects of aging process. For this reason, D- β -aspartic acid-containing proteins are regarded as one of the molecular markers of aging process. In the present study, we investigated the localization of D- β -aspartic acid-containing proteins in cases of pterygium, one of the most prominent age-related ocular conditions.

Methods: Surgical specimens of pterygium-containing conjunctivae from 14 patients and specimens of pterygium-free conjunctivae from 4 patients were obtained. Immunohistochemical localization of D- β -aspartic acid-containing proteins was investigated using a polyclonal antibody against a D- β -aspartic acid-containing peptide.

Results: Strong immunoreactivity to D- β -aspartic acid-containing proteins was observed in subepithelial elastotic lesions and surrounding collagenous lesions from all surgical specimens with pterygia. In contrast, no immunoreactivity to D- β -aspartic acid-containing proteins was seen in pterygium-free specimens.

Conclusions: D- β -aspartic acid-containing proteins are produced in the body during the course of aging process. In addition, conversion of L- to D-aspartyl residues is accelerated by ultraviolet (UV) irradiation. Since aging and UV exposure are the aggravating factors of pterygia,, it is reasonable to find D- β -aspartic acid-containing proteins in specimens with pterygia.

Commercial Interest, None.

Commercial Relationships: Yuichi Kaji, None; Tetsuro Oshika, None; Ryouhei Nejima, None; Saiyo Mori, None; Kazunori Miyata, None; Noriko Fujii, None

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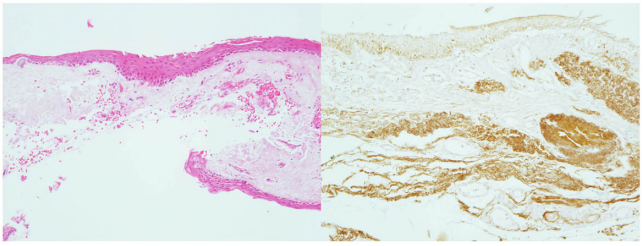

H-E staining　　D-β-Asp-containing proteins

531 - P62-51

Downregulation of IL-8, ECP, and total IgE in the tears of patients with atopic keratoconjunctivitis treated with rebamipide eyedrops

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Purpose: Rebamipide eyedrops are approved in Japan for the treatment of dry eye disease. Some patients with allergic conjunctival diseases also manifest dry eye. Earlier we reported that rebamipide suppressed polyI:C-induced inflammatory cytokines in human conjunctival epithelial cells. In the current study we examined the effect of rebamipide eyedrops on the level of interleukin-8 (IL-8), eosinophil cationic protein (ECP), and total IgE on the ocular surface.

Methods: We prescribed rebamipide eyedrops to patients with atopic keratoconjunctivitis (AKC) who presented with dry eye (6 eyes in 4 AKC patients) and measured the IL-8, ECP, and total IgE levels in their tears before- and 2, and 4 - 6 weeks after the start of rebamipide treatment. To measure the IL-8 and total IgE levels in their tears we used BD™ CBA Flex sets; ECP measurements were with ELISA.

Results: The level of IL-8, ECP, and total IgE in the tears of AKC patients was reduced significantly 4 - 6 weeks after the start of rebamipide treatment. We also recorded subjective symptoms associated with AKC, e.g. itching, foreign body sensation, and eye mucus discharge, by using a patient questionnaire. Their subjective symptoms associated with AKC were also significantly ameliorated at 2 and 4 - 6 weeks.

Conclusions: Our observations suggest that the anti-inflammatory effects of rebamipide eyedrops help to combat human ocular surface inflammation and that they may be a new effective therapy in patients with AKC.

Commercial Relationships: Mayumi Ueta, None; Jun Shoji, None; Chie Sotozono, None; Shigeru Kinoshita, otsuka pharmacial company (F)

Clinical Trail: UMIN000009677

532 - P63-52

A case with refractory scleritis remitted by abatacept treatment

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Purpose: Scleritis is a common complication of rheumatoid arthritis (RA). It is difficult to manage scleritis accompanied with RA when it resists to topical streroid treatments. The aim of this study is to report a case with refractory scleritis accompanied with malignant RA who gained remission of scleritis by abatacept treatment.

Methods: Case report. A 65-year-old woman with a 5-year history of malignant RA was treated at Toyama University Hospital in Japan.

Results: The patient showed scleritis, interstitial pneumonitis, and positive RA particle aggregation and she was first treated with methotrexate and oral steroid by a rheumatologist. The scleritis was simultaneously treated with topical betamethasone and tacrolimus, but the scleritis was persisted.

In February 2011, a biological agent, etanercept was added at the dosage of 25mg twice a week. Then, her joint symptoms improved, but the scleritis repeated remissions and relapses. In July 2012, since she got pneumonia, she had to discontinue etanercept treatment and to taper oral predonine. At the same time, the scleritis was worsened. In January 2013, another biological agent, abatacept treatment was started, which resulted in an improvement of all symptoms. After that, she remained complete remission of the scleritis.

Conclusions: Of biological agents available for RA, abatacept may be effective for a refractory scleritis accompanied with RA.

Commercial Relationships: Akio Miyakoshi, None; Tomoko Nakamura, None; Koichiro Shinoda, None; Atsushi Hayashi, None

533 - P64-53

Comparative Study of Eyelid Hygiene Solutions in Meibomian Gland Dysfunction

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Purpose: To compare the effectiveness between 2 eyelid hygiene solutions(diluted baby shampoo and normal saline solution) for the treatment of stage 2 meibomian gland dysfunction (MGD).

Methods: Twenty patients (male 6, female 14) with symptomatic dye eyes and stage 2 MGD were enrolled. The mean age was 50.35 years. All patients were treated with preservative free artificial tears and eyelid hygiene with diluted baby shampoo in one eye and normal saline solution in the fellow eye for 4 weeks. Dry eyes symptoms by ocular surface disease index (OSDI), stage of MGD, tear film break up time (TBUT), conjunctival and corneal

staining were assessed.
Results: The mean of OSDI was significantly improved after treatment (p<0.001). MGD in all patients were improved from stage 2 to stage 1. The median of TBUT in each group was significantly improved 1 second (p=0.001). Conjunctival and corneal staining in each group was significantly improved from grade 1 to grade 0 (p=0.031). There were no significant difference in the improvement of the stage of MGD, the median of TBUT, and conjunctival and corneal staining between two groups.
Conclusions: Eyelid hygiene with diluted baby shampoo and normal saline solution are equally effective in improving the stage of meibomian gland dysfunction, TBUT, and conjunctival and corneal staining in patients with grade 2 MGD.
Commercial Relationships: Jatuporn Duangpatra, None; Theeratep Tantayakom, None; Wiwan Sansanayudh, None; Raveewan Choontanom, None; Kanaspana klanarongran, None
Clinical Trail: R143h/56

534 - P65-54

Vernal keratoconjunctivitis, clinica and therapeutic aspects

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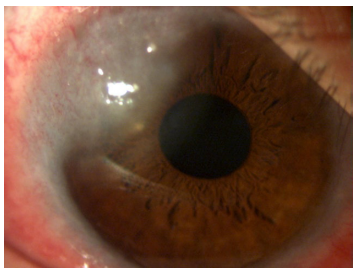
Purpose: To evaluate clinical aspects, specific sensitization, epidemiological and therapeutic characteristics of vernal keratoconjunctivitis (VKC)
Methods: Retospective clinical case series included 300 VKC patients,between may 2008 and july 2014, data included patients and family histories, results of ocular surface exams, allergic tests and response to corticosteroids assocated to mast stablizers (NAAGA) treatment.
Results: The great majority of VKC patients were male(87%), 36% of limbal forms, 56% of corneal complications, 23 keratoconus associated, a skin prick tests and specific serum IgE was positive in 58% and 64% of patients respectively. therapeutic results were good in 73% cases, satisfactory with frequent relapses treated with corticosteroids in 21% cases, 5% of steroid dependent and 1% of several unresponsive forms.
Conclusions: Vernal keratoconjunctivitis, very common in our climate remains a severe form by they corneal complications and diffficult managment .
Commercial Relationships: Sihem Lazreg, None



535 - P66-55

Comparison of SLET(simple limbal epithelial transplantation) with CLAU (conjunctival limbal autograft) in unilateral chronic ocular burns

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Purpose: To compare the efficacy and safety of SLET and CLAU in the management of chronic ocular burns with 360^0 limbal stem cell deficiency(LSCD).
Methods: 20 patients of unilateral chronic ocular burns of more than 3 months duration with symblepharon , with total LSCD and a healthy fellow eye were included in the study. Patients were randomly divided into two groups of 10 each, to undergo either SLET (2 clock hour limbal harvesting) or CLAU (6-8 clock hour limbal harvesting) from the fellow eye with symblepharon release and amniotic membrane transplantation. They were evaluated postoperatively upto 6 months for ocular surface stability based on corneal epithelialisation, neovasularization and fornicial reconstruction. Secondary outcome measures included Corneal clarity and BCVA(Best Corrected Visual Acuity).
Results: Ocular surface stability and forniceal reconstruction was achieved in sixteen (80%) of the twenty patients at six months follow up and was comparable between two groups. Four cases had recurrence of symblepharon and vascularisation. Persistent corneal epithelial defect was not seen in any case at six weeks. None of the healthy donor eyes suffered any complications.
Conclusions: SLET is an effective alternative to CLAU for ocular surface reconstruction in chronic ocular burns
Commercial Relationships: Ritu Arora, None; Pallavi Dokania, None; J L Goyal, None; Parul Jain, None; Aditi Manudhane, None; Pooja Jain, None; Richa Agarwal, None

536 - P67-56

Role of Matrix Metalloproteinase in Stevens - Johnson syndrome (SJS) Ocular Sequelae Patients

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Purpose: SJS is a mucocutaneous immuno bullous condition with prolonged ocular complications involving

chronic conjunctivalization and keratinization of corneal surface. Matrix metalloproteinases (MMP) have essential role in tissue remodeling and inflammation in diseased tissues and metastasis in various cancers through extracellular matrix alterations. Out of all the MMPs known, role of MMP-11 in SJS ocular sequelae remains to be explored. Therefore, this study aims to evaluate the status of MMP-11 in SJS ocular sequalae patients.

Methods: Pannus tissues excised from eighteen SJS patients during ocular surface reconstruction surgeries were subjected to clinical and histopathological examination. Immunohistochemistry (IHC) was performed on all cases for MMP-11 and was evaluated on the basis of staining intensity. Immunoexpression of MMP-11 was correlated with clinicopathological features

Results: Mean age of SJS patients was 25.6 ± 13.10 year with a male preponderance (77.7%). Antipyretics (38%) were most common causative of SJS found. Mean time duration from onset of systemic episode to first presentation was 8.3 years. Mean BCVA was 2.72 logmar ± 0.47, mean Schirmer' s test was 0.27 ± 1 mm. Epithelial hyperplasia was found histologically in 16/18 (88.8%), stromal vascularisation in 10/18 (55%) and stromal fibrosis in 4/18 cases (22%). MMP-11 expression was detected in 14/18 cases and was found correlating significantly with epithelial hyperplasia (p=0.0114), keratinization (p=0.0359) and stromal inflammation (p=0.0474).

Conclusions: This is the first report showing the expression of Matrix Metalloproteinase 11 in Stevens - Johnson syndrome ocular sequalae. This indicates that MMP-11 may contribute towards severity of histopathological changes seen during pannus formation. MMP-11 expression will help in elucidating the underlying pathogenesis in SJS.

Commercial Relationships: Renu Venugopal, None; Seema Sen, None; Seema Kashyap, None; Tushar Agarwal, None; Namrata Sharma, None

537 - P68-57

Is Vitamin D Receptor Knockout Mice a Qualified Model of Meibomian Gland Dysfunction?

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Study Group: Kawashima Group

Purpose: To analyze the eyelids of vitamin D receptor (VDR) knockout mice and to evaluate whether this phenotype can be used as a model to study Meibomian gland dysfunction (MGD).

Methods: A total of 20 eyelids with the phenotype of VDR knockout mice aged 3 months (n=5) and 7 months (n=5) were investigated and compared with 20 normal eyelids from wild-type C57/B6J mice of the same age (n=10). All subjects underwent microscopy examination, tear volume test, fluorescein staining, HE staining of eyelid and lacrimal gland (LG), counting of cilia length and cilia number, immunofluorescent staining for keratinization marker CK1, CK5 and CK6.

Results: All the VDR knockout mice aged 3 months or 7 months developed thinning and whitening of cilia, cystic alteration within the Meibomian glands (MGs). With aging, cysts inside the MGs became severe while the area of MGs on conjunctiva surface shrinked and wrinkled. Lid margin of VDR knockout mice showed more expression of CK1 compared with wild-type controls. In addition, there were no significant difference of corneal fluorescein staining, body weight, LG/tear volume between the VDR knockout mice and wild-type controls.

Conclusions: Among all the mice with VDR deficiency, morphological changes including reduced area of MGs, pathological cystic formation on the orifice of or inside the MGs, marginal zone shift and hyper-keratinization of lid margin, the thinning and whitening of cilia strongly suggest that the VDR knockout mice meet the criteria of MGD to some extent, but the functional changes such like the changes of lipid production or lipid component of that VDRKO knockout mice should be further studied.

Commercial Relationships: Kai Jin, None; Motoko Kawashima, None; Masataka Ito, None; Kazuo Tsubota, None

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538 - P69-58

Determination of tegafur and 5-fluorouracil in tear by HILIC-MS/MS

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Purpose: Tegafur (FT) is a prodrug of 5-fluorouracil (5-FU) as a constituent of S-1, and that is widely used for cancer chemotherapy. However, many cases are reported that patients prescribed S-1 experienced ophthalmic adverse effects, such as lacrimal passage impairment and corneal epitheliopathy. We established a rapid, simple and sensitive method for the analysis of FT and 5-FU in tear fluids by HILIC-MS/MS.

Methods: Tear fluids (10 μ l) spiked with the FT, 5-FU and 5-chlorouracil (IS) were diluted with 40 μl of 2M ammonium acetate followed by a simple protein precipitation with 250 μl of 2% formic acid in acetonitrile. After centrifugation, the clear supernatant extract (15 μ l) was directly injected into the HILIC-MS/MS, and separated on a Unison UK-Amino column (50 mm × 3 mm i.d., particle size 3 μ m) with a linear gradient elution system.

Results: Quantification was performed by multiple reaction monitoring (MRM) with negative-ion atmospheric pressure chemical ionization (APCI), and provided high specificity for determinations of the FT, 5-FU and IS in tear. Distinct peaks appeared for the drugs on each channel within 1

min. Recoveries of FT and 5-FU in tear were 74 – 97%. The regression equations showed excellent linearities in the range of 1.0 – 8.0 μg/ml and 0.04 – 0.5 μg/ml for FT and 5-FU, and the limits of detection were 0.1 μg/ml and 0.02 μg/ml for FT and 5-FU, respectively. Intra- and interday coefficients of variation for all the drugs were not greater than 6.6%. The accuracies of quantitation were 96 – 122%.

Conclusions: The present method will be useful for high-throughput determination of FT and 5-FU in clinical analyses. The method was successfully applied to determination of the levels of FT and 5-FU in tear fluids after oral administration to the patients prescribed S-1.

Commercial Relationships: Ritsuko Obuchi, None; Xiao-Pen Lee, None; Makiko Hirosawa, None; Hiroo Ishida, None; Ken-ichi Fujita, None; Susumu Nittono, None; Masato Yoshida, None; Yasutsuna Sasaki, None; Keizo Sato, None; Haruo Takahashi, None

539 - P70-59

3D meibography for diagnosis of dry eye

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Purpose: The dysfunction of meibomian glands which secrete components of lipid layer in tears is currently pointed out as one of the main causes occuring dry eye. The distribution of that is more than 70% in Asian, especially. This brought out the importance for the dysfunction of meibomian glands. Our study was aimed to confirm the efficacy of 3D meibography to evaluate the structures of meibomian glands.

Methods: This study is a cross sectional study for patients who had diagnosed as dry eye disease associated with the dysfunction of meibomian glands at Seoul Saint Mary's Hospital from July to October, 2014. To confirm the structure of dry eye patients, 3D images using 3D OCT (optical coherence tomography) and 2D images using infrared camera were obtained. Patients who had the drop-out lesion in 3D and 2D images were devided as two groups, and differences between them were analyzed. At the same time, to find the clinical signification for structural changes of meibomian glands, all patients had an ocualr surface and a tear function examination to define the degree of dry eye.

Results: As compared between 3D and 2D images for patients who had the drop-out lesion on meibomian glands, the more they had severe dry eye, the more the structure of meibomian glands got irregular ($p < 0.05$). But, the tendency of disappering glands focally or totally was similar between them. The structural difference such as the degree of disappering in mebomian glands and the accesory organ of eyelids surrounding mebomian glands made drop-out lesions which be visible through 3D images, not 2D.

Conclusions: Our study confirmed that the structural change of meibomian glands is proportional to the degree of ery eye. The change of meibomian glands which are not visible in 2D images founded through 3D images. Especially, 3D meibography was more powerful than 2D infrared camera to find out the real state of drop-out lesion on meibomian glands.

Commercial Relationships: Young-Sik Yoo, None; Kyung-Sun Na, None; Choun-Ki Joo, None

Retina - Poster

596 - P01-1

Current Changes in Treatment and Medical Expense of Age-Related Macular Degeneration in Japan

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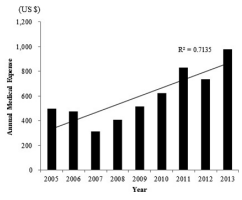
Purpose: To investigate changes in the number of patients, treatment, and medical expense of age-related macular degeneration (AMD) in Japan in these nine years.
Methods: Among 2,710,284 Japanese who were registered in Health Insurance Society database from 2005 to 2013, patients with AMD were extracted based on diagnosis codes of the International Classification of Diseases version 10. Investigated parameters were changes in patient number, treatment, and medical expense during the study period.
Results: The prevalence of AMD among Japanese of same or older than 20 years old significantly increased from 0.06% to 0.19%. A photodynamic therapy was a predominant therapy from 2005 to 2008. Anti-vascular endothelial growth factor (VEGF) therapy started in 2008 and became a predominant therapy since 2009. Medical expense for AMD increased 5.1 times during the study period. Patients with medical treatment were required approximately $3,860 as an annual medical expense, which was approximately 7.4 times higher than those without medical treatment in 2013.
Conclusions: Patient with AMD and medical expense for AMD care significantly increased in these nine years in Japan. It is important to consider AMD care from the medical economical aspect.
Commercial Relationships: Atsuki Kume, None; Kenji Kashiwagi, None; Tomohiro Ohshiro, None; Yoichi Sakurada, None



597 - P02-2

Structural predictors of the development of choroidal neovascularisation in early age-related macular degeneration

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Purpose: To determine structural predictors of subsequent neovascularisation in eyes with early atrophic age-related macular degeneration (AMD).
Methods: Seventy eyes of 70 participants with early atrophic dry AMD (Age-Related Eye Diseases Study grades 2-3), including 4 eyes of 4 participants who later developed neovascular AMD (nAMD) were cross-sectionally assessed at baseline and followed every 3 months. Structural parameters assessed with spectral-domain optical coherence tomography (SD-OCT) included, central retinal thickness (CRT), subfoveal pigment epithelial detachment (PED) presence and height, retinal pigment epithelium (RPE) thickness and inner segment ellipsoid (ISe) band integrity. Two-sampled t-tests were used to assess differences in mean parameters between eyes that remained dry and eyes that developed nAMD.
Results: Compared to participants who remained dry, participants who developed nAMD had similar mean CRT (287.9 ± 28.1μm vs. 329 ± 108.3μm, respectively, p=0.5) and mean RPE thickness (32.2 ± 5.1μm vs. 32.8 ± 8.0μm, respectively, p=0.9) at baseline. Among 21 participants with a subfoveal PED, participants who remained dry (n=19) had a smaller mean PED height compared to participants who developed nAMD (n=2) (107 ± 31.9μm vs.296.5 ± 29μm, respectively, p=0.045). Among participants who remained dry over the study period, 3 (4.5%) participants had disruption of the ISe band compared to the 4 (100%) participants who developed nAMD. Mean time to development of nAMD was 5.25 months.
Conclusions: Disruption to ISe band integrity and larger subfoveal PED may be significant structural markers in predicting subsequent development of nAMD among people with early AMD. Patients with these characteristics may warrant closer monitoring to assess for possible development of nAMD.
Commercial Relationships: Thomas Hong, None; Geoffrey Broadhead, None; Andrew Chang, None
Clinical Trail: ACTRN12612000729820

598 - P03-3

Reliability of Smartphone-Based Electronic Visual Acuity Testing, (Applications in Remote Monitoring and Clinical Research of Macular Pathology)

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Purpose: To assess the feasibility and accuracy of a smartphone-based electronic method of visual acuity (VA) testing for remote monitoring and clinical research, and to evaluate its test-retest reliability and concordance with standard Early Treatment for Diabetic Retinopathy Study (ETDRS) testing.
Methods: Visual Acuity was measured with the smartphone-based electronic visual acuity (SEVA) testing algorithm (SightBookTM, DigiSight Technologies, Inc. Portola Valley, CA) at a test distance of 30 centimeters, twice, and both ETDRS distance chart and LEA numbers

near vision chart, once, on one eye of each of 69 normal, 35 cataract, 55 diabetic, and 41 age-related macular degeneration (AMD) patients (n=200). Reliability and concordance were calculated using the Bland-Altman limits of agreement, the coefficient of repeatability (COR), and the intraclass correlation coefficient (ICC).
Results: For the SEVA testing, test-retest reliability was high (ICCs= 0.977 of all patients, 0.980 of normal subjects, 0.957 of AMD, 0.966 of cataract, 0.970 of DR; 95% limits of agreement ± 0.20 logMAR). SEVA and near VA with LEA numbers chart were highly correlated (r2=0.671 of initial test in SEVA; r2=0.714 of repeated test in SEVA). There were no differences between SEVA and near VA with LEA numbers in all diseases and visual acuity groups. SEVA and distance VA by ETDRS were highly correlated (r2=0.757 of initial test in SEVA; r2=0.746 of repeated test in SEVA). There were no differences between VA with SEVA and distance VA with the ETDRS chart in all diseases and VA groups except the AMD group (p=0.003).
Conclusions: Smartphone-based electronic VA testing using the SightBookTM application has high test-retest reliability and good concordance with ETDRS distance visual acuity and standard near vision testing
Commercial Relationships: Seung-Young Yu, None; Hyung Woo Kwak, None

599 - P04-4

Proteomic analysis of exudative age-related macular degeneration

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Purpose: Genome wide association study of age-related macular degeneration (AMD) identified that the gene polymorphisms of CFH, HTRA1, and TNFRFS10A were disease susceptibility genes. It had previously been shown that CFH was associated with drusen formation through oxidative stress whereas HTRA1 and TNFRFS10A was associated with the inflammatory aspect of the disease. However, it remains to be unknown how these genetic biomarkers would be involved in the pathophysiology of AMD. In this study, we performed proteomic analysis to identify potentially specific biomarkers of exudative age-related macular degeneration.
Methods: Twenty-seven aqueous humor samples (24 from patients with native AMD and 3 control samples from patients with cataract, average age of 72.3 years old) were analyzed with capillary electrophoresis coupled to mass spectrometry to define potential candidate protein markers of AMD. Aqueous humor was extracted after informed consent at the time of anti-VEGF injection.
Results: In the analysis by MASCOT SEARCH RESULTS, 20 known disease proteins and 17 potentially new proteins were identified.
The seven proteins were identified from two or more AMD cases, and Prostaglandin H2 D-isomerase was identified by 11 cases.
Conclusions: Since oxidative stress and chronic inflammation are involved in the development and progression of AMD, the identification of prostaglandin H2 D-isomerase is intriguing findings.
These results indicate that proteomic analysis using aqueous humor may be useful for the identification of specific biomarkers of exudative type AMD.
Commercial Relationships: Kiichiro Okano, None; Tsutomu Sakai, None; Hideo Kohno, None; Takeo Iwamoto, None; Hiroshi Tsuneoka, None

600 - P05-5

Quantitative Analysis of Fundus Autofluorescence in Exudative Age-related Macular Degeneration, Long-term Follow-up

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Purpose: To evaluate and quantify fundus autofluorescent (FAF) changes occurring in patient with exudative age-related macular degeneration (AMD) after treatment with anti-vascular endothelial growth factor (VEGF).
Methods: This is a retrospective study. Forty eyes of 37 consecutive patients with naïveexudative AMD who had received treatment with anti-VEGF therapy and followed for more 48 months were enrolled. All patients received three initial monthly injections of ranibizuambor bevacizuamb, followed by PRN treatment. At all visits, FAF images were analyzed using semiautomated software (RegionFinder, Heidelberg Engineering, Heidelberg, Germany). A stepwise multivariate linear regression analysis was used to investigate factors that may be associated with the enlargement of hypofluorescence at follow-up.
Results: The mean age of the patients was 69.1 years, the mean number of anti-VEGF injections received was 10.1, and the mean number of photodynamic therapy (PDT) received was 1.6. At baseline, the mean size of hypofluorescent area was 1.293 square millimeter. The mean size of hypofluorescent area was decreased after loading phase of anti-VEGF (1.007 square millimeter, P = .101), but significantly increased by final follow-up of 48 months (3.861 square millimeter, P < .001). The significant predictors in multivariate linear regression modeling for the dependent variable hypofluorescent area at 48 months was the hypofluorescent area after loading phase of anti-VEGF (P = .001). Hypofluorescent area at baseline, the number of anti-VEGF injections and PDT were not significant (P = .320, P = .610, and P = .457, respectively).
Conclusions: Increase in hypofluorescence was observed in patients with exudative AMD undergoing anti-VEGF therapy. Hypofluorescence after loading phase of anti-VEGF was a significant predictor of hypofluorescence at final follow-up.
Commercial Relationships: Kyung Hoon Seo, None; Min Seok Kang, None; Ki Young Kim, None; Seung-Young Yu, None; Hyung Woo Kwak, None

601 - P06-6

Structural and Functional Relationship between Optical Coherence Tomography and Custom Developed Manipal E-Amsler Screening Test Inpatients with Macular Diseases

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Purpose: To compare the structural and functional measures of retinal macular diseases between Optical Coherence tomography (OCT- Carl Zeiss Meditec, Germany and custom developed Manipal E Amsler© screening chart.

Methods: Manipal E-Amsler© (ME) software is a custom developed software, in collaboration with Apps2Ease Pvt. Ltd, Manipal, Karnataka, India. We reported that the sensitivity of this chart was 90.2% in detecting functional deficits due to macular diseases and structural assessment was performed with OCT). This case control study included patients diagnosed with maculopathies and age related macular degeneration along with age matched controls. 230 eyes were recruited from secondary and tertiary eye care centres in Manipal and Mangalore, Karnataka, India. All subjects underwent comprehensive eye examination and OCT and ME chart were also administered with proper instructions. . Macular thickness map and the ME score were recorded. From the map, central foveal thickness, cube average thickness and the ME score were included for the analysis using SPSS ver 16.0

Results: Median Age (Inter-quartile range) was 58 (17) and the median score was 0.00 (38.50). Mean ± SD of central foveal thickness showed 299.64µm ± 123.36 and mean ± SD of cube average thickness showed 288.34µm ± 45.95. The correlation between the central foveal thickness and the ME score showed a good relation or association, R^2 0.409 and a slightly lower R^2 0.315 in comparison of cube average thickness along with ME score. Kruskal Wallis test showed a p value 0.001 stating that the data is statistically significant.

Conclusions: The ME Amsler© scores worsened with increasing central foveal thickness, representative of more significant disease stages. The relationship weakened for Cube thickness and ME Amsler©, this could probably because of the summative assessment of both structural and functional measures.

Commercial Relationships: Shailaja S, None; Swetha M, None; Ramesh S Ve, None; Avik Ray, None; Abhishek Bhaduri, None

602 - P07-7

Inner choroidal layer thickness in central serous chorioretinopathy by enhanced depth imaging optical coherence tomography

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1. Kansai Medical University, Hirakata, Japan.

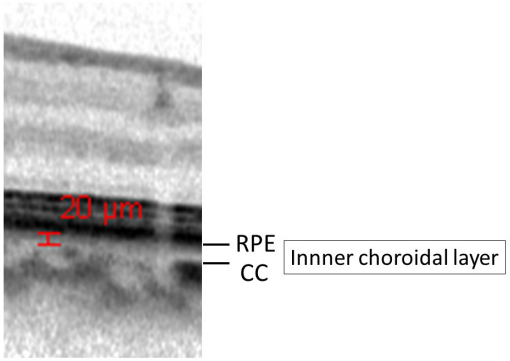
Purpose: To compare the inner choroidal layer〔between retinal pigment epithelim (RPE) and choriocapillalis〕thickness between the area of choroidal vascular hyperpermeability and normal choroidal vascular permeability on indocyanine green angiography (ICGA) by enhanced depth imaging optical coherence tomography (EDI-OCT) in the same eye of central serous chorioretinopathy (CSC) patients.

Methods: All CSC patients were examined on fluorescein angiography (FA), ICGA, EDI-OCT horizontal raster scan (30° x 35° ; ART 12 scan) at the same period. EDI OCT was performed by Heidelberg spectralis®. The measurement of the inner choroidal layer thickness were performed by hand. For 18 eyes of 16 patients diagnosed with CSC. The age of all CSC patients was under 55 years old (mean age 46.2 ± 5.2 :male 10, female 6). The clinical phase of CSC were 4 eyes with acute CSC, 4 eyes with chronic CSC, and 10 eyes with recurrent CSC.

Results: The inner choroidal layer thickness of the area of choroidal vascular hyperpermeability was 3.6 ± 0.23 μ m, which was significantly thinner than 18.8 ± 0.56 in the area of normal choroidal vascular permeability ($P<0.01$:t-test) in CSC eyes. The area of choroidal vascular hyperpermeability on ICGA in eyes with CSC corresponded with choroidal vessel dilatation of sattler`s and haller`s layer by EDI-OCT.

Conclusions: We hypothesized that thinning of the inner choroidal layer thickness of the area of choroidal vascular hyperpermeability on ICGA was caused by pressing of choroidal vessel dilatation of satter`s and haller`s layer.

Commercial Relationships: Katsuaki Miki, None; Yoshimi Nagai, None; Tomoyuki Chihara, None; Motoki Kimura, None; Masayuki Onaka, None; Kanji Takahashi, None



603 - P08-8

Factors Influencing Need for Retreatment and Long-term Visual Outcome After Intravitreal Bevacizumab For Myopic Choroidal Neovascularization

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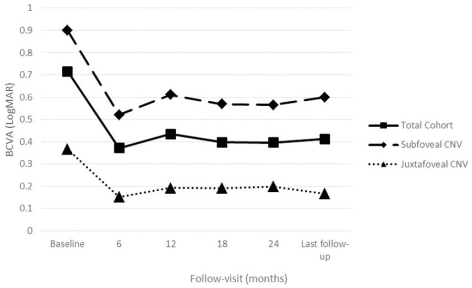
Purpose: To evaluate the efficacy and the predictive factors associate with the need for retreatment and long term visual outcome after intravitreal bevacizumab (IVB) for myopic choroidal neovascularization (CNV).

Methods: Retrospective cohort study of 69 eyes with subfoveal or juxtafoveal myopic CNV. Eyes with follow-up of at least 24 months were included. All eyes were treated with 3 initial monthly IVB injections followed by pro re nata retreatment.

Results: The mean logarithm of the minimum angle of resolution (logMAR) BCVA at baseline was 0.71 +/- Standard Deviation (SD) 0.55 and at 24 months was maintained at 0.40 +/- SD 0.46 ($p < 0.001$) and 0.41 +/- SD 0.50 at the last follow-up. The mean number of injections was 3.85 +/- 1.75 (range, 3 – 10) and a total of 25 eyes (36.2%) required retreatment after the initial 3-monthly loading doses. Both subfoveal and juxtafoveal myopic CNV eyes had significant improvement in BCVA (0.30 +/- 0.48 vs 0.20 +/- 0.27, $p = 0.324$), and juxtafoveal myopic CNV eyes had significantly better BCVA at baseline and at the last follow-up than the subfoveal group (Figure 1). Treatment naïve eyes had significant improvement from baseline BCVA and the amount of improvement was significantly more than those that received previous photodynamic therapy (PDT) (0.32 +/- 0.47 vs 0.07 +/- 0.14, $p = 0.012$). Multivariate stepwise regression analysis showed that the baseline CNV size ($p < 0.05$), baseline BCVA ($p < 0.001$) and duration of symptoms ($p < 0.001$) were significant predictive factors for final BCVA, and BCVA improvement. Multiple logistic regression analysis identified CNV size ($p = 0.038$) and follow-up duration ($p = 0.016$) were significant predictive factors for retreatment. No significant association was found for number of injections.

Conclusions: IVB seems to be an effective treatment for both subfoveal and juxtafoveal myopic CNV and for eyes with prior PDT in the long term for at least 24 months. Patients presented with shorter duration of symptoms and smaller CNV size before treatment are significant prognostic factors that predict better visual outcome. Eyes with smaller CNV size may have better chance for no retreatment needed.

Commercial Relationships: Danny Ng, None; Alvin Kwok, None; Walton Li, None



Mean logMAR BCVA following IVB in 69 eyes and in subgroup of eyes diagnosed with subfoveal and juxtafoveal myopic CNV.

604 - P09-9

Diurnal blood pressure variation type in diabetic patients with macular edema

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Purpose: Diurnal blood pressure variation has not been well known in patients with diabetic macular edema (DME). The purpose of this study was to investigate the correlation between blood pressure variation patterning and severe DME. In addition, to estimate the risk factor of severe DME.

Methods: Twenty four-ambulatory blood pressure monitoring (ABPM) of 43 patients (20 females) with type 2 diabetic mellitus (DM) was examined and several clinical parameters were evaluated. Variation in diurnal BP was compared between two groups, DM patients without retinopathy (NDR group, n=20), those with severe macular edema needed surgical treatment (DME group, n=23). DME was evaluated by optical coherence tomography (OCT). Each patient of both groups was categorized into the following 4 types based upon the blood pressure (BP) variation type; reduction rate of BP (1 - night-time / day-time) (%): ≧ 20% as "extreme-dipper" , >10-20% as "dipper" , > 0-10% as "non-dipper" , and < 0% as "riser" . The dominant type in both groups was examined and compared .

Results: 17patients (85%) in NDR group and 19 patients (83%) in DME group took one or more antihypertensive agents. With regard to BP variation, more than half of the NDR group showed normal variation (dipper) in both systolic BP (SBP) and diastolic BP (DBP), while abnormal diurnal blood variation patterning of BP increased in DME group. (dipper yes/no (SBP), 8/12, 4/19, NDR vs. DME , p= 0.1, dipper yes/no (DBP):10/10, 2/21, NDR vs. DME , p= 0.0033, Fisher's exact test). Hb (g/dl) was statistically significant lower in DME (14.2 ± 1.28, 12.7 ± 1.7,p = 0.0020, unpaired-t test) .

Conclusions: In patients with DME, the decline in nocturnal BP would not be anticipated and that abnormal diurnal blood pressure variation increased, though antihypertensive agents were used.

Abnormal diurnal blood pressure variation and lower Hb could be significantly
related to the severeness of DME.

Commercial Relationships: Kihoko Kato, None; Norihito Doi, None; Tomonori Sakoguchi, None; Tetsurou Kamada, None; Taiji Sakamoto, None

605 - P10-10

Relationship between outer retinal microstructural changes and visual acuity in diabetic macular edema

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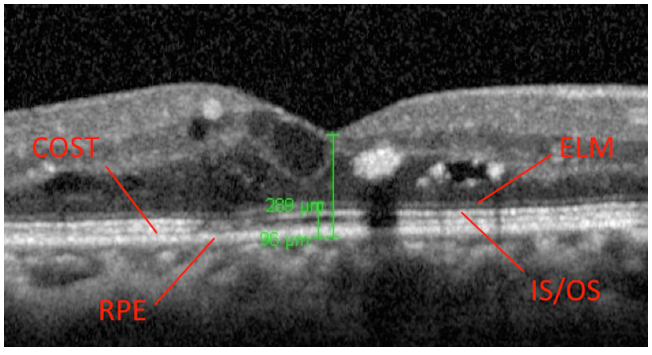
Purpose: To investigate the correlation of outer retinal layers (ORL) thickness and visual acuity (VA) in patients with diabetic macular edema

Methods: Consecutive patients presented with diabetic macular edema seen at the retina clinic of The University of Hong Kong were recruited for optical coherence tomography (OCT) assessment. The outer retinal layers (ORL) thickness was defined as the distance between external limiting membrane (ELM) and retinal pigment epithelium (RPE) at the foveal center. The correlation between total retinal thickness, ORL thickness and vision was calculated.

Results: 78 patients with diabetic macular edema were recruited. The mean age was 58.1 years (+/- 11.5 years) and their mean visual acuity measured with Snellen chart was 0.51 (+/- 0.18). The correlation coefficient between total retinal thickness and visual acuity was 0.34 ($p < 0.001$) whereas the correlation coefficient was 0.65 between outer retinal layers thickness and visual acuity ($p < 0.001$).

Conclusions: Outer retinal layers thickness represents the total length of photoreceptor inner segments and outer segments, which consists of mitochondria for energy production and disks with opsin for photons absorption. Outer retinal layers thickness correlates better with vision than the total retinal thickness. Its correlation with visual acuity is comparable to the photoreceptor outer segment (PROS) length with a theoretically higher repeatability. To conclude, outer retinal layers thickness is a novel optical coherence tomography parameter in the assessment of diabetic macular edema. Moreover, it could potentially be a long term visual prognostic factor for patients with the condition.

Commercial Relationships: Raymond Wong, None; Ian Wong, None



Representative optical coherence tomography (OCT) scan showing segmentations for outer retinal layers (ORL) thickness measurement in diabetic macular edema (DME). ELM, eternal limiting membrane. IS/OS, photoreceptor inner segment/outer segment junction. COST, cone outer segment tips. RPE, retinal pigment epithelium.

606 - P11-11

Quantitative analysis of automated retinal layer segmentation in ischemic and non-ischemic diabetic macular edema using spectral domain optical coherence tomography

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Purpose: To determine the decrease in thickness of various segmented layers of retina in patients with ischemic and non-ischemic diabetic macular edema (DME) after regular intravitreal injections of anti-vascular endothelial growth factors (anti-VEGFs) over a period of 1 year. Whereas there are many studies measuring total retinal thickness, this study investigated the effect of anti-VEGF treatment on individual layers of the retina.

Methods: 41 eyes with DME of 33 patients with no previous anti-VEGF treatment were imaged by spectral domain optical coherence tomography (SD-OCT) at baseline. Patients were then treated with intravitreal anti-VEGF (ranibizumab) adhering to a "treat and extend" protocol. OCT imaging was obtained again after 1 year of continuous treatment. Retinal layers were determined using segmentation software and thickness changes of individual layers were quantitatively analyzed.

Results: In eyes with non-ischemic DME a significant decrease of layer thickness in the central area of the ETDRS grid was found for the retinal nerve fiber layer (RNFL) (42.5 ± 31.5 vs 29.5 ± 18.5µm, p=0.0244), the ganglion cell layer (GCL) (44 ± 28 vs 32.5 ± 21.5µm, p=0.0167) and the inner plexiform layer (IPL) (56.5 ± 35.5µm vs 47 ± 30µm, p=0.0063). A similar and significant reduction in the same layers was also detected for the inner and outer rings of the ETDRS grid. In contrast, in eyes with ischemic DME, a significant reduction was only found for the total retinal thickness of the inner ring of ETDRS grid (p=0.0010).

Conclusions: After 1 year of continuous intravitreal treatment with ranibizumab according to a "treat and extend" protocol, we observed a more pronounced decrease in layer thickness of the RNFL, the GCL and the IPL in eyes with non-ischemic DME than in eyes with ischemic DME. Nuclear layers and the photoreceptor layer did not show a significant reduction. The studied parameters might be useful tools to monitor intravitreal anti-VEGF treatment for DME in clinical practice and future clinical trials.

Commercial Relationships: Abhishek Jain, None; Wolf Sebastian, None; Martin Zinkernagel, None; Andreas Ebneter, None

607 - P12-12

Evaluating the Outcome of National Health Insurance (NHI)-Approved Three-Dose Intravitreal Ranibizumab Injection in Eyes with Diabetic Macular Edema (DME) in Taiwan

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Purpose: To analyze the functional and structural results of the three-dose intravitreal Ranibizumab injection in diabetic macular edema (DME) patients.

Methods: The criteria for National Health Insurance (NHI) application approval of Ranibizumab for DME in Taiwan were BCVA between 0.05 and 0.5, HbA1C <10% within the past 3 months from application, evidence of diabetic retinopathy on fundus exam, and central retinal thickness (CRT) ≧ 300 μm. 97 eyes (71 patients) from July 2013 to March 2014 in Taipei Veterans General Hospital were retrospectively collected. They were divided into groups by baseline visual acuity (VA), durations from the first to the third injections, and eyes managed with laser before or either kinds of anti-VEGF within 6 months. Patient's characters, VA, CRT, as well as patterns of DME were documented by detailed chart review. Functional outcome was VA change after three injections. CRT reduction with different injections' durations and the effect of additional two complying with NHI application standard were also analyzed.

Results: Mean baseline VA in these patients was 0.7 ± 0.34 in logMAR. Ninety-one percent of eyes suffered from advanced NPDR and PDR. Diffused intra-retinal edema composed 64% of the patterns of DME. Fourty-nine eyes (51%) presented stable VA change (within 1 line), and 36 eyes (37%) improvement (more than 1 line). The worse the baseline VA leaded to better improvement (P=0.005). Duration of the three injections and with or without additional two had no significant influences on the VA change. Eyes pretreated with both anti-VEGF and laser had the greatest percentage (45%) of VA improvement than Bevacizumab alone, laser alone, and no treatment before. Average baseline CRT (460.11 μm) showed a 29.9% thickness reduction after three injections. Duration of injection within or above three months revealed similar thickness reduction. Eyes with additional two injections had some beneficial effects but without significance. Reduction of thickness was greater in eyes with thicker CRT.

Conclusions: In functional outcome, 88% of the eyes remain stable or improve in VA, and worse baseline VA leads to greater improvement. For the structural outcome, thicker baseline CRT can perform greater reduction. VA improvement after three shots of Ranibizumab for DME in our study was comparable to that in other clinical trials.

Commercial Relationships: Yu-fan Chang, None; Tai-Chi Lin, None; Chang-Sue Yang, None; Po-Kang Lin, None; An-Fei Lee, None; Ling-Ing Lau, None; Fang-Yi Tsai, None; Fenq-Lih Lee, None; Shih-Jen Chen, None

608 - P13-13

Intravitreal bevacizumab increases intraocular inflammatory factor levels after injection in patients with proliferative diabetic retinopathy

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Purpose: We evaluated the cytokine levels in the aqueous humor and fibrovascular membranes (FVMs) of patients with proliferative diabetic retinopathy (PDR) after intravitreal injection of bevacizumab (IVB).

Methods: This was a retrospective cross-sectional study, and patients with PDR (n = 69) were included. 42 aqueous humor samples of 42 patients affected by PDR and 8 aqueous humor samples of 8 patients with age related cataract were investigated. 28 eyes received an intravitreal bevacizumab injection (1.25 mg) 2 or 7 or 14 days before pars plana vitrectomy (PPV). The blank control group included 14 eyes that underwent PPV without previous IVB. The angiogenic and inflammatory cytokines included VEGF, IL-6, IL-8, and MCP-1. Using suspension array technology (Luminex system), the concentrations of cytokines were measured. Immunohistological studies were performed on 27 surgical specimens obtained during PPV. 18 eyes received an intravitreal bevacizumab injection (1.25 mg) 7 or 21 days before PPV. 9 eyes underwent PPV without previous IVB. The expressions of VEGF, IL-6R, IL-8R, and CCR2 in the FVMs were detected through immunostaining.

Results: Significantly higher concentrations of VEGF, IL-6, IL-8, and MCP-1 were found in the aqueous humor of PDR patients than in the aqueous humor of cataract patients. Concentrations of IL-8 ranged from 13.92 ± 14.62pg/mL to 15.49 ± 8.45pg/ml (2 day) to 22.18 ± 20.71pg/ml (7 day) to 35.03 ± 39.9pg/ml (14 day). A significant correlation was observed that the concentration of IL-8 after IVB for 14 days (p=0.029). Concentrations of IL-6 ranged from 604.56 ± 1054.48pg/ml to 774.93 ± 1472.79pg/ml (2 day) to 328.57 ± 438.1pg/ml (7 day) to 271.26 ± 228.1pg/ml (14 day). Concentrations of MCP-1 ranged from 475.72 ± 385.43pg/ml to 618.22 ± 359.10pg/ml (2 day) to 438.69 ± 194.90pg/ml (5 day) to 466.95 ± 208.01pg/ml (14 day). The levels of IL-6 and MCP-1 slightly increased 2 day after IVB, but missed statistical significance. The percentages of IL-8R positive fibroblasts significantly increased from 7.48 ± 2.46% to 14.28 ± 7.68% (7 day, p=0.012) to 11.37 ± 3.03% (21 day, 0.038).

Conclusions: These results suggest that IVB aggravates intraocular inflammatory cytokines. Intravitreal bevacizumab injection may lead to an increase of intraocular concentrations of IL-8, which may act as an inflammation marker in PDR eyes.

Commercial Relationships: Jing Wen, None; Yanrong Jiang, None

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609 - P14-14

Evaluation of ganglion cell-inner plexiform layer thickness according to nerve conduction velocity and autonomic nerve function in diabetes

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Purpose: To evaluate the numerical correlation of ganglion cell-inner plexiform layer (GCIPL) thickness with diabetic peripheral neuropathy and autonomic nerve function in diabetic patients.
Methods: The 160 eyes with diabetes who presented no diabetic retinopathy or mild non-proliferative diabetic retinopathy were retrospectively reviewed using spectral-domain optical coherence tomography (Cirrus HD-OCT), peripheral nerve conduction study and autonomic nerve function test. The average GCIPL thickness of macular 6 regions was analyzed using Ganglion Cell Analysis (GCA) algorithm. The peripheral nerve conduction study was measured at peroneal, post. tibial motor nerve and sural sensory nerve. The autonomic nerve function test score was acquired with assessment of Valsalva maneuver, lying heart rate, interval change of heart rate, postural hypotension, and sustained handgrip. The results were classified into 3 groups based on each criteria respectively, and correlations with macular GCIPL thickness and average retinal thickness of parafoveal area within 1-3mm were analyzed.
Results: The mean age was 63.61 ± 12.52. The mean macular GCIPL thickness was 79.89 ± 4.70 ㎛ and mean average retinal thickness of parafovea was 315.05 ± 12.70 ㎛ . The peripheral nerve neuropathy base on nerve conduction study was significantly correlated with macular GCIPL thickness (No peripheral neuropathy vs definite peripheral neuropathy, 80.8 ± 5.8 ㎛ vs 77.2 ± 4.2 ㎛ , p=0.001). The autonomic nerve function was also significantly correlated with macular GCIPL thickness (No or mild dysfunction vs Severe dysfunction, 81.2 ± 6.6 ㎛ vs 77.6 ± 5.9 ㎛ , p=0.005). There were no significant correlations between average retinal thickness of parafovea and neither peripheral nerve neuropathy (p=0.873) nor autonomic nerve function (p=0.674)
Conclusions: The decrease in peripheral nerve velocity and severity of autonomic nerve dysfunction are significantly associated with decrease in GCIPL thickness in patient with diabetes.
Commercial Relationships: Ki Young Kim, None; Min Seok Kang, None; Seung-Young Yu, None; Hyung Woo Kwak, None

610 - P15-15

Short Pulse Duration High-Power Laser Photocoagulation During Vitrectomy for Diabetic Retinopathy Reduces Postoperative Inflammation

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Purpose: To determine the effectiveness of short pulse duration, high-power laser photocoagulation (PC) during vitrectomy for diabetic retinopathy (DR).
Methods: The effects of short pulse duration PC with power of 340–360 mW and duration of 0.02 second were compared to conventional PC with power of 120–150 mW and duration of 0.2 second. The degree of inflammation was quantified by laser flare cell photometry before and at 1 day, 1 week, 4 week, and 12 weeks postoperatively.
Results: Twenty-two eyes of 22 consecutive patients were studied. Ten eyes were treated with short pulse duration PC and 12 eyes with conventional PC. The total energy was significantly lower in the short pulse duration PC group than in the conventional PC group (P=0.02). The laser flare cell values were not significantly different between the groups after 1 day, but at 1 week, the flare cell value was significantly lower in the short pulse duration PC group than in the conventional PC group (P=0.04). This difference was also present at 4 and 12 weeks (P<0.05).
Conclusions: The reduced inflammation after vitrectomy for DR after short pulse duration PC than conventional PC indicates that the short pulse duration PC protocol should be considered.
Commercial Relationships: Masahiko Sugimoto, None; Atsushi Ichio, None; Mineo Kondo, None
Clinical Trail: UMIN000015342

611 - P16-16

Comparison of Navigated and Conventional Panretinal Pattern Scan Laser Photocoagulation for Diabetic Retinopathy

Yuichi Toriyama [1] Takao Hirano [1] Toshinori Murata [1]
1. Ophthalmology, Shinshu-university, Matsumoto, Japan.

Purpose: To compare the pain and treatment time between conventional and navigated pattern scan laser with eye-tracking system for panretinal laser photocoaguration (PRP).
Methods: Eyes underwent PRP with either conventional pattern scan laser (MC-500 Vixi®:NIDEK) or navigated pattern scan laser (Navilas®:OD-OS). Each of three eyes of three patients were treated in both group. At the end of treatment sessions, the intra-operative pain level that the patients felt was assessed using a visual analogue scale (VAS), with 0 being no pain and 10 being unbearable pain. Treatment time were also recorded for both groups. Before and after PRP treatment, central subfield thickness were evaluated by optical coherence tomography (Cirrus:Zeiss).
Results: The navigated pattern scan laser caused less pain according to a visual analogue scale (1.5 ± 1.7) than a conventional pattern scan laser (5.0 ± 1.7). The navigated

pattern scan laser required longer treatment time (89 ± 12sec/100 shots) than the conventional patter scan laser (49 ± 10sec/100 shots), but navigated laser treatment time got shortened with learning curve. In both group, there was no significant change in central subfield thickness in one month after treatment.
Conclusions: Navigated pattern scan laser treatment enables less treatment associated pain in comparison with conventional pattern scan laser. Treatment time was comparable with both lasers.
Commercial Relationships: Yuichi Toriyama, None; Takao Hirano, None; Toshinori Murata, None
Clinical Trail: UMIN000007535

612 - P17-17

Navilas® navigated laser system treatment for diabetic macular edema

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Purpose: To evaluate one-month outcomes following treatment with the Navilas® navigated laser photocoagulator (OD-OS GmbH, Teltow, Germany) for diabetic macular edema (DME).
Methods: We prospectively evaluated 7 diabetic patients who underwent focal/grid laser treatment with Navilas® for DME. Statistical analysis included descriptive and continuous variables (spectral-domain optical coherence tomography [OCT] parameters and best-corrected logMAR visual acuity [BCVA]) that were compared using paired t-tests. A P value of < 0.05 was considered to be statistically significant.
Results: Seven eyes of 7 patients (mean age, 66.9 ± 15.9 years; 4 men and 3 women) were examined. Central retinal thickness in OCT decreased significantly from 351 ± 48 μm to 314 ± 38 μm ($P = 0.0156$) and total macular volume in OCT decreased significantly from 11.3 ± 0.7 mm^3 to 11.0 ± 0.6 mm^3 ($P = 0.1563$). No significant difference was observed between BCVA before (0.18 ± 0.28) and one month after (0.16 ± 0.30) treatment ($P = 0.5000$). No patients experienced complications.
Conclusions: Focal/grid laser treatment with Navilas® was safe and effective in treating DME according to OCT results one month after treatment. Visual acuity remained stable. A longer follow-up period is needed to fully assess the effects of Navilas® on DME treatment.
Commercial Relationships: Takao Hirano, None; Yuichi Toriyama, None; Toshinori Murata, None
Clinical Trail: 7535

613 - P18-18 Withdrawn

Clinical and microbiological profile of patients with Corynebacterium endophthalmitis , Review of a decade

Savitri Sharma [1] Joveeta Joseph [1] Khemlal Nirmalkar [1] Annie Mathai [2]
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Purpose: To report the clinical settings, antibiotic susceptibilities, management strategies, and visual outcomes of patients with endophthalmitis caused by *Corynebacterium* species.
Methods: This is a retrospective, consecutive, non-comparative case series. We reviewed case records of patients with culture-proven *Corynebacterium* endophthalmitis seen between August 2004 and July 2014. Data included the laboratory findings, predisposing factors, presenting visual acuity, treatment given, clinical response, secondary interventions, antibiotic sensitivity of the organism, and final visual acuity.
Results: Of 5439 patients clinically diagnosed as infective endophthalmitis during the study period; vitreous samples were culture positive for bacteria in 1488 (27.3%). Sixteen patients (1.07%) were identified as *Corynebacterium* endophthalmitis. The clinical settings included trauma (n=10), post cataract surgery (n=5), and post-penetrating keratoplasty (n=1). In 7/16 (43.7%) patients, the organisms were identified in direct microscopy. Tested by disc-diffusion method, all isolates were vancomycin and cefazolin sensitive. Presenting VA ranged from 20/30 to no light perception. Initial treatment strategies included pars-plana vitrectomy with intravitreal antibiotic (vancomycin and ceftazidim) injection (n=9) and pars plana lensectomy with intravitreal antibiotic (vancomycin and ceftazidim) injection (n=7). Final VA outcomes were 20/200 or better in 10 (62.5%) of 16 patients, counting finger in three and light perception in 3 patients.
Conclusions: The prevalence of corynebacterial endophthalmitis is low. The organisms are usually susceptible to vancomycin which is the primary intravitreal drug used for the treatment of endophthalmitis. Early presentation and treatment leads to favorable outcomes. Based on a PubMed literature search and to the best of our knowledge, the current study is the largest series of culture-positive endophthalmitis cases caused by *Corynebacterium* species.
Commercial Relationships: Savitri Sharma, None; Joveeta Joseph, None; Khemlal Nirmalkar, None; Annie Mathai, None

614 - P19-19

Implications of Visually Symptomatic vs Asymptomatic Fungal Eye Disease

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Purpose: Current practice guidelines mandate a dilated ocular fundus exam within 2 weeks of a positive fungal blood culture. However, in light of diminishing incidence of positive ocular findings as well as the initiation of early treatment with anti-fungals medications the utility of this

mandate has come into question. We evaluated cases of fungemia and fungal eye disease over a 5-year period at a tertiary care center to isolate those cases of fungemia that would be most benefited by a dilated ocular fundus exam.
Methods: We evaluated cases of positive fungal cultures from Jan 2007 through July 2012 at Scott & White Memorial in Temple, Texas. Data collected during a retrospective chart review included demographic information, dates of positive cultures, organisms isolated, drug sensitivities, systemic antifungal medications, risk factors for fungemia, ophthalmology consultation, visual symptoms, and clinical course.
Results: 231 patients with positive fungal cultures were identified. 81% received antifungal treatment. 50% (126) of all cases received a dilated fundus exam. 83 patients were able to provide a reliable history, 13 (15.7%) reported visual complaints (blurry vision, floaters, eye pain). 4 (3%) patients were unable to be examined. 7 (6%) patients had nonspecific findings (Roth spots, cotton-wool spots) attributable to underlying comorbidities. 4 patients (3%) were found to have presumed positive fungal chorioretinitis. These patients with positive fungal chorioretinitis were asymptomatic or unresponsive at the time of exam, and did not require procedural intervention. Of these 4 cases, 1 was lost to follow-up, 1 was placed on hospice, 1 died approximately 1 mo after ophthalmologic exam, and 1 was followed as outpatient, maintained on systemic antifungals with resolution of disease. Interestingly, 2 patients developed presumed fungal eye disease after their initial positive blood fungal culture. Both patients presented to clinic after their hospital stay with visual symptoms. These patients required procedural intervention with intravitreal injection and vitrectomy.
Conclusions: Based on our findings, we suggest that dilated eye exams may be of most benefit in cases of fungal eye disease that are symptomatic. Thus, modification of current guidelines that would preferentially focus on symptomatic patients may be possible while maintaining a favorable visual prognosis.
Commercial Relationships: Shagun Dhaliwal, None; Johnathan Kim, None; Luke Potts, None; Jason Smart, None; Kevin Lai, None; Robert Rosa, None; Angela Hochhalter, None; Juhee Song, None

615 - P20-20

Optical coherence tomographic highly reflective line in eyes with resolved macular edema associated with branch retinal vein occlusion

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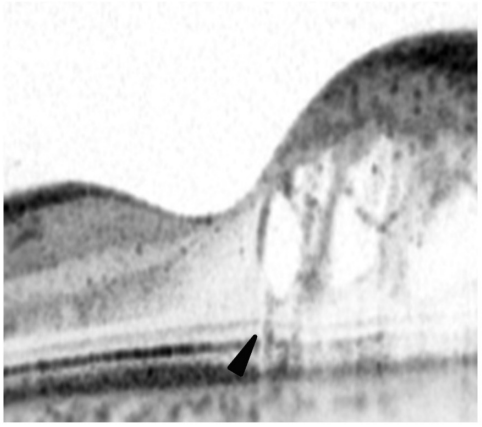
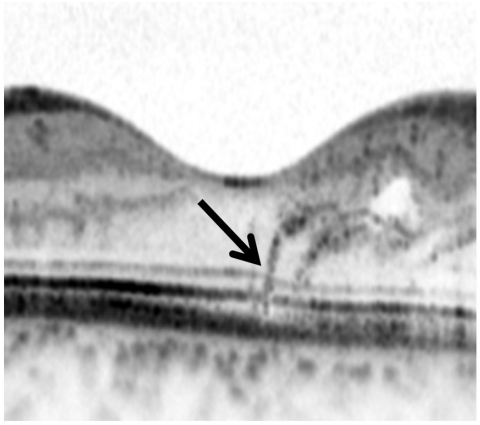
Purpose: To determine whether the highly refletive line which was vertically seen in outer retina after resolution of macular edema associated with branch retinal vein occlusion (BRVO) is correlated with the integrity of photoreceptors at the fovea.
Methods: We retrospectively reviewed the medical records of 59 eyes with a resolution of macular edema associated with BRVO in the spectral-domain optical coherence tomographic (SD-OCT) images. Fifty-nine eyes were classified by the presence or absence of the highly reflective line after resolution of macular edema. We investigated the characteristics of the highly reflective line based on the SD-OCT images.
Results: SD-OCT showed that 21 eyes (35.6%) had the highly reflective line after resolution of macular edema. At the final visit, seventeen (80.9%) of 21 eyes with the highly reflective line had a disrupted ellipsoid zone at the fovea, whereas 3 eyes (14.3%) had a complete ellipsoid zone, and one eye (4.8%) had an absent ellipsoid zone ($P < .0001$). The percentage of eyes with a disrupted external limiting membrane at the initial visit was significantly higher in eyes with the highly reflective line (90.5%) than that in eyes without highly reflective line (63.2%; $P = .0324$).
Conclusions: The presence of the highly reflective line after resolution of macular edema is associated with initial disrupted ELM. Determining the presence of the highly reflective line is also a useful predictor of localized damage of photoreceptors.
Commercial Relationships: Taiji Hasegawa, None; Naonori Masuda, None; Nahoko Ogata, None



Spectral-domain optical coherence tomography (SD-OCT) images of eyes before and after resolution of macular edema.
(Figure 1A) At the initial visit, an eye with macular edema had a disrupted external limiting membrane (ELM) at the fovea. The SD-OCT image showed the highly reflective line beneath the cystoid space associated with macular edema (arrowhead).



(Figure 1B) After resolution of macular edema, the SD-OCT image showed that highly reflective line remained even after resolution of macular edema (arrow).

616 - P21-21

The occurrence and progression of outer retinal tubulation in Chinese patients after intravitreal injections of ranibizumab

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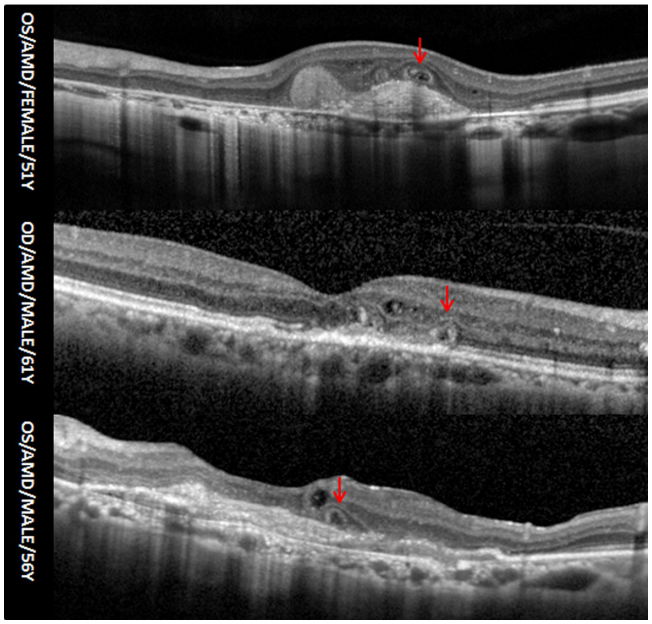
Purpose: To investigate the occurrence and progression of outer retinal tubulation (ORT) in Chinese patients after intravitreal ranibizumab injections, using spectral domain optical coherence tomography (SD-OCT) with eye tracking function.

Methods: 15 age related macular degeneration (AMD) and 6 polypoidal choroidal vasculopathy (PCV) eyes of 21 patients were enrolled and assessed by SD-OCT. One patient received photodynamic therapy (PDT) previously, and all patients received intravitreal injections of ranibizumab.

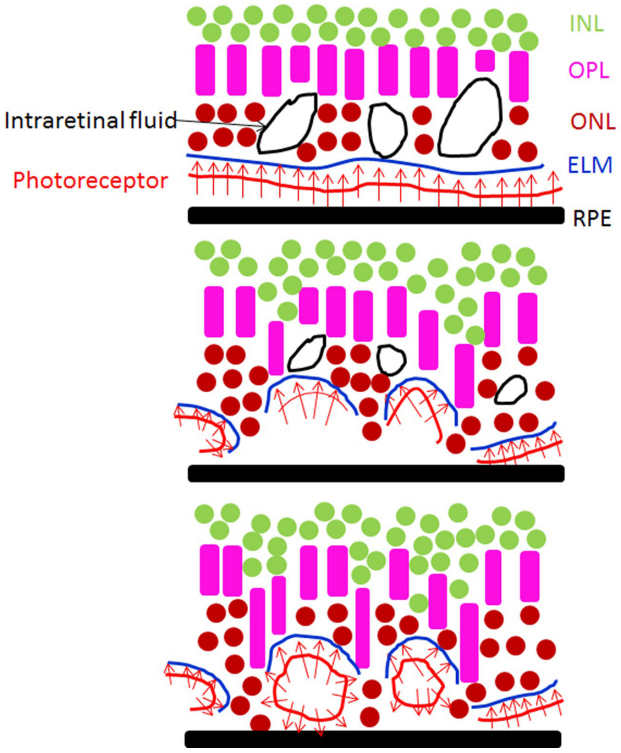
Results: At baseline, only one AMD eye (4.8%) showed ORT, which appeared as round or ovoid hyporeflective spaces with hyperreflective borders. During the follow ups, ORT was identified in nine of 15 AMD eyes (69.2%) and one of six PCV eyes (16.7%). These new ORTs in 10 eyes were originated from the intraretinal fluid. Inner nuclear layer (INL), outer plexiform layer (OPL) and outer nuclear layer (ONL) were pulled down to form "cynapsis" , separating each ORT. However, ORT in 3 eyes disappeared after intravitreal ranibizumab injections.

Conclusions: This is the first observation on the occurrence and progression of ORT in Chinese AMD and PCV patients, in a point to point manner. The ORT could become stable or disappear after ranibizumab injections, and outer retina involved in the process of ORT formation.

Commercial Relationships: Rui Hua, None; Lei Chen, None



The ORT on OCT profiles.



The schema of the ORT formation.

617 - P22-22

Spectral Domain Optical Coherence Tomography of occult optic disc pit

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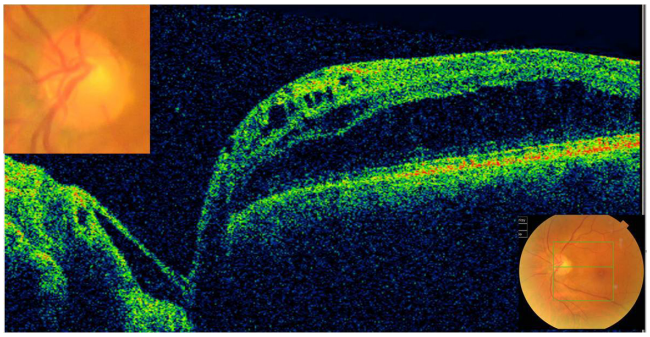
Purpose: To report a patient with normal appearing optic disc with macular schisis , in whom SD-OCT revealed a hidden optic disc pit obscured by a membrane and to highlight the role of SD- OCT in detection of occult optic pits.

Methods: Descriptive case report of a 62 year old female patient with defective vision in left eye of ten years duration .There was shallow serous elevation of macula and papillomacular area with ILM folds and a normal appearing optic nerve head.SD OCT showed large cystoid spaces at the fovea with focal loss of photoreceptor layer., prominent schisis of inner retinal layers and a wide split of outer plexiform layer, nasal to fovea. Scan of optic nerve showed a moderately reflective membrane spanning the optic disc with an underlying pit temporally, which communicated with the outer retinal schisis.

Results: Inner retinal schisis, wide split of outer plexiform layer and large cystoid spaces seen only nasal to fovea with normal architecture of the retina temporal to fovea made us suspect optic pit maculopathy though the disc appeared normal . SD-OCT did confirm the presence of a pit located deep in the stroma of the optic nerve and also showed a prominent hyper reflective membrane spanning the disc and obscuring the pit on clinical examination thus enabling a diagnosis of occult optic pit causing maculopathy.

Role of membranes in the pathogenesis of maculopathy in cavitary disc anomalies is widely debated. While some

reports suggest that absence,defect or collapse of such membranes may be associated with maculopathy, there is also a report of a patient with congenital , "bilateral peri-pappilary hyporeflective cavities" in whom maculopathy was seen in the eye without a membrane while the other eye with an intact membrane developed no maculopathy.
Also a case of maculopathy with normal optic disc appearance , where OCT showed no pit but a membrane over the disc with a fenestration in it, has been reported.
Conclusions: SD –OCT is a simple, non invasive imaging modality in detecting occult optic disc pits and membranes associated with it.
The role of such membranes in the development of maculopathy needs further elucidation.The source of the intra-retinal fluid and the role of the membrane in the pathogenesis of maculopathy in our case remains unanswered.
Commercial Relationships: Jyothsna Rajagopal, None; Chandra Kumar H V , None; Sri Ganesh, None



scan of normal appearing optic nerve showing membrane spanning an underlying pit which communicates with the outer retinal schisis

618 - P23-23

Fundus autofluorescence and onset time in central serous chorioretinopathy

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Purpose: Although fundus autofluorescence (FAF) has been usually used for the evaluation of the function in retinal pigment epitelium, the hyperautofluorescence associated with subretinal fluid in case of such as central serous chorioretinopathy is reported recently. Our purpose in the current study is to evaluate the relationship between FAF and onset time in CSC.
Methods: Forty-four eyes of 44 patients (male, female = 30, 14, average age, 49.8 years) in newly diagnosed cases with CSC were examined retrospectively. Fundus autofluorescence was obtained by the Heidelberg Retina Angiograph 2 (Heidelberg Engineering). Subretinal fluid was confirmed by optical coherence tomography. Durations of disease from subjective onset time in CSC were derived by careful medical interview at initial visit.
Results: Mean durations of disease in the current study were 2.8 months. Hypofluorescent FAF corresponding to the area of subretinal fluid was observed in 31 eyes, diffuse hyperfluorescent FAF was in 9 eyes, patchy or granular hyperfluorescent FAF was in 4 eyes. Durations of disease in each group were 1.5 months, 5.3 months, and 7.3 months, respectively. Duration of disease in hypofluorescent FAF group was significantly shorter than other 2 groups (both $P<0.01$).
Conclusions: The current study confirmed hyperfluorescent FAF due to the subretinal fluid was observed in about 6 months past from subjective symptom. FAF might be useful to estimate the duration of disease and helpful to determine the optimal timing of treatment.
Commercial Relationships: Ichiro Maruko, None; Yutaka Ishikawa, None; Kanako Itagaki, None; Yukinori Sugano, None; Tetsuju Sekiryu, None; Iida Tomohiro, None

619 - P24-24

Peripapillary choroidal thickness in central serous chorioretinopathy, Is choroid outside of the macula also thick?

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Purpose: To investigate peripapillary choroidal thickness (CT) outside of the macula in central serous chorioretinopathy (CSC).
Methods: We reviewed the medical records of 34 patients with unilaterally symptomatic idiopathic CSC and 34 age-matched controls. Subfoveal and peripapillary CT were measured from images obtained by spectral domain optical coherence tomography. The nasal peripapillary CT (PCT) of choroid outside of the macula was determined.
Results: The subfoveal CT of CSC (369.74 ± 54.17 μm) and fellow eyes (316.18 ± 54.68 μm) of the patient group were thicker than those of the normal controls (281.90 ± 40.97 μm, all, $P < 0.05$). The subfoveal CT in CSC was significantly thicker than those in the fellow eyes. Nasal PCT was also thicker in CSC (217.59 ± 62.03 μm) and fellow eyes (206.66 ± 59.35 μm) of the patient group compared to the normal controls (179.52 ± 39.64 μm, all, $P < 0.05$). However, there was no difference in nasal PCT between CSC and fellow eyes ($P = 0.150$).
Conclusions: This result may suggest that manifest CSC occurs in patients with thick choroids both within and outside of the macula, especially when subfoveal CT is increased.
Commercial Relationships: Jaeryung Oh, None; Young-Ho Kim, None; Cheolmin Yun, None
Support: a grant (2012-108) from AJU Pharm. Co., Seoul, Korea and a grant from the Korean Health Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A102024)

620 - P25-25

A Report On Fluorescein Angiographies Done At A Tertiary Institution Since The Approval Of PHIC Circular No. 0035 S. 2013 (17 DEC 2013)

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Purpose: The purpose of this study is to observe if 1) there was a change in the number of fluorescein angiographies done at a private tertriary institution in the Philippines, before and after full subsidy by a national health insurance corporation. By determining the number of normal and abnormal scans, this paper further seeks to shed light on prescribing behavior of ophthalmologists that may be related to this policy change.

Methods: This is a single-center observational study which compares the number of FA's done at a private tertriary hospital in the Philippines from January to August of 2013, with those after the circulation of PHIC circular No. 0035, from January to August of 2014. Readings were labeled as either normal or abnormal. The same comparison was done with fundus photos and macular OCT' s. Data was analyzed with an unpaired t-test assuming unequal variances using Microsoft Excel 2007, to determine statistically significant differences between means of the two study years, for the different tests.

Results: More angiographies were requested in 2014 than in 2013 (591 vs. 486), which was statistically significant at p=0.10. In both 2013 and 2014, there were more abnormal FA' s than normal readings (84 and 170 for 2013 and 2014, respectively, vs. 402 and 421). The number of normal readings done between 2013 and 2014 is statistically significant (84 from January to August in 2013 vs. 170 in from the same months of 2014), with a p value at <0.01 (p=0.002). There was a statistically significant (at p<0.001) drop in the number of fundus photos and macular OCT' s done in 2014 compared with 2013 (566 from 888, and 279 from 815, respectively).

Conclusions: Any causal relationships the data may represent merits exploration because 1) the procedure implies an increased risk of adverse reactions due to the more invasive FA, compared to less invasive imaging options, 2) it may lend insight into uncovering physician prescribing behavior for diagnostic procedures, and 3) although alleviation of the financial burden associated with FA' s is seemingly in favor of the patient, a continuation of the current trend in prescribing behavior may actually drive the demand for corporate spending up, resulting in an inflated end-of-fiscal-year total expenditure, prompting the PHIC to demand higher contributions from its member patients, thus shifting the burden back to them.

Commercial Relationships: Bryan Jason Atienza, None; Sherman Valero, None

621 - P26-26

En face imaging of Optical Coherence Tomography in a case of choroidal rupture

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Purpose: To clarify the usefulness of en face imaging of optical coherence tomography (OCT) to detect lesions in a case of choroidal rupture.

Methods: En face imagings were performed during the follow up a case of choroidal rupture. The case was a 22-years old man who had a trauma to his right eye. En face image was obtained by OCT (RT vue-100, Optovue, Inc., USA). At the initial examination, fluorescein angiography (FA) and indocyanine green angiography (IA) were performed simultaneously by a scanning laser ophthalmoscope (HRA2, Heidelberg Engineering., Germany).

Results: At the initial examination, his best-corrected visual acuity (BCVA) of right eye was 0.1. By fundus examination, four lesions with subretinal hemorrhage were observed at the macular area including a fovea. At those lesions, background findings showed hypo fluorescence by FA and the blurred findings were observed by IA. There were no hyper fluorescence findings by FA and IA. On en face images between retinal pigment epithelium (RPE) and lamina choloidcapillaris, a crescent-shaped low signal line was observed at each subretinal hemorrhage lesions except for the foveal area.On OCT standard tomographic images, retinal detachment and low signal lesions under the retinal detachment at these four lesions were observed. Furthermore, very fine findings of rupture of RPE and Bruch membrane were observed corresponding to the crescent-shaped lesions detected by en face imaging. With these findings, we diagnosed this case as a choroidal rupture. At one month after the trauma, his BCVA of right eye became 0.2. The subretinal hemorrhages were getting to resolve but still remained. No other findings were observed by fundus examination, although the crescent-shaped low signals at the layer of RPE were observed by en face imaging. At four months after the trauma, his right BCVA was 0.4. The subretinal hemorrhages were resolved. Even by the fundus examination, the crescent-shaped findings were observed, which were corresponding to the en face images.

Conclusions: En face imaging of OCT clearly showed the crescent-shaped lesions with the subretinal hemorrhage in a case of choroidal rupture, which could not be observed by other ophthalmic examinations. En face imaging was useful to detect the lesions and their extent. In addition to other fundus examinations, en face imaging would be useful for the evaluation of diseases.

Commercial Relationships: Tomoko Mase, None; Satoshi Ishiko, None; Tomofumi Tani, None; Kazuhiro Sugawara, None; Akitoshi Yoshida, None

622 - P27-27

Normal Macular Thickness in Healthy Indian Eyes using Spectral-Domain Optical Coherence Tomography

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Purpose: To determine the macular thickness and its variation by age and gender in healthy Indian eyes using Spectral Domain Optical Coherence Tomography (SD-OCT).

Methods: In a prospective, cross-sectional study in a medical college in India, 400 healthy adult subjects (≥18 years) underwent macular cube scanning using Zeiss SD-OCT. Macular thickness from all the 9 regions of the Early Treatment Diabetic Retinopathy Study map was documented for each subject. Variations in macular thickness by age and gender were determined.

Results: The mean age of the subjects was 38.05 ± 12.13 (range 18-78) years. The mean age in males and females were 39.19 ± 12.16 and 37.13 ± 12.05 years respectively (P>0.05). The mean central subfield thickness (CST) of all subjects was 240.40 ± 18.26 µm and mean macular thickness was 287.87 ± 18.07 µm. The CST in right and left eyes were 240.40 ± 18.25 µm and 239.65 ± 17.73 µm respectively (P=0.55). There was strong correlation for all measured values between right and left eyes (P=0.87 for CST). Male gender was associated with greater mean CST and mean macular thickness compared to females (P<0.05). There was association of mean CST with sex (adjusted r^2=0.095, P<0.05) but not with age. Overall, the nasal quadrant was the thickest followed by superior, inferior and temporal subfields.

Conclusions: This is a normative data for macular thickness in healthy Indian eyes using Zeiss SD-OCT. This will serve as a baseline for diagnosing and treating macular pathologies in Indian eyes, since such data using Zeiss SD-OCT in Indian eyes are lacking.

Commercial Relationships: Dr. Tanie Natung, None

623 - P28-28

Experimental visualization and quantification of vitreous contamination following intravitreal injections

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Purpose: To visualize and quantify vitreous contamination following intravitreal injection (IVI) using an experimental vitreous contamination model (EVCM).

Methods: First, we used enucleated porcine eyes as a type1 EVCM. Fluoresbrite carboxylate microspheres (2.5% Solids-Latex) (Polysciences, Inc.) were applied to the conjunctival surface at the site of injection. Then, 0.05ml of saline solution was injected into the porcine eye using 27-, 30-, and 32-gauge (G) needles. The injection procedure was carefully monitored employing an intraocular fiber catheter (AS-611, FiberTech). We repeated this procedure ten times. Second, for experimental quantification, condensed microspheres were applied to an excised sheet of porcine sclera serving as type 2 EVCM. Then, 0.05ml of saline solution was injected from the top of an applied condensed microsphere through the sclera using 27-, 30-, and 32-G needles, and the injected saline solutions were collected as samples. This procedure was also repeated ten times. The fluorescence strength of each sample was measured using fluorophotometry (Multi Label Counter ARVOmx 1420, PerkinElmer). The excitation and emission wavelengths were 485 and 535 nm, respectively. The *Steel-Dwass test* for multiple comparisons was used for statistical analysis.

Results: We visually detected fluorescent microspheres in 10/10 eyes injected with a 27G, 9/10 with a 30G and 9/10 with a 32G needle. In the experimental quantification study, the means of fluorescence strength in the ten samples were, 30.4 ± 8.3 X 10^5 for 27G, 6.17 ± 3.03 X 10^5 for 30G and 2.78 ± 1.72 X 10^5 for 32G (mean ± standard deviation). These values were all significantly higher than that of the control (7.68 X 10^3) (P<0.01). Furthermore, the strength of fluorescence in 27G group was significantly higher than those in the 30 and 32G groups (P < 0.01, P < 0.01, respectively). There was no difference between the 30 and 32G groups.

Conclusions: EVCMs are useful for evaluating vitreous contamination. IVI carries the risk of introducing contamination directly into the eyes even when a 32G needle is used. Furthermore, the 27G needle has a much higher risk of contamination.

Commercial Relationships: Hiroyuki Nakashizuka, Fiber Tech (F); Jun Shoji, None; Akiko Ishimori, None; Mitsuko Yuzawa, None

624 - P29-29

Half-dose Verteporfin Photodynamic Therapy for Aquired Vitelliform Lesions associated with central serous chorioretinopathy

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Purpose: To report the results of half-dose photodynamic therapy (half-dose PDT)for acquired vitelliform lesions (AVLs) associated with chronic central serous chorioretinopathy(CSC).

Methods: Four eyes of 4 patients(3 males and 1 female) with AVLs associated with chronic CSC were received half-dose PDT. The mean age was 59 years(range, 37 to 74years). The patients were followed with best-corrected visual acuity (BCVA), optical coherence tomography (OCT) and autofluorescence after half-dose PDT. The measurement of choroidal thickness using enhanced-depth imaging (EDI)-OCT was performed in 2 of 4 eyes.

Results: The mean follow-up was 19months(range, 6 to 36 months). Serous retinal detachment (SRD) remained in the all eyes except 1 eye which SRD absorbed one year later. Although two of four cases which obtained the data of choroidal thickness showed decreased choroidal thickness after half dose PDT, SRD has no changed. The best corrected visual acuity (BCVA) at final visit has no

changed or slightly decreased.
Conclusions: Half-dose PDT has no effect AVLs associated with CSC. The results were different from the results of half-dose PDT for CSC in the previous reports. In AVLs associated with CSC, the retinal pigment epithelium dysfunction might be underlying primarily, and disturb the function of pumping subretinal fluid to the choroid.
Commercial Relationships: Hajime Onoe, None; Kyoko Fujita, None; Mitsuko Yuzawa, None

625 - P30-30

Bilateral central retinal vein occlusion associated with mantle cell lymphoma

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Purpose: We present our findings in a case of bilateral central retinal vein occlusion that developed in a patient with mantle cell lymphoma.
Methods: A 74-year-old woman developed primary tonsil mantle cell lymphoma in March 2009 and was in complete remission after R-CHOP chemotherapy. In July 2010, she had a recurrence of the lymphoma in the gastric mucous membrane, tonsils, and pharynx but no additional treatment was given. In January 2014, a metastasis to the lymph nodes was detected.
Results: In March 2014, the vision in her right eye was reduced, and two weeks later at the first visit to our hospital, the BCVA declined to 0.01 OD and 1.0 OS . She was found to have a right central retinal vein occlusion with macula edema and left impending central retinal vein occlusion. Although malignant cells were not detected in the cerebrospinal fluid, methotrexate and dexamethasone were injected intraspinally and chemotherapy by bendamusutine was given both prophylactically. One month later, the vision in her left eye decreased, and her BCVA declined to 0.02 OD and 0.03 OS . She was found to have a left central vein occlusion with macula edema. Hematological and systemic evaluations eliminated hypercoagulability and autoimmune disorders. Although MRI and CT showed no central nervous infiltrations, the clinical features suggested that the central retinal vein occlusion was related to the recurrence of the lymphoma. She was treated with an intravitreal injection of anti-VEGF (aflibercept) and panrential photocoagulation. The macula edema improved and neovascular glaucoma was prevented. Her vision did not recover because of the retinal ischemia.
Conclusions: Bilateral central retina vein occlusion can be associated with mantle cell lymphoma although it is extremely rare. Nevertheless, clinicians should be aware of this association because a central retinal vein occlusion is a clinical sign of a relapsing malignant ocular lymphoma.
Commercial Relationships: Nana Furuya, None; Toshiyuki Oshitari, None; Eiju Sato, None; Shuichi Yamamoto, None

626 - P31-31

Comparison of Subfoveal Choroidal Thickness Results according to Photodynamic Therapy Spot Size for Chronic Central Serous Chorioretinopathy

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Purpose: To retrospectively evaluate subfoveal choroidal thickness 1 year after photodynamic therapy (PDT) and determine the correlation between subfoveal choroidal thickness and PDT spot size in patients with chronic central serous chorioretinopathy (CSC).
Methods: Thirty-eight eyes of 36 patients with chronic CSC who had received verteporfin PDT were enrolled in the present study. The laser spot size of 18 patients was chosen to cover the localized hyperfluorescent area on indocyanine green angiography (ICGA) (Group 1). The other 18 patients were treated with PDT to cover wide area including leaking point and detachment of retinal pigment epithelium because the boundary of hyperfluorescent area is unclear (Group 2). The subfoveal choroidal thickness between two groups was measured using enhanced depth imaging OCT before PDT at baseline and months 1, 3, 6, 12.
Results: The mean spot size for PDT was 1944.44 ㎛ in group 1 and 2922.22 ㎛ in group 2. The mean baseline subfoveal choroidal thickness was 341.44 ± 81.62 ㎛ in group 1 and 352.33 ± 108.52 in group 2. There were no significant differences between two groups (p=0.673). The mean subfoveal choroidal thickness decreased significantly to 312.56 ㎛ at 1 month, 293.33 ㎛ at 3 month, 280.72 ㎛ at 6 month and 273.33 ㎛ at 12 month in group 1 (p<0.001, p<0.001, p<0.001 and p<0.001, respectively compared with baseline). In group 2, it decreased significantly to 324.89 ㎛, 305.39 ㎛ , 288.39 ㎛ , 275.89 ㎛ at 1, 3, 6, 12 month (p<0.001, p<0.001, p<0.001 and p<0.001, respectively compared with baseline). There were no significant differences between two groups with regard to subfoveal choroidal thickness according to PDT spot size at 1, 3, 6, 12 month (p=0.696, p=0.719, p=0.839 and p=0.864, respectively).
Conclusions: Verteporfin PDT for CSC resulted in thinner subfoveal choroidal thickness for 1 year significantly. However, there were no significant differences between two groups with regard to subfoveal choroidal thickness according to PDT spot size.
Commercial Relationships: Min Seok Kang, None; Seung-Young Yu, None; Hyung Woo Kwak, None

627 - P32-32

Regional Macular Function, Evaluating Repeatability in Tractional Epiretinal Membranes

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Purpose: To evaluate the repeatability of regional

functional measures calculated from perimetric macular integrity assessment (MAIA-MP) in eyes with tractional epiretinal membrane (tERM).

Methods: 30 patients with tERM underwent MAIA-MP, Snellen visual acuity (VA) measurement, fundus examination and spectral domain optical coherence tomography (SD-OCT) at two time points. Snellen VA was converted to early treatment of diabetic retinopathy study (ETDRS) letters for statistical analysis. Fellow eyes with VA≥70 ETDRS letters and normal macular morphology on SD-OCT (n=25) were recruited as controls.

MAIA-MP threshold maps were overlaid with SD-OCT thickness maps based on an ETDRS grid using ImageJ software. Overall function (AMS) as well as mean perimetric sensitivities and retinal thicknesses defined by the ETDRS grid regions (foveal point, CFS & CTF, central macula, CMS & CMT, inner nasal, INS & INRT, inner superior, ISS & ISRT, inner temporal, ITS & ITRT, inner inferior, IIS & IIRT) were calculated. Pearson's correlation coefficient (r) was used to evaluate repeatability of all measures between visits.

Results: Between visits, there was no significant change in mean VA, CFS, CTF, CMT, INRT, ISRT and IIRT in either group ($p>0.05$ for all). Statistically significant increases in CMS, AMS, INS, ISS, ITS ($p<0.05$ for all) were comparable in both groups ($p>0.05$). Mean ITRT reduced by 9.2um ($p=0.01$) in tERM group only. Table 1 shows correlation (r) values between baseline and follow up visits, which reflects the repeatability of the measurements between two time points.

Conclusions: In healthy controls, the repeatability of some MAIA-MP measures may be higher than VA and comparable to SD-OCT. In tERM eyes, whilst the repeatability of VA and SD-OCT is higher than MAIA-MP. Most regional MAIA-MP measures retain a degree of repeatability comparable to controls. Further evaluation of these relationships is warranted.

Commercial Relationships: Adil Syed, None; Meidong Zhu, None; Andrew Chang, None

Table 1: Correlations (r values) between baseline and follow up results reflecting repeatability of measurements.

	Study Group r value (p value)	Control Group r value (p value)
VA	0.85 (<0.01)*	0.67 (<0.01)*
Perimetric Measures		
- CFS	0.33 (0.34)	0.57 (<0.01)*
- CMS	0.66 (<0.01)*	0.75 (<0.01)*
- AMS	0.70 (<0.01)*	0.79 (<0.01)*
- INS	0.29 (0.12)	0.61 (<0.01)*
- ISS	0.50 (0.01)*	0.56 (0.01)*
- ITS	0.68 (<0.01)*	0.65 (<0.01)*
- IIS	0.70 (<0.01)*	0.71 (<0.01)*
Retinal Thicknesses		
- CTF	0.83 (<0.01)*	0.61 (<0.01)*
- CMT	0.94 (<0.01)*	0.34 (0.11)*
- INRT	0.79 (<0.01)*	0.78 (<0.01)*
- ISRT	0.82 (<0.01)*	0.69 (<0.01)*
- ITRT	0.92 (<0.01)*	0.57 (<0.01)*
- IIRT	0.85 (<0.01)*	0.85 (<0.01)*

Interpretation: Weak, $0.00\leq r<0.40$; Moderate, $0.40\leq r<0.60$; Strong, $0.60\leq r<0.80$; Very Strong, $0.80\leq r\leq 1.00$. * = $p<0.05$, denotes statistically significant correlation.

628 - P33-33

Discrepancy in Fluorescein Angiography and Optical Coherence Tomography Findings of Macular Edema in Intermediate Uveitis

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Purpose: To assess discrepancies between fluorescein angiography (FA) and optical coherence tomography (OCT) findings for macular edema in intermediate uveitis, and to investigate differences in treatment responses according to the discrepant patterns

Methods: Patients were classified into three groups after evaluating macular edema on FA and OCT images. Discrepant results for macular edema detection on either FA or OCT or both were evaluated to calculate discrepancy frequencies. After 6 months of anti-inflammatory treatment, differences in treatment response were analyzed depending on the discrepant patterns.

Results: Discrepant findings for macular edema between FA and OCT were found in 21 (48%) of 44 eyes, 7 eyes (16%) in the FA+/OCT– group, and 14 eyes (32%) in the FA–/OCT+ group. The mean disease duration was significantly longer in FA+/OCT– group than the FA+/OCT+ and FA-/OCT+ groups (32.1 ± 30.8 months vs. 26.1 ± 21.7 and 10.2 ± 12.0 months $p=0.033$). Best corrected visual acuity (BCVA) was significantly improved after treatment in the FA+/OCT+ group, ($p=.01$) but it did not significantly improve in the FA+/OCT– and FA-/OCT+ groups. Central retinal thickness (CRT) was significantly decreased after treatment in FA+/OCT+ and FA–/OCT+ groups ($p=.000$, $p=.029$) but not in the FA+/OCT– group despite treatment

Conclusions: Discrepancy in FA and OCT findings for macular edema was common in patients with intermediate uveitis. Our results suggest that the treatment outcomes may be different according to the discrepant patterns.

Commercial Relationships: Bohyuck Kim, None; Hyeong Gon Yu, None

629 - P34-34

Correlation between OCT findings and visual outcomes after anti-VEGF therapy for RVO

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Purpose: To evaluate correlation between optical coherence tomographic (OCT) findings and visual outcomes after anti-vascular endothelial growth factor (VEGF) therapy for retinal vein occlusion (RVO).

Methods: Fifty-one eyes of 51 patients (mean age of 67 years) with macular edema secondary to central or branch retinal vein occlusion who underwent intravitreal injections of 2.5mg bevacizumab or 0.5mg ranibizumab were studied. Measurement of the best-corrected visual acuity (BCVA), spectral-domain OCT (SD-OCT) and microperimetry were performed before and 1, 3, and 6 months after the treatment. Foveal thickness, total

macular volume, and presence of external limiting membrane (ELM), photoreceptor inner/outer segment junction (IS/OS) and foveal bulge on the SD-OCT images were examined.
Results: The mean follow-up period was 9.9 ± 5.7months. In 40 of the 51 eyes, a complete resolution of macular edema was achieved 1.6 ± 0.9 months after the treatment. The other 11 eyes did not have a resolution of macular edema. The foveal thickness and total macular volume before the treatment were significantly correlated with the BCVA and retinal sensitivity 6 months after the treatment and the final BCVA ($P < 0.005$). Presence of ELM, IS/OS and foveal bulge at the time of resolution of the macular edema was significantly correlated with the BCVA and retinal sensitivity at 6 months and the final BCVA ($P < 0.05$).
Conclusions: Eyes with the severe macular edema before the treatment or with outer retinal impairment at the time of resolution of macular edema had worse visual prognosis after anti-VEGF therapy for RVO.
Commercial Relationships: Akiko Mino, None; Yoshinori Mitamura, None; Takashi Katome, None; Takeshi Naito, None

630 - P35-35

Role of scleral buckling in addition to vitrectomy for the treatment of rhegmatogenous retinal detachment, systematic review and meta-analysis

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Purpose: The role of scleral buckling (SB) in addition to pars plana vitrectomy (PPV) for the treatment of rhegmatogenous retinal detachment (RRD) has been studied, but yet remained unclear to date. We aimed to elucidate the issue through systematic review of literature and meta-analysis.
Methods: MEDLINE, Embase, and Cochrane CENTRAL have been systematically searched. Eligible are studies comparing surgical outcomes between PPV and PPV with SB for the treatment of rhegmatogenous retinal detachment. The outcome measures were primary reattachment, final reattachment, and postoperative complications. Meta-analysis was performed using a random effect model, and pooled odds-ratio (OR) with 95% confidence interval (CI) was calculated for each outcome measure.
Results: Six studies with 1432 subjects were included for analysis. Primary reattachment rate was significantly higher in PPV+SB compared to PPV alone group (OR 1.69; 95% CI 1.15–2.48, $P = 0.007$). Final reattachment was achieved in 328 out of 329 subjects in PPV+SB group and 293 out of 294 subjects in PPV alone group. Postoperative epiretinal membrane was more frequently observed in PPV+SB group (OR 1.72; 95% CI 1.23–2.38, $P = 0.001$). Statistically non-significant increase was observed in PPV+SB group for the frequency of postoperative elevation of intraocular pressure/glaucoma (OR 2.59; 95% CI 0.69–9.73, $P = 0.16$) and postoperative cystoid macular edema (OR 3.31; 95% CI 0.96–11.38, $P = 0.06$).
Conclusions: Use of SB adjunct to PPV is considered helpful to achieve higher primary reattachment rate. The tendency of higher postoperative complication rate in PPV+SB compared to PPV alone needs to be carefully interpreted due to non-randomized nature of the included studies.
Commercial Relationships: Takashi Ueta, None; Kiyohito Totsuka, None; Murilo Roggia, None; Hiroko Inui, None; Yasuo Noda, None

631 - P36-36

Peripheral Prosthetic Vision in the Inferior Visual Field as a Means to Improve Mobility Performance

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Purpose: Retinitis pigmentosa (RP), a degenerative disorder of the retina, is characterized by progressive visual field (VF) constriction from the periphery to the center. Current retinal prosthesis designs for therapeutic treatment of late RP seek to restore vision by eliciting a limited number of artificial percepts, called phosphenes, densely arranged in the central VF. While phosphene density determines visual acuity, the size of the VF is a dominant factor in mobility. We therefore propose novel device designs to expand the given phosphene count into inferior and lateral VF areas. The efficacy of phosphene layouts was evaluated using simulated prosthetic vision (SPV).
Methods: SPV was presented to eight normally sighted subjects on a head-mounted display (HMD). 100 phosphenes were arranged in the VF to either provide a relatively dense but narrow field (high-acuity) or a sparse but wide field of view (wide-angle). Subjects were asked to navigate pedestrian scenarios in a photorealistic virtual obstacle course. Tasks included low-lying obstacle avoidance, path following, and stationary and moving pedestrian avoidance. Subject motion and number of collisions were recorded and analyzed to quantify potential benefits of peripheral phosphenes on mobility performance.
Results: Subjects were able to successfully navigate with the peripheral phosphene vision provided via the HMD. Movement speed increased, and both task time and distance walked were reduced using high-acuity phosphene vision. Conversely, a wide-angle VF facilitated obstacle avoidance. Wide-angle phosphene vision proved to be more useful in spacious environments, while high-acuity vision yielded better results in confined spaces such as alleyways. Discerning fine scene details like wall-floor boundaries and marking lines as visual cues for navigation required a dense phosphene field.
Conclusions: Peripheral prosthetic vision in the inferior and lateral VF allows for successful mobility in virtual outdoor tasks. In future designs, redistributing electrodes responsible for producing central perception to the retinal periphery might benefit implant recipients. As to whether

or not it is advisable to relocate a portion of or the whole electrode array remains to be evaluated. Future research should compare task-dependent performance of central vs. peripheral phosphene vision, as well as hybrid grids spanning a larger VF area.
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632 - P37-37

Autosomal dominant cone-rod dystrophy with GUCY2D gene mutations in three Japanese families

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Study Group: Department of Ophthalmology, Juntendo University Graduate School of Medicine

Purpose: Cone-rod dystrophies (CRDs) are a group of inherited eye disorders that affect both the cone and rod cells of the retina. There were ten causative genes of autosomal dominant (ad) CRDs. Here we report on guanylate cyclase 2D (*GUCY2D*) and RAB28, member RAS oncogene family (*RAB28*) gene analysis eighteen subjects in fifteen families suspected adCRD.
Methods: Genomic DNA was extracted from peripheral blood leukocytes of the eighteen subjects according to standard procedures, after obtaining informed consent. We amplified the coding regions of *GUCY2D* and *RAB28* genes from the genomic DNA of the patients by PCR, while dye terminator method was used for sequencing. The sequence data were referred to dbSNP and HGMD. The amino acid substitutions were analyzed with PolyPhen-2 and *SIFT* for prediction software of functional effects of GUCY2D and RAB28 proteins.
Results: Two heterozygous missense mutations p.R838H and p.R838C of *GUCY2D* were detected in three families . These mutations were considered to be the causative mutations of our adCRD families from the results of "pathogenic" with PolyPhen-2, SIFT and other report (Ito et al. 2004). *GUCY2D* gene Arg838 was reported as a mutation hotspot related to adCRD (Kohl et al. 2010).
Conclusions: Identification of causative gene mutations of adCRD is often difficult with genetic heterogeneity and clinical heterogeneity. Both complete ophthalmologic examination and steady gene analysis may be required for mutation detection.
Commercial Relationships: Takuro Fujimaki, None; Ai Yanagawa, None; Keiko Fujiki, None; Akira Murakami, None
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633 - P38-38

Attempt neonatal fundus blood flow measurement using a Laser speckle flowgraphy (LSFG)

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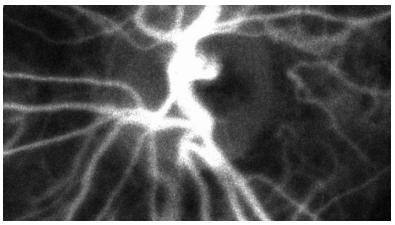
Purpose: Retinopathy of prematurity (ROP) is thought to accompany ocular circulation disturbances. Laser speckle flowgraphy (LSFG) is used to measure ocular blood flow in adults, but there have been no reports of using this diagnostic technique for neonate. We used LSFG-NAVI that was modified for use with the subject in the spine position to measure retinal blood flow in neonate and investigated whether it could be used for cases of ROP in the future.
Methods: The test was performed on a neonate referred to the ophthalmology department for examination with the consent of the parents. The neonate was 36 weeks of gestation with a body weight of 2,300 g, and was placed outside the incubator for the examination. After mydriasis, the eyelids of the sleeping infant were opened, and care was taken not to place any pressure on the eyeball. The infant was carefully placed close to a LSFG-NAVI for use in the spine position, and imaging was conducted for 4 s with the optic nerve head as a reference. Heart rate was 152 beats/min during the test.
Results: Three physicians oversaw the infant' s fixation, opening of eyelids, and imaging. No eyelid speculum was used, and ocular blood flow could be measured while the infant was resting. The test was completed in approximately 10 min. Although the acquired images were slightly defocused, it was possible to analyze blood flow volume parameters and pressure waveform by placing a rubber band over the entire optic nerve head region.
Conclusions: Despite the fact that in this case, body movement and poor vision fixation made imaging difficult in contrast to an adult patient, we were able to conduct imaging. The images may have been defocused because of differences in imaging conditions such as different axial length and refraction. Other possible issues faced during imaging procedures of premature infants include time restrictions for the infant being outside of the incubator and test duration, optic media opacity, and blepharophimosis. We believe that further modification of the LSFG-NAVI for use in the spine position could bring us one step closer to clarifying ocular hemodynamics in ROP.
Commercial Relationships: Tadashi Matsumoto, None; Yukinobu Okajima, None; Yuichi Hori, None


36w neonatal fundus blood flow

634 - P39-39

Parental Awareness of the Need for ROP Screening of Premature Infants in North China

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Purpose: To analyze the level of informed and awareness about retinopathy of prematurity (ROP) among parents in North China.

Methods: The questionnaire was given to 230 parents known to be involved in the treatment of ROP infants and collected in person between January and April 2013 in the People Eye Center, People's Hospital of Peking University, which is one of the most prestigious ROP referral eye center in North China.

Results: In all, 221/230 (96.1%) were returned. 73 (33.0%) ROP patients were female, 69 (31.2%) were twins, and 3 (1.4%) were triplets. The mean GA was 30.3 ± 2.2 weeks. The mean BW was 1524.5 ± 1.0 g. Of the 221 completed questionnaires, 128 (57.9%) premature infants received screening during pediatric hospital stays. 100 (45.2%) premature have more than 6 weeks stays in pediatric hospital, of which 33 (33.0%) did not receive any screening during hospital stays. 208 (94.1%) parents were informed and received recommendation for screening by pediatricians, 13(5.9%) did not receive any recommendation for screening. Only 159 (71.9%) parents were aware of ROP, while 62 (28.1%) were not aware of the disease. Since ROP stage 4, 5 demonstrated poor prognoses, we found whether parents were informed by pediatricians were closely associated with this (P<0.001). However, we found no association between the grade of hospital and whether the parents were informed (P=0.625).

Conclusions: The awareness among parents and pediatricians in North China about ROP still need to improve. Better communication, educations and promotion from the pediatricians and ophthalmologists, timely informed the parents will help.

Commercial Relationships: Jing Feng, None; Yi Chen, None; Xiaoxin Li, None

635 - P40-40

Characteristics and outcomes of fall-related open-globe injuries in pseudophakic patients

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Purpose: To describe the demographic and clinical characteristics of fall-related open globe (OG) injuries in pseudophakic patients and to identify prognostic indicators of poor visual and anatomic outcome.

Methods: Charts of 206 consecutivepatients who presented to the Yale-New Haven Hospitalwith open globe injury between January 1, 2003, and December 31, 2013 were reviewed. Of these, 26 pseudophakic patients with fall-related OG injury were identified for inclusion in the series. Demographics, ophthalmic history, circumstances of injury, initial best-corrected visual acuity (BCVA), ocular exam findings at the time of injury, surgical interventions, follow-up BCVA, and ocular exam findings were tabulated.

Results: Nineteen patients (73%) were women. Mean age (± SD) was 80.6 ± 4.6 years (range 61-97). Falls occurred predominantly indoors and at home. BCVA at time of injury was ≤ 20/200 in all patients and was < 20/400 in 24 of 25 patients (96%). Mean ocular trauma score(OTS) and wound size were 38.54 ± 10.95 and 9.81mm ± 7.20, respectively. OTS was lower (p = 0.0017, <0.0001, 0.0240) and wound size was larger (p = 0.0440, 0.0145, 0.0026) in patients with final BCVA <20/40, <20/400, and phthisis at final follow-up; compared to patients with BCVA ≥ 20/40, 20/400, and no phthisis at final follow-up, respectively. Final BCVA < 20/400 was associated with 360 ° subconjunctival hemorrhage (SCH), retinal detachment and proliferative vitreoretinopathy (p = 0.0498, 0.0181, 0.0310). Total hyphema, intraocular lens (IOL) damage, and IOL expulsion were associated with needing multiple surgical interventions (p = 0.0345, <0.0001, 0.0023).

Conclusions: Fall-related OG injuries in pseudophakic patients result in profound visual disability with damage to multiple ocular structures. Large wound size, low OTS, 360° SCH, total hyphema, posterior injury, and IOL damage are common findings that are also prognostic of poor visual and anatomic outcome.

Commercial Relationships: Shaheen Kavoussi, None; Martin Slade, None; Seth Meskin, None; Ron Adelman, None

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636 - P41-41

Evisceration Or Reconstruction In Case Of Globe Rupture With No Light Perception

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Study Group: nil

Purpose: Evisceration is performed only when the eye is completely destroyed and cannot be reconstructed anatomically or there is no hope for any vision (i.e., most of globe's contents have been lost).

Methods: A 40 year old male presented with loss of vision in his right eye after an injury with bamboo stick. He developed supra orbital laceration and sudden loss of vision. He went to the eye hospital where primary suturing of supraorbital laceration was done and was advised evisceration. He was given similar advice at 2 other hospitals. As he didn't want to lose his eye at any cost, he came to our hospital for the third opinion.

On examination there was no light perception in the right eye.There was lid ecchymosis, conjunctival congestion and chemosis. He had hyphema with flat anterior chamber making examination of the posterior segment difficult.

Investigations done CT scan showed distortedand proptotic globe with 10-15 mm focus of vitreous bleed. **B Scan** showed posterior dislocation of lens with dense vitreous haemorrhage in posterior vitreous cavity. Incarceration of vitreous was present inferiorly.**Large scleral tear superonasally and superotemporally extending up to optic disc posteriorly**.

He was planned for right eye primary scleral tear

repair with Intravitreal antibiotics.
He was followed closely and after ten days following the surgery, lid swelling was decreased, extra ocular movements were full. Anterior chamber was formed with organized hyphema and mild corneal edema.
Plan was not stopped there. He was planned for 23G Pars plana vitrectomy + Lensectomy using anterior chamber maintainer with PFCL + Endolaser + FGE + Densiron injection. Intra operatively superior retina was incarcerated in scleral wound and inferior giant retinal tear was noted which was repaired**.**
Results: On the first postoperative day retina was looking attached with perception of light present and projection of rays present in superior, temporal and nasal quadrant.
Conclusions: Evisceration is performed only when the eye is completely destroyed and cannot be reconstructed anatomically or there is no hope for any vision (i.e., most of globe' s contents have been lost)1. Though there is risk of sympathetic ophthalmia but preservation of the globe may have positive psychological impacts on the patient and his relatives.
Commercial Relationships: Sundaram Natarajan, None; Arun Pandey, None; Gauri Khare, None

637 - P42-42

The application of pulsed water jets in vitreoretinal surgery and relevant retinal findings

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Purpose: To report on an intraocular piezoelectric actuator-driven pulsed water jet (ADPJ) system and results of the experimental application of ADPJ retinal massage.
Methods: We determined the voltage at which ADPJ could optimally induce intravascular displacement of the blood column (DBC) through massage of the outer retinal vessels, while not causing retinal complications such as tearing or hemorrhage. After the application of ADPJ massage, we measured retinal thickness near the massaged vessels using optical coherence tomography (OCT) on days 1, 3 and 7 (n =4). Finally, we enucleated the eyes and evaluated the pathological condition of the retinas.
Results: We found that the minimum voltage to achieve intravascular DBC without causing retinal tearing or hemorrhage was 40 V. At this voltage, although mild retinal swelling occurred in the inner retinal layer near the massaged vessels, there was no swelling in the outer retinal layer. Furthermore, this retinal swelling resolved within 1 week (day 1, 116%, day 3, 107%, and day 7, 102%). ADPJ massage at voltages greater than 60 V caused retinal tearing and hemorrhage.
Conclusions: Though retinal swelling occurred in the inner retinal layer after ADPJ massage at 40 V, ADPJ massage at 40 V successfully induced DBC without causing irreversible retinal complications. ADPJ at an appropriate power level therefore holds promise as a safe instrument for the intraocular surgical treatment of retinal vascular diseases.
Commercial Relationships: Hiroshi Kunikata, None; Yuji Tanaka, None; Naoko Aizawa, None; Atsuhiro Nakagawa, None; Teiji Tominaga, None; Toru Nakazawa, None
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638 - P43-43

In Vivo Emulsification Analysis of 1000 and 5000 Silicone Oil After Retinal Detachment Surgery

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Purpose: To compare in vivo emulsification of silicone oil 1000 and 5000 centistokes (cSt) after retinal detachment surgery
Methods: Silicone oils from patients who underwent retinal detachment surgery were investigated using Spectrophotometer UV-Vis. FTIR was used to determine functional groups of silicone oil. Spectroscopically changes beetween original and after use silicon was compared for 100cSt and 5000 cSt Silicon oil. The phisically changes also corelated with the duration of tamponade, inflammation factors, patients age and severity of retinal detachment
Results: The absobance 1000 cSt Silikon group was higher than 5000 cSt group. The transmitance was lower on 1000 cSt. On 770 nm wave lenght spectrophotometry, there was statitical significance different on emulsification beetween 1000 cSt and 5000 cSt
Conclusions: In vivo emulsification was higher in silicone oil 1000 compared to 5000 cSt silicone oil after vitreoretinal tamponade 4-16 weeks due to retinal deatchment surgery. Emulsification related to higher absorbance and lower transmitance in 1000 cSt silicone oil. Inflammation may became a factor affecting this condition
Commercial Relationships: Arief Kartasasmita, None; Widi Astuti, None; Rova Virgana, None; Risdi Risdiana, None

639 - P44-44

Influences of 27-gauge transconjunctival sutureless vitorectomy on postoperative corneal topographic conditions

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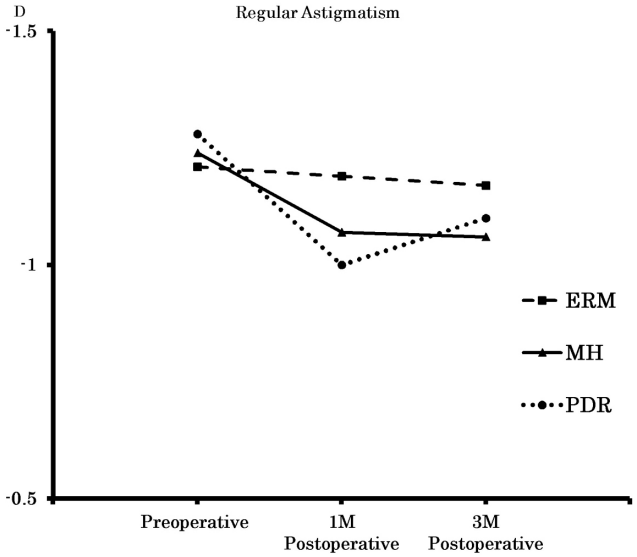
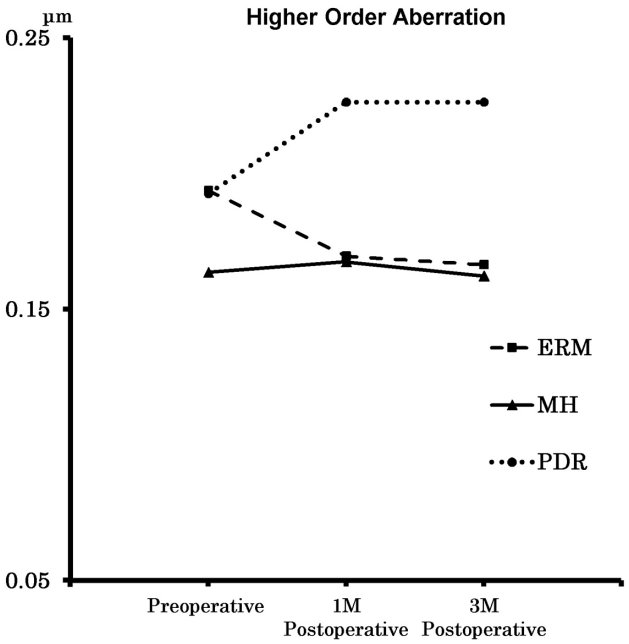
Purpose: To evaluate the influences of 27-gauge transconjunctival sutureless vitorectomy (TSV) on postoperative corneal topographic conditions.

Methods: Fifty three eyes of 53 patients undergoing 27-gauge TSV were retrospectively studied. 22 eyes with epiretinal membrane (ERM), 23 eyes with macular hole (MH), and 8 eyes with proliferative diabetic retinopathy (PDR) were included. Corneal topography was obtained with wave-front analyzer (KR-1W®, Topcon) preoperatively and at 1 and 3 months postoperatively. The corneal topographic parameters were the average corneal power (CP), regular astigmatism (RA), spherical aberration (SA), and higher order aberration (HOA).
Results: All of the 4 corneal topographic parameters (CA, RA, SA, and HOA) remained stable up to 3 months after the surgery (P > 0.5 for all parameters). There was no significant difference in the RA between the gas tamponade group (ERM) and the non-gas tampnodane group (MH) at 1 month after the surgery (-1.07 ± 0.52 D vs -1.12 ± 0.36 D, P = 0.82, Unpaired t-test). There were also no significant differences between the two groups in regard to the other 3 parameters. When compared between macular diseases (MH/ERM) and other diseases (PDR) a1 month after the surgery, no significant difference were observed for all parameters, including HOA (0.17 ± 0.07 μm / 0.17 ± 0.05 μm vs 0.22 ± 0.11 μm, P = 0.21 and P =0.20, respectively, Unpaired t-test).
Conclusions: Corneal topographic conditions were not significantly affected by 27-guage TSV.
Commercial Relationships: Takafumi Hirashima, None; Yohei Satou, None; Takeshi Moriya, None; Takao Utsumi, None; Miou Hirose, None; Hideyasu Oh, None



In each of ERM group, MH group and PDR group, there were no significant changes in the regular astigmatism at both 1 and 3 months after the surgery, compared with preoperative levels



In each of ERM group, MH group and PDR group, there were no significant changes in the higher order aberration at both 1 and 3 months after the surgery, compared with preoperative levels

640 - P45-45

Novel surgical technique for the treatment of the massive subretinal hemorrhage

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Purpose: The intravitreal gas injection with or without the tissue plasminogen activator (tPA) is the standard procedure for the treatment of the massive subretinal hemorrhage (SRH), but it takes time to displace SRH. We report the efficacy of the new surgical technique using the subretinal injection of tPA and air for the treatment of SRH.
Methods: A retrospective, non-randomized consecutive case series included 4 eyes in 4 patients with the massive SRH (mean age, 74.8 ± 5.4 years old; 2 male and 2 female; the mean follow-up, 91.3 ± 11.3 days; 2 eyes with micro aneurythms and 2 eyes with age-related macular degeneration). Main outcome included the time from the displacement of SRH, the mean best-corrected visual acuity (BCVA), the retinal thickness at the fovea and the complications related to the surgery.
Results: SRH displacement was achieved in all eyes (100%). SRH was displaced from the fovea within an average duration of 2 ± 1.2 days postoperatively. The mean BCVA improved significantly from 0.02 preoperatively to 0.07 and 0.2 at 1 and 3 months after the surgery respectively (P <0.05). The mean retinal thickness at the fovea improved from 1639 ± 202 μm preoperatively, to 413 ± 316 μm at 1 month (P <0.05). There was no intraoperative complications. The postoperative complication included one case with macular hole (MH) and one case with retinal pigment epithelium tear. MH closed after the additional gas injection.

Conclusions: This novel technique using the subretinal injection of tPA and air achieved the quick displacement of SRH and visual recovery. Further study is needed to clarify the safety and the long-term outcome.
Commercial Relationships: Takahiro Sogawa, None; Kaori Sayanagi, None; Yasushi Ikuno, None; Noriyasu Hashida, None; Chikako Hara, None; Yoko Fukushima, None; Miki Sawa, None; Kohji Nishida, None

641 - P46-46

The comparison of surgical outcome of idiopathic epiretinal membrane using ICG(IndoCyanine Green) or BBG(Brilliant Blue G)

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Purpose: To examine the difference of outcomes after the vitrectomy using ICG or BBG for ILM peeling.
Methods: Forty-one cases performed the vitrectomy with ILM peeling [ICG 9 cases ; BBG 32 cases] at Juntendo University Nerima Hospital from June 2008 to July 2014 were retrospectively studied. Visual acuity, foveal thickness, GCIPL (Ganglion Cell + Inner Plexiform Layer) and the frequency of complications after the vitrectomy with ILM peeling using ICG or BBG, were retrospectively compared.
Results: The average preoperative visual acuity(logMAR) improved from 0.78(± 0.21) to 0.93 and from 0.66(± 0.23) to 0.87(± 0.21) in ICG cases and BBG cases respectively. However, there was no significant difference between the two gropus (p=0.72). The average postoperative foveal thickness were 366.7(± 70.2) μm and 393.5 (± 60.2) μm in ICG cases and BBG cases respectively. There was no significant difference between the two gropus (p=0.34). The average postoperative GCIPL were 45.0(± 17.1) μm and 45.9 (± 19.2) μm in ICG cases and BBG cases respectively. There was no significant difference between the two gropus (p=0.85). Postoperative complications were as follows. Transient vitrous hemorrhage; 3 eyes and 4 eyes in ICG cases and BBG cases respectively. Transient macula edema; one eye in each case.
Conclusions: There seems to be no difference of outcomes after the vitrectomy with ILM peeling between the use of ICG and the use of BBG in epiretinal membrane.
Commercial Relationships: Atsuhide Takesue, None; Kouki Kanbayashi, None; Takahide Suzuki, None; Toshiyuki Yokoyama, None

642 - P47-47

Prediction of spontaneous closure and visual outcome in traumatic macular hole with spectral domain optical coherence tomography

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Purpose: To investigate the nature history of traumatic macular hole, and the factors predicting the anatomical and functional outcomes.
Methods: In this retrospective study, patients with traumatic macular hole and followed up for at least 3 month without surgery were included. Retinal thickness, diameters of hole and base were measured by spectral domain optical coherence tomography (SD-OCT), which also identified conditions such as retinal atrophy, retinal detachment, intraretinal cyst, vitreous traction The relationship between the baseline clinical and tomographic factors with spontaneous closure and final best-corrected visual acuity (BCVA) were analyzed.
Results: Among 22 traumatic macular holes, 8 (36.4%) closed spontaneously. The holes with spontaneous closure had smaller size (median 234.5 vs. 401 um, p=0.013) and less intraretinal cyst (12.5% vs. 78.6%, p=0.006) compared to holes that did not close. The final BCVA was marginally associated with base diameter (r=0.468, p=0.028) and initial BCVA (r=0.526, p=0.012). Multivariate regression analysis showed that the intraretinal cyst and base diameter were independent predictive factors for anatomical and functional outcomes respectively.
Conclusions: Absence of intraretinal cyst predicted the spontaneous closure of traumatic macular hole, and small base diameter predicted better visual outcome.
Commercial Relationships: Haoyu Chen, None; Weiqi Chen, None; Kangkeng Zheng, None
Support: This study was supported by the National Nature Science Foundation of China (30901646 and 81170853), Guangdong Science and Technology Project (2011B031300013)

643 - P48-48

Unexplained entitiy of CNVM following VR surgery

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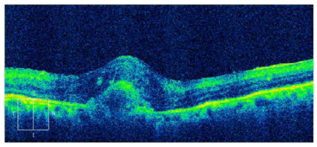
Purpose: Very little is found in literature about the CNVM occurring in patients following vitrectomy for macular pathology (hole and ERM). No cases have been reported till date on CNVM post vitrectomy for Retinal detachment. We hereby report two cases of Classical subfoveal CNVM occurring following VR surgery in retinal detachment patients.
Methods: With retrospective analysis of databases we at our center operated 800 (300 macular surgeries) VR surgeries over period of 3 yrs. from 2011 to 2013. All cases operated by a single surgeon with 3 port 23-gauge vitrectomy with settings of 5000 cut rate, 50 to 300 vacuum with endo illuminator (LED white light).
As tamponad silicon oil / C3F8 gas on choice depending on case basis was used. Endolaser is used with no laser being applied in the macular area. The average time taken for the surgeries 1 1/2 hours (1-2 hrs.)
Post operatively patients are followed up on first week, third week, 1st month and monthly till the first year.
The two cases reported here met the same surgical protocol. Silicon oil used as tamponad, was removed in both cases after 8 months. Post SOR the patients who had stable vision and normal fundus, presented with sudden diminution of vision in the operated eye.

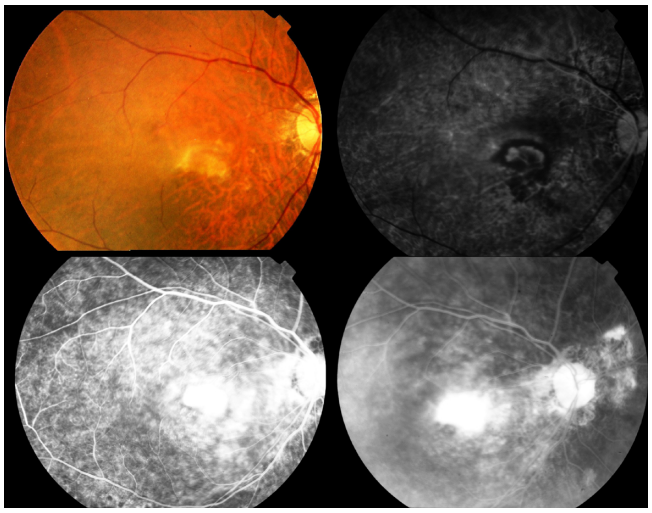
Pre operatively both the patients were mild myopic (-2 and -3D) with no RPE changes, no myopic macular changes and no ARMD changes.

Results: With clinical examination a diagnosis of CNVM was made which was confirmed with Fundus angiography (classical) and OCT (Sub foveal). With no ARMD / Myopic macular degenration changes pre operatively the diagnosis of Post VR CNVM was made. Both patients were treated with anti VEGF injections 3 doses every month for 3 months with completed regression of CNVM with formation of scar and a stable vision in subsequent follow up.

Conclusions: The probable hypothesis to the etiology behind the diagnosis would be the inflammatory process following surgical intervention might have contributed to the additional oxidative stress to the neurosensory retina and that a preexisting surgically induced abnormal RPE activation (subclinical) resulted in an unfamiliar VEGF up regulation which may explain the expected response to anti-VEGF treatment. Our patients being a mild myopic with slight thinned retina as visible on fundus photo might be susceptible for photo toxicity. Further research is needed to evaluate the possible mechanisms and risk factors that lead to formation of CNVM after VR surgery.

Commercial Relationships: Krishna Nagaradh, None; Chandra Kumar H.V, None; Naveen K, None; Divya Chandran, None; Jyothsna Rajagopal, None





Eye Movements/Strabismus/Amblyopia/Neuro-ophthamology - Poster

644 - P49-1

A new anti-suppression approach improves the contrast sensitivity and visual evoked potential in anisometropic amblyopia

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Purpose: To evaluate the efficacy of Eyetronix Flicker Glasses therapy in contrast sensitivity and visual evoked potential in anisometropic amblyopia through breaking the interocular suppression and promoting the binocular fusion.

Methods: Thirty subjects with anisometropic amblyopia aged from 7 to 13 were enrolled in our study. A novel stimulus of Eyetronix Flicker Glasses, which was preprogrammed to alternatively close and open each eye at the temporal frequency of 7 Hz, was used in our study. Visual acuity, contrast sensitivity of 3, 6, 12 and 18 cycles per degree spatial frequencies and binocular function were measured at the baseline and each follow-up visiting to assess the improvement of amblyopia. In addition, pattern-reversal visual evoked potential was also used to record the activity of visual cortex before and after the treatment.

Results: After 3 months anti-suppression treatment, the visual acuity significantly improved from 0.42 to 0.60. The contrast sensitivity of 3, 6, 12 and 18 cycles per degree spatial frequencies showed a significant improvement at the second, third, forth and last follow-up visiting. The mean stereoacuity had a significant improvement from 248 seconds to 84 seconds. The stereoacuity of 11 children had recovered to 60 seconds. In addition, there is a decreased P100 latency and increased N75-P100 amplitude of the visual evoked potential after the treatment.

Conclusions: EyeTronix Flicker Glasses could provide an alternative method for amblyopia treatment. It is effective in improving monocular and binocular function of amblyopia. Additionally, it may have an effect on the recovery of the visual cortex of amblyopia.

Commercial Relationships: Ying Yuan, EyeTronix, Inc. (F); Jiangnan He, None; Min Li, EyeTronix, Inc (F); Huiqin Cheng, EyeTronix, Inc (F); Bilian Ke, EyeTronix, Inc (F)

Support: This work was supported by the National Natural Science Foundation of China (Grant No.81200713), the foundation of Interdisciplinary Research of "Medical and Engineering or Science" of Shanghai Jiaotong University (Grant No.YG2011MS62), the Natural Science Foundation of Shanghai, China (Grant No.09ZR1425400), the Hospital Training Plan of Medical Talent in 2012 (12RC06) and Shanghai Key Laboratory of Fundus Disease.

Clinical Trail: 2013-010

645 - P50-2

Objective assessment of visual functions in individuals with severe motor and intellectual disabilities

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Purpose: It has been reported that evaluation of visual functions is quite difficult in individuals with severe motor and intellectual disabilities (SMID) because of their poor responsiveness. To assess how they utilize visual input effectively, we attempt to record optokinetic nystagmus (OKN) by using a non-contact eye tracker.

Methods: Twenty-two persons with SMID at the institute for developmental disabilities were examined. OKN responses were recorded binocularly at a sample rate of 300 Hz. To enhance accuracy in calibration of gaze position, attractive cartoon TV program, subtending 6.0 x 3.8 deg, was presented on the monitor as fixation targets. The OKN stimuli comprised of a square wave grating at a linear velocity of 5 deg/sec with spatial frequency of 0.3 cycle/deg and move rightward or leftward direction. The duration of the stimulus was 5 sec, and there was interval of 5 sec between each test stimulus. Interval was considered as a control. The velocities were computed using differential processing from the data of acquired eye movement, and those of 100 deg/sec or less were extracted. The gain of OKN was calculated using only for data that showed a statistically significant difference in velocity between slow-phase OKN and control findings (the Mann-Whitney test).

Results: There were significant differences between slow-phase OKN and control findings in all persons. Figure 1 shows the distribution of slow-phase OKN. The mean of the gain for rightward, leftward was 0.30 and 0.32, respectively.

Conclusions: OKN is a visually driven eye movement for stabilizing retinal image against moving field. The results suggest that OKN analysis using an eye tracker is non-invasive and is benefit for objective assessment of visual functions in persons with SMID.

Commercial Relationships: Kenji Suzuki, None; Takahiro Niida, None; Yuma Shinomiya, None; Hitoshi Uchiyama, None; Yuko Komachi, None; Hiroshi Mochizuki, None

Support: Grant-in-Aid no.22330260 for Scientific Research from Japan Society for the Promotion of Science.

Figure1

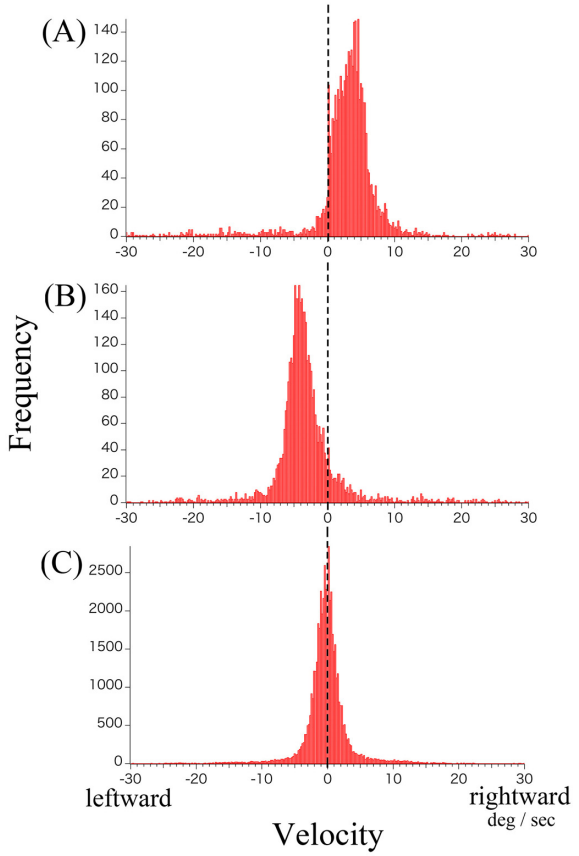

Figure 1: An example of the representative data. The histograms show the distribution of slow-phase velocity at an interval of 0.2deg/sec. Both histograms of rightward (A) and leftward direction (B) exhibit a peak of about±5 deg/sec, whereas histogram of control (C) exhibits a peak of about 0 deg/sec.

646 - P51-3

Dynamics of Distractor and Target Spatial Averaging in the Global Effect of Saccades

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Purpose: In the global effect, saccades are displaced towards a distractor that is near in location to the target, an effect that is thought to reflect neural averaging in the superior colliculus. The temporal profile of this averaging process has not yet been investigated, however. We studied how the global effect varied with the degree of temporal dissociation between distractor and target appearance.

Methods: 12 subjects performed the experiment in which there were three distractor conditions. In *no-distractor* condition, there was no distractor, and the target was flashed for 100ms at 19 ° eccentricity on the horizontal meridian on either the left or right side randomly. In *upper distractor* condition, a distractor appeared at 19° visual angle eccentricity but displaced upwards by 20° of polar angle from the horizontal meridian. In *lower distractor* condition, a distractor appeared at 19° visual angle eccentricity but displaced 20° downwards in polar angle. The distractor was presented on either the left or right side randomly. The distractor was either flashed for 100ms (*flashed distractor*) or continuously present till the end of trial (*persistent distractor*). On distractor trials, we introduced a temporal offset between the distractor and target appearance, ranging from 0ms (simultaneous onset) to 800ms. The target was flashed for 100ms at 19° eccentricity on the horizontal meridian, on the same side as the distractor. We analyzed saccade amplitude data in terms of temporal offsets, distractor type (flashed versus persistent) and saccade latencies.

Results: Our results showed that the global effect in presence of temporally offset distractor and target depended on the offset, the saccade latency, and the distractor type. The global effect decreased significantly with 100ms of offset between the distractor and target, but was still evident. The global effect was stronger when the distractor was continuously present throughout the trial. The global effect occurred only in saccades with latencies between 150 and 350ms.

Conclusions: Our results demonstrate that the global effect can occur despite separation of the distractor and target in time, suggesting that there is substantial persistence of distractor-related activity that is available for spatial averaging in a putative neural structure such as the superior colliculus.

Commercial Relationships: Woo Young Choi, None; Jayalakshmi Viswanathan, None; Jason Barton, None

Support: CIHR grant MOP-81270, Canada Research Chair and Marianne Koerner Chair in Brain Diseases (JB)

647 - P52-4

Evaluation of macular inner retinal layer in optic tract syndrome using swept-source optical coherence tomography

Katsutoshi Goto [1, 2] Atsushi Miki [1, 3] Tsutomu Yamashita [1, 3] Syunsuke Araki [1] Go Takizawa [1] Kenichi Mizukawa [1] Junichi Kiryu [1]

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Purpose: To report an evaluation of macular inner retinal layer using swept-source optical coherence tomography (SS-OCT) in patients with homonymous hemianopia due to optic tract syndrome.

Methods: Fourteen eyes of 7 patients with homonymous hemianopia due to optic tract syndrome. The duration of the lesions from onset ranged from 4 to 324 months. The age-matched control group was composed of 30 eyes of 30 normal subjects. All patients underwent an ophthalmic examination including SS-OCT (DRI OCT-1 Atlantis®). SS-OCT examination was performed on the same day of the visual field test. The scanning protocol used was macula 3D scan pattern with a scan density of 512 × 256 covering 12 × 9 mm area. The 4 × 3 mm area around the fovea was divided into as follows, 4 quadrants; superotemporal (ST), inferotemporal (IT), superonasal (SN), inferonasal (IN)

and 2 hemiretina; temporal (T), nasal (N). The macular inner retinal layer thickness was defined as the macular retinal nerve fiber layer (RNFL), ganglion cell layer (GCL) and inner plexiform layer (GCL+IPL), RNFL and GCL+IPL (ganglion cell complex, GCC). Each layer thickness was compared with the control group. The T/N ratio and N/T ratio were calculated, and area under the receiver operating characteristic curve (AUC) was used. The eyes on the same side of the optic tract lesion were defined as the ipsilateral eyes, and the eyes on the opposite side the contralateral eyes.

Results: The RNFL, GCL+IPL, GGG thicknesses were significantly smaller than control group ($p<0.001$, for each). Especially, nasal quadrants were reduced preferentially in the contralateral eyes and temporal quadrants were reduced preferentially in the ipsilateral eyes. The AUC of T/N ratios were 0.88 for GCC, 1.00 for GCL+IPL, 0.85 for RNFL in the contralateral eyes. The AUC of N/T ratios were 0.97 for GCC, 0.89 for GCL+IPL, 1.00 for RNFL in the ipsilateral eyes.

Conclusions: Our findings demonstrate that thinning of the GCC, GCL+IPL, RNFL in hemiretina corresponds to the optic tract lesion. The measurement of GCL+IPL thickness in the contralateral eyes and the RNFL thickness in the ipsilateral eyes is useful to evaluate the retinal ganglion cell atrophy in hemiretina due to optic tract syndrome.

Commercial Relationships: Katsutoshi Goto, None; Atsushi Miki, None; Tsutomu Yamashita, None; Syunsuke Araki, None; Go Takizawa, None; Kenichi Mizukawa, None; Junichi Kiryu, None

648 - P53-5

Clinical measurement of ocular counter-rolling during head tilt using fundus photography

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Purpose: To measure the degree of ocular counter-rolling (OCR) during head tilt using a new fundus photographic method.

Methods: **Design:**An evaluation of diagnostic technology; a prospective observational study.

Participants: We enrolled 30 healthy subjects, 20 to 40 years of age.

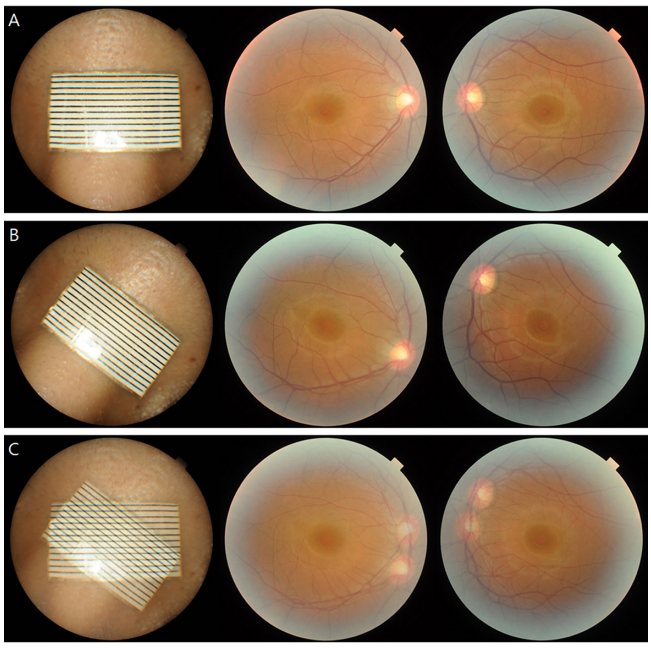
Methods: Fundus photographs were obtained in presumed baseline position and in stepwise head tilt positions to evaluate ocular torsion. Horizontal marks on nose were photographed simultaneously for evaluation of head tilt. The images were analyzed using Photoshop to measure the degree of ocular torsion and head tilt. Reproducibility or interobserver variability was assessed by Bland-Altman plots and by calculation of the intraclass correlation coefficient (ICC).

Results: The degree of OCR continuously increased during head tilt. The mean value of OCR was 2.4 ± 1.7° , 4.7 ± 2.3° , 6.6 ± 2.4° , 7.2 ± 2.9° , and 7.7 ± 2.9° at 0~10° , 10~20° , 20~30° , 30~40° and 40~50° head tilt respectively. The OCR gains decreased progressively as the head tilt angle increased. The mean gains of OCR were 35.9%, 32.6%, 27.2%, 20.8% and 17.7% at 0~10° , 10~20° , 20~30° , 30~40° , and 40~50° head tilt respectively. The 95% limit of agreement of interobserver variability for the angle of OCR was 0.1 ± 1.6, and an ICC was 0.97.

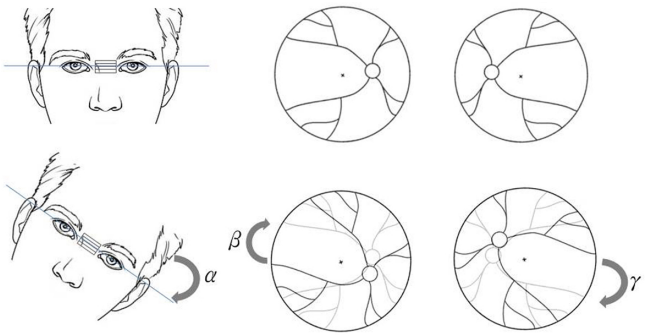
Conclusions: Fundus photographic technique was a reproducible and accurate tool for measuring OCR. Considering its simplicity, ease of use, and comfortability, it hasclear applications in clinical practice.

Commercial Relationships: Han Woong Lim, None; Minjee Lee, None; Seung Hun Park, None; Sei yeul Oh, None; Yumi Song, None; Sang Won Moon, None

Support: This study was supported by a grant of the South Korea Healthcare technology R&D Project, Ministry of Health and Welfare, South Korea. (HI10C2020).



1. Image processing by Photoshop for quantitative measurement of the degree of ocular counter-rolling. A, Photograph of horizontal marks on nose and fundus photographs of both eyes obtained in presumed baseline position. B, Photographs of horizontal marks and both fundus at left head tilt position. C, The image of head tilt position was converted to a semi-transparent image and it overlapped with the baseline image.



α = Angle of head tilt
β , γ = Angle of right, left ocular torsion

OCR = $\alpha - \beta, \gamma$

2. Schematic of both ocular torsions according to head tilt. The degree of ocular counter-rolling (OCR) is defined as the difference between the angle of head tilt (α) and the angle of an ocular torsion (β = angle of right ocular torsion, γ = angle of left ocular torsion)

649 - P54-6

Retinal ganglion cell atrophy in homonymous hemianopia due to acquired occipital lesions using swept-source OCT

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1. Sensory Science, Faculty of Health Science and Technology, Kawasaki University of Medical Welfare, Kurashiki, Japan. 2. Ophthalmology, Kawasaki Medical School, Kurashiki, Japan. 3. Doctoral Program in Sensory Science, Graduate School of Health Science and Technology, Kawasaki University of Medical Welfare, Kurashiki, Japan.

Purpose: To report a reduction in macular retinal thickness of inner retina detected with swept-source optical coherence tomography (SS-OCT) in patients with homonymous hemianopia following acquired cerebral damage.

Methods: Nine patients (3 men and 6 women), ranging in age from 38 to 74 years (mean 58.0 years) with unilateral occipital damage, were examined using SS-OCT. The duration of the lesions from the onset ranged from 1 month to 8.0 years (mean 3.7 years). The age-matched control group was composed of 56 eyes of 56 normal subjects. All patients underwent an ophthalmic examination including SS-OCT (DRI OCT-1 Atlantis®). The scanning protocol used was macula 3D scan pattern with a scan density of 512 × 256 covering 12 × 9 mm area. The 4 × 3 mm area around the fovea was divided into hemianopic and unaffected sides. Three macular inner retinal layer thicknesses were determined, 1) retinal nerve fiber layer (RNFL) thickness, 2) ganglion cell layer and inner plexiform layer (GCL+IPL) thickness, and 3) ganglion cell complex (GCC; RNFL+GCL+IPL) thickness. Each layer thickness was compared with the control group. The areas under the receiver operating characteristic curves (AUCs) were compared among these parameters. The relationship between the time after stroke and macular retinal layer thickness in hemianopic sides was evaluated.

Results: Although optic atrophy and retinal nerve fiber layer defects were not discernible on funduscopy, statistically significant thinning of inner retina was demonstrated in the hemi-retinae corresponding to the affected hemifields ($p < 0.05$). Total retinal thickness was not found to be significantly affected. Area analysis revealed striking thinning in the most central macula. The AUCs for the RNFL, GCL+IPL, and GCC thickness in the hemianopic side were 0.742, 0.976, and 0.973, respectively. Regression analysis revealed a negative linear relationship (linear regression, $R^2 = 0.533$, $p = 0.026$) between the time after stroke and GCC thicknesses in hemianopic sides.

Conclusions: Our findings confirmed our previous observation using spectral-domain optical coherence tomography (Yamashita T et al. Jpn J Ophthalmol 2012; 56, 502-510) that degeneration of retinal ganglion cells can occur after an occipital stroke. The change may be slowly progressive at least for 8 years. It is also suggested that central retina is preferentially affected in these patients.

Commercial Relationships: Tsutomu Yamashita, None; Atsushi Miki, None; Katsutoshi Goto, None; Syunsuke Araki, None; Yoshiaki Ieki, None; Akio Tabuchi, None; Junichi Kiryu, None

650 - P55-7

Intraosseous orbital cellular hemangioma with intracranial involvement

Tun Hang Yeo [1] Bernard C Ho [2] Boon Chuan Pang [3] Eugenie W Poh [1]

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Purpose: To report a rare case of intraosseous orbital cellular hemangioma that involved the intraconal compartment, which extended intracranially, and had evidence of similar lesions elsewhere in the cranium

Methods: Case report

Results: A 72-year-old female presented with a month's history of poor left eye vision with associated redness and discomfort. On examination, she had 2mm proptosis of the left eye and inferior dystopia with limitation on all gazes. She also had a grade 1 RAPD with left nasal optic disc swelling. Gadolinium enhanced MRI showed a multi-lobulated expansile mass involving the left lateral frontal bone extending into extraconal space. There was also dural enhancement over the frontoparietal regions and two other similar enhancing lesions in the skull. A left frontal craniotomy with tumor resection was performed together with the neurosurgical team. Histologically, the tumor was an unusual vascular proliferation with marked cellularity and mitotic figures. However, there were no other cytological and architectural features to suggest angiosarcoma. This finding was consistent with an intraosseous cellular hemangioma. The patient's symptoms resolved 3 months after excision.

Conclusions: Intraosseous hemangiomas of the facial bones can extend to the orbit, giving rise to ocular symptoms. Capillary and cavernous hemangiomas are common histological subtypes. Intraosseous orbital hemangiomas are uncommon and rarely do they extend intracranially. The cellular subtype presented in this case is a unique entity and has only been reported once previously. Morever, the presence of similar lesions elsewhere in the skull renders this case the first of its kind. Although not considered malignant, cellular hemangiomas have the potential for recurrence. Surgical removal is the management of choice for symptomatic patients. The potential for recurrence and presence of similar lesions elsewhere calls for regular monitoring of these patients.

Commercial Relationships: Tun Hang Yeo, None; Bernard C Ho, None; Boon Chuan Pang, None; Eugenie W Poh, None

651 - P56-8

Case of blepharospasm and cellulitis following facial plastic surgery

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Purpose: The number of cases of plastic surgery is increasing in Japan, and as might be expected, the number of complications from the plastic surgery is also increasing. We report a case of blepharospasm and facial cellulitis after plastic surgery to the nose. <div id="UMS_TOOLTIP" style="position, absolute; cursor, pointer; z-index, 2147483647; background-color, transparent; top, -100000px; left, -100000px; background-position, initial initial; background-repeat, initial initial;">
Methods: A 24-years-old woman developed blepharospasm of her left eyelid right after the first injection of Radiesse®, a volumizing filler, to the dorsum of the nose. Radiesse is composed mainly of calcium hydroxyapatite. She had a second injection 1 week after the first, and developed cellulitis on her right buccal region, her nose, and forehead 2 days after the second injection. She received intravenous antibiotics for 3 days. <div id="UMS_TOOLTIP" style="position, absolute; cursor, pointer; z-index, 2147483647; background-color, transparent; top, -100000px; left, -100000px; background-position, initial initial; background-repeat, initial initial;">
Results: She first visited our clinic 3 weeks after the first injection. Her best-corrected visual acuity was 1.2 in both eyes, and there were no signs of inflammation in the anterior chamber, and no abnormality in the retina and optic disc. The blepharospasm recovered spontaneously 1 week after the first visit to our clinic, and the cellulitis followed a self-limiting course with scabbing after the antibiotic treatment. <div id="UMS_TOOLTIP" style="position, absolute; cursor, pointer; z-index, 2147483647; background-color, transparent; top, -100000px; left, -100000px; background-position, initial initial; background-repeat, initial initial;">
Conclusions: Our clinic had previously reported a case of ophthalmic artery occlusion following the injection of hyaluronic acid into the glabellar area (Nonomura S, et al. Nihon Ganka Gakkai Zasshi 2015;118(9):783-7). It was fortunate that the patient we report this time had no visual function impairments, but there were extensive facial cellulitis and blephalospasm. These cases suggest that plastic surgery should be undertaken only after the patient is informed on the risk of complications. <div id="UMS_TOOLTIP" style="position, absolute; cursor, pointer; z-index, 2147483647; background-color, transparent; top, -100000px; left, -100000px; background-position, initial initial; background-repeat, initial initial;">
Commercial Relationships: Ryutaro Akiba, None; Toshiyuki Oshitari, None; Shuichi Yamamoto, None

652 - P57-9

Buccal mucous membrane grafting for cicatricial entropion

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Purpose: To evaluate the use of anterior lamellar recession or posterior lamellar resection with buccal mucous membrane grafting for treatment of cicatricial entropion.

Methods: A retrospective chart review was performed of all cases of anterior lamellar recession or posterior lamellar resection with buccal mucous membrane grafting performed by one surgeon for cicatricial entropion or trichiasis of the upper or lower eyelids from June 2008 to September 2014.
Results: 12 eyelids underwent anterior lamellar recession, 14 eyelids underwent posterior lamellar resection and all patients had procedure of buccal mucosal graft. 26 eyelids (14 upper lids, 12 lower lids) were identified in 16 patients. The patients' mean age was 56.3 years. The mean follow-up period was 7.9 months (range 2-24 months). One eyelid underwent repeat grafting for recurrent entropion secondary to graft dislocation. There was a reduction in the frequency of patients reporting discomfort, foreign body sensation, tearing and pain following treatment in 23 eyelids. 2 eyelids had recurrent entropion that was managed by electrolysis or cilia epilation.
Conclusions: Anterior lamellar recession or posterior lamellar resection with buccal mucous membrane grafting in patients with cicatricial entropion is a simple and cosmetically effective procedure without complications.
Commercial Relationships: Seoung Hyun An, None; Woo seok Choi, None; Eun Jung Sohn, None; Yoon Hyung Kwon, None; Won Yeol Ryu, None; Woo Jin Jeong, None; Hee bae Ahn, None

653 - P58-10

Suture infection suspected to be caused by silk thread used in suspension surgery for ptosis

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Purpose: This report describes a case of suture infection suspected to be caused by silk thread used in suspension surgery for ptosis.
Methods: The patient, a 63-year-old male, underwent frontalis suspension using 4-0 silk thread for right ptosis in September 2009. He initially progressed well following surgery but approximately one year postoperatively, reddening and swelling appeared from the eyelid to the forehead region.
Results: The patient was administered cefcapene pivoxil (CFPN-PI) and ofloxacin (OFLX) eye ointment but showed no improvement and was subsequently examined for suspected suture infection. The 4-0 silk thread was removed from the surgical wound site and debridement

was performed to the area. *Enterobacter aerogenes*, *Streptococcus agalactiae* (GBS) were detected from a culture taken from the suture thread.
Conclusions: A scanning electron microscope revealed a cluster of bacteria that appeared to be producing a biofilm. Postoperatively, the eyelid swelling was quickly reduced and the patient is currently progressing favorably.
Commercial Relationships: Yukinobu Okajima, None; Tadashi Matsumoto, None; Yuichi Hori, None

654 - P59-11

Noninvasive Assessment of Extraocular Muscle Insertion Using 3-Dimentional Anterior Segment Optical Coherence Tomography before Strabismus Reoperation

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Purpose: To investigate the location of extraocular muscle insertion using 3-dimentional anterior segment optical coherence tomography (AS-OCT) before strabismus reoperation.
Methods: A report of three cases
Results: Case 1, A 9-year-old male with recurred intermittent exotropia of 30 prism diopter (PD), who had undergone bilateral lateral rectus recession 6 years ago. AS-OCT demonstrated normal location of medial rectus insertion, while the lateral rectus insertion was located 10mm from the limbus OU. By re-recessing the left lateral rectus 4 mm, his eye position became orthophoric postoperatively.
Case 2, A 6-year-old female with residual esotropia of 25 PD after bilateral medial rectus recession at the age of 8 months. AS-OCT showed normal location of lateral rectus insertion, but medial rectus insertion was not detected within 16 mm from the limbus OU. The left lateral rectus was resected 5 mm. Her esophoria improved to 8 PD postoperatively.
Case 3, A 57-year-old male with recurred constant exotropia of 40 PD, who had undergone strabismus surgery about 45 years ago. AS-OCT indicated normal location of medial rectus insertion, but lateral rectus insertion was not visible within 16 mm from the limbus. The right medial rectus was resected 7 mm, making the eye position orthophoric postoperatively.
Conclusions: AS-OCT can noninvasively visualize the insertion of both intact and postoperative extraocular muscles. Such ability is useful for the assessment of previous surgery, which may make strabismus reoperation less invasive.
Commercial Relationships: Yuka Sato, None; Shinichi Fukuda, None; Takahiro Hiraoka, None; Tetsuro Oshika, None

655 - P60-12

Effect of horizontal transposition of vertical rectus muscle for treatment of acquired trochlear nerve palsy

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Purpose: Trochlear nerve palsy is one of the most common forms of paralytic strabismus, resulting in ipsilateral hypertropia and excyclotorsion. Horizontal transposition of the vertical rectus muscles is performed for treatment of excyclotropia as well as superior oblique muscle tucking "Harada-Ito operation". The extent of excyclotropia that can be corrected and the impact of this technique on the vertical muscle balance are not well known. Here we report the surgical results of seven patients with acquired trochlear nerve palsy.
Methods: The records of patients who visited our hospitals in 2004 to 2011 and were diagnosed with acquired trochlear nerve palsy were evaluated. Seven patients (2 males and 5 females, average age at surgery; 59.0 ± 17.1 years) underwent a surgery by a single surgeon. The extents of deviation before and after surgery were assessed with synoptophore. All surgeries were performed on paretic eyes (nasal transposition of inferior rectus muscle with resection for 4 cases, temporal transposition of superior rectus muscle with recession for 1 case and only nasal transposition of inferior rectus muscle for 2 cases).
Results: The preoperative extents of cyclodeviation and vertical deviation were 7.2 ± 1.8 ° and 4.0 ± 3.5 °, respectively, in primary position. The postoperative extents of cyclodeviation and vertical deviation were 3.0 ± 2.0 ° and 2.1 ± 2.3 °, respectively. The average effect of horizontal transposition of vertical rectus muscle for cyclodeviation was 4.6 ± 1.7 ° per one tendon width and 0.8 ± 1.1 ° per 1mm for vertical deviation. An additional operation was required for two of seven cases, one case with excyclodeviation and vertical deviation and the other with esodeviation and vertical deviation.
Conclusions: Ipsilateral horizontal transposition of vertical rectus muscle is effective for treatment of excyclotorsion due to acquired trochlear nerve palsy. Additional resection or recession of the translocated muscle is available for the hypertropia complicated by excyclotorsion.
Commercial Relationships: Mayo Nozaki, None; Ryo Ando, None; Sayaka Ishigaki, None; Yasuhiro Shinmei, None; Ayako Mizoguchi, None; Hiroshi Shimizu, None; Shinki Chin, None; Susumu Ishida, None

656 - P61-13

Effects of divergence with base-in prisms on near visual function

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Purpose: To investigate the visual function at near fixation during divergence with base-in prisms.

Methods: Six visually normal subjects, aged 23-28 years (26.3 ± 2.0; mean ± SD) were included in this study. The dominant eye was determined using the hole-in-card test. The subjects were induced into 4 degrees of esophoria (0~2, 4, 8, 10~16 Δ E') by putting the base-in prism on non-measurement eye at near fixation. The objective refraction and the pupil diameter were measured using WAM-5500 (Grand Seiko Co, Fukuyama, Japan), open field autorefractor. And near binocular visual acuity was measured in each degree of esophoria. We calculated the amount in change of the refraction and in the rate of variability of pupil diameter (V.P.D.) for each degree of esophoria and assessed the relationship between them using the data of non-dominant eye.

Results: No significant differences in the objective refraction were found between the dominant and non-dominant eye for any degree of esophoria. A 2-factor ANOVA found that the object refraction significantly change with differing degree of esophoria ($p<0.01$). Difference in the objective refraction was observed significantly between 0~2 Δ E' (-2.02 ± 0.21D) and 10~16 Δ E' (-1.34 ± 0.54D) ($p<0.01$), 4 Δ E' (-1.99 ± 0.24D) and 10~16 Δ E' (-1.34 ± 0.54D) ($p<0.01$), 8 Δ E' (-1.82 ± 0.47D) and 10~16 Δ E' (-1.34 ± 0.54D) ($p<0.05$). A positive correlation was found significantly between the amount in change of the refraction and the degree of esophoria ($r=0.75$ $p<0.01$). No correlation was found between the rate of V.P.D. and the degree of esophoria. A negative correlation was found significantly between near binocular visual acuity and the degree of esophoria ($r=0.78$, $p<0.01$).

Conclusions: As the degree of divergence increased, the objective refraction increased and near binocular visual acuity decreased. It is suggested that accommodation may decrease depend on increasing the degree of divergence even if the fusion is possible. These changes may be related to the decreased near binocular visual acuity during divergence with base-in prisms.

Commercial Relationships: Manami Kawai, None; Satoshi Ishiko, None; Noriko Nishikawa, None; Ikuko Yamaga, None; Kazuhiro Sugawara, None; Akitoshi Yoshida, None