

Symposium 1
Myopia
Saturday, 3 March 2007

S1.1
THE EFFECT OF SHORT PERIODS OF POSITIVE LENS DEFOCUS IN PREVENTING EXPERIMENTALLY-INDUCED AXIAL MYOPIA IN TREE SHREW

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Introduction: Recent results in the tree shrew model of ocular growth have demonstrated that, with use of a sequential increasing power lens paradigm, positive lens defocus can reliably induce a relative hyperopia compared to untreated control animals. This is in keeping with findings in primates (Smith and Hung, 1999). The present study addressed the question of whether short periods of positive lens defocus were more effective at preventing experimentally-induced myopia in a mammalian model than short periods of plano lens wear.

Methods: Maternally reared tree shrews (*Tupaia Belangeri*) were fitted with dental acrylic pedestals on the 14th day after eye opening. All tree shrews received treatment with -9.5D lenses binocularly fitted at 15 days after eye opening. Animals were randomly allocated to one of three groups based on whether negative lens wear was left uninterrupted ($n = 5$), or interrupted with Plano (0.0D) lenses worn binocularly ($n = 5$) or +4.0D lenses worn binocularly ($n = 5$) for a one-hour period in the middle of the light cycle each day. Measurements of refractive error and ocular dimensions were taken at the start of treatment and at 5 days and 12 days of the lens treatment.

Results: Refractive status or ocular component dimensions at baseline were not significantly different between the three groups ($p > 0.05$). Tree shrews that had binocular treatment with -9.5D lenses underwent 10.6D of myopic refractive change after 12 days of treatment, relative to baseline measures ($p < 0.01$). Animals whose negative lens wear was interrupted by 1 hour of plano lens wear each day underwent 3.6D of myopic refractive change after 12 days of treatment when compared to baseline ($p < 0.01$). Animals whose negative lens wearing treatment was interrupted by 1 hour of +4.0D lens wear each day underwent 0.4D of myopic refractive change after 12 days of treatment, compared to baseline (not significant). Refractive differences from baseline measures within groups were due predominantly to elongation of the vitreous chamber depth.

Conclusions: Short periods of positive lens wear proved slightly more effective in preventing negative lens-induced axial myopia than short periods of plano lens wear in the tree shrew model of ocular growth and myopia.

S1.2
OPTICAL OPTIONS FOR HUMAN MYOPIA CONTROL: NEW IDEAS FROM ANIMAL STUDIES OF DEFOCUS EFFECTS ON EYE GROWTH

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Purpose: Clinical trials of multifocal spectacle lenses for the control of myopia progression in children report very limited benefit and there are reports that undercorrection exacerbates myopia progression. These

findings contrast with the strong inhibitory effects on ocular growth of imposed myopic defocus (positive lenses) in animal model studies. This paper will examine alternative optical therapies reported to have improved efficacy as myopia control treatments and look for parallels in animal studies.

Methods: Data from clinical studies involving progressive addition spectacle lenses (PALs), bifocal contact lenses (BCLs), and alternating monovision spectacles (AML, i.e. undercorrection of each eye in turn on alternate days), for myopia control in children will be reviewed along with data from animal studies involving short-term, simultaneous and alternating defocus stimuli.

Results: In studies involving chicks, the inhibitory effects on eye growth of short, repeated periods of imposed myopic defocus (positive lenses) tend to dominate over those of equivalent exposure to hyperopic defocus (negative lenses), except when exposure is very limited in duration. In tree shrew, interruption of imposed hyperopic defocus for a short period with either minimal or low myopic defocus also is inhibitory. These studies have parallels in two studies in children involving BCLs for myopia control and one involving AML, reporting greater slowing of myopia progression than in previous studies using PALs.

Conclusions: Experimental myopia studies predict the results of recent studies using novel optical manipulations for myopia control in children, which are of a magnitude to be efficacious. Understanding more about the mechanisms underlying the effects of these treatments will allow their refinement and exploitation.

S1.3
CHOROIDAL REGULATION OF SCLERAL REMODELING DURING VISUALLY GUIDED OCULAR GROWTH

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In chicks and primates, post-natal visually guided ocular growth is highly correlated with the rate of proteoglycan synthesis in the posterior sclera. Our lab is interested in identifying the intraocular mechanism(s) involved in the regulation of scleral proteoglycan synthesis. Significant changes in choroid morphology, blood flow, vascular permeability and gene expression also occur in association with changes in the rate of ocular elongation. Choroids isolated from chick eyes recovering from induced myopia demonstrate significant increases in thickness, protein synthesis and vascular permeability, as well as changes in the expression of numerous genes encoding ion channel proteins, serum proteins, growth factors and extracellular matrix components. Similarly, choroids isolated from marmoset eyes demonstrate significant changes in gene expression during compensation for plus and minus lenses. In vitro, choroids and suprachoroidal fluid isolated from recovering and myopic eyes demonstrate significant negative and positive regulation (respectively) of scleral proteoglycan synthesis when tested on normal chick sclera in vitro. Based on these results, we hypothesize that the choroid synthesises and releases bioactive growth factors into the suprachoroidal space that act to regulate scleral proteoglycan synthesis and the rate of axial elongation. The identification of these bioactive choroidal factors will help to elucidate the mechanism of visually guided ocular growth and emmetropisation.

S1.4

IS DOPAMINE AN IMPORTANT REGULATOR OF EYE GROWTH IN ANIMALS AND HUMANS

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Eye growth is regulated by signals generated by the retina in response to form-deprivation or the sign of defocus of out-of-focus images. One of the most studied potential signal molecules is dopamine. Dopamine release is reduced in form-deprivation myopia (FDM), and dopamine agonists block the development of FDM, suggesting that dopamine acts as an inhibitor of eye growth. Whether it is involved in the effects of imposed defocus on eye growth is not clear. The ability of brief periods of normal vision to prevent FDM appears to involve increased dopamine release and D2-dopamine receptors, further supporting a role for dopamine. In form-deprived eyes, dopamine agonists rapidly increase the expression of retinal ZENK mRNA, an immediate early gene associated with changes in eye growth. Atropine, which has similar effects on retinal ZENK, may inhibit eye growth by stimulating the release of dopamine. The dependence of dopamine release on flicker and light intensity may be relevant to the development and prevention of myopia. The reduced temporal contrast in form-deprivation may lead to reductions in dopamine release, while the protective effects of time spent outdoors may be related to increased dopamine release in the high ambient light intensity typical of day-light hours outdoors.

S1.5

EXPERIMENTAL STUDIES OF EMMETROPISATION AND MYOPIA IN THE MARMOSET MONKEY: LENS INDUCED CHANGES IN EYE SHAPE AND REFRACTIVE STATE

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Myopic human eyes are reportedly longer than they are wide (prolate) with relatively less myopia in the periphery. Conversely, hyperopic eyes tend to be wider than they are long (oblate) with relatively less hyperopia in the periphery. It is unclear whether these shapes and peripheral refractions are a consequence of ametropic eye growth - not all ametropic eyes have these shapes, and the shapes and peripheral refractions of emmetropic eyes are variable as well. Posterior eye shape is also likely to be inherent to some degree, and a given shape might make the eye more susceptible to developing a particular refractive error because of the associated peripheral refractions.

This presentation will describe our current studies exploring these possibilities. We are examining the relationship of eye shape and peripheral refraction in marmoset monkeys raised with contact lenses to induce anisometropia. Aniso contact lens rearing paradigms in marmosets effectively alter eye growth and refractive state in a predictable manner. The pattern of peripheral refractions is measured with an IR videorefractor and eye shape is determined using MR imaging.

Our results indicate that changes in eye shape and peripheral refraction accompany experimentally induced refractive errors in marmosets, and are similar to those reported in humans with naturally occurring refractive errors. We have also noted nasal-temporal asymmetries in eye shape and peripheral refractions in marmosets with induced myopia that may reflect constraints on eye growth.

S1.6

THE UNMET NEED FOR REFRACTIVE CORRECTION IN CHILDREN OF SCHOOL AGE: A GLOBAL PUBLIC HEALTH PROBLEM

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Refractive error is recognised as the principal cause of visual impairment in school-age children. Visual impairment and refractive error data from 11 RESC (Refractive Error Study in Children) surveys of 50,000 school-age children in seven countries are summarised. Although correction of refractive error is easy, safe, and effective, it is shown that half of those in need of first-time or updated spectacles are without them.

S1.7

ETHNICITY-SPECIFIC PREVALENCES OF REFRACTIVE ERRORS VARY IN ASIAN CHILDREN IN NEIGHBORING MALAYSIA AND SINGAPORE

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Aim: To compare the prevalences of refractive errors in Malay, Chinese and Indian children in Malaysia and Singapore.

Methods: Children aged 7-9 years from three schools in the Singapore Cohort study of the Risk factors for Myopia ($n = 1962$) and similarly aged children from a random cluster sample in the metropolitan Kuala Lumpur area in the Malaysia Refractive Error Study in Children ($n = 1752$) were compared. Cycloplegic autorefractometry was conducted in both countries.

Results: The prevalence of myopia (spherical equivalent of at least -0.5 diopters (D) in either eye) was higher in Singapore Malays (22.1%) than in Malays in Malaysia (9.2%; 95% confidence interval (CI) 11.2 to 14.7; $p < 0.001$). Similarly, Singapore Chinese (40.1%) had higher prevalences than Malaysian Chinese (30.9%; 95% CI 1.5 to 16.9). Singapore Indians had a higher prevalence (34.1%) than Malaysian Indians (12.5%; 95% CI 17.4 to 25.9). The multivariate odds ratio of astigmatism (cylinder at least 0.75 D in either eye) in Singapore Malays compared with Malaysian Malays was 3.47 (95% CI 2.79 to 4.32). Ethnicity-specific hyperopia rates did not differ in Singapore and Malaysia.

Conclusions: The ethnicity-specific prevalence of myopia in Singapore Malays, Chinese and Indians are higher than those in Malaysian Malays, Chinese and Indians. As Malays, Chinese and Indians in Malaysia have a genetic make-up similar to that of Malays, Chinese and Indians in Singapore, environmental factors may contribute to the higher myopia rates.

S1.8

ENVIRONMENTAL FACTORS ASSOCIATED WITH THE DEVELOPMENT OF REFRACTIVE ERROR: IS TIME SPENT OUTDOORS CRUCIAL

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Purpose: To examine the associations between refractive error and risk factors for myopia, in two age cohorts of Australian school children.

Methods: The Sydney Myopia Study invited participation from all Year 1 and Year 7 students enrolled in 51 randomly selected schools, stratified by socio-economic status. Participants underwent a comprehensive eye examination including cycloplegic autorefractometry and measurement of ocular biometry using an IOLMaster. Information on hours of near work and time outdoors was obtained by questionnaire. Myopia was defined as spherical equivalent of $-0.5D$ in the right eye.

Results: In the 1765 Year 1 students (participation 78.9%) of mean age 6.7, the prevalence of myopia was 1.5% (95% CL: 1.0-2.3). In the 2353 Year 7 students (participation 75.3%) of mean age 12.7, the prevalence of myopia was 12.8% (CL: 11.2-14.2). There was no significant impact of near work on spherical equivalent, but Year 7 students in the highest tertiles of outdoor activity had the most hyperopic refractions, irrespective of the amount of near work performed. The Year 7 students who engaged in the lowest tertile of outdoor activity and the highest tertile of near work had an odds ratio for myopia of 2.6 compared to those doing the least near work and spending the most time outdoors.

Conclusion: The prevalence of myopia in Australian children is low by international standards. Time spent on near work had little effect on refraction, whereas increased time spent outdoors was associated with more hyperopic refractions and less myopia. High levels of engagement in outdoor activity may explain the low prevalence of myopia in Australian school children, despite high educational standards. This conclusion is supported by comparison with data from age and ethnicity matched children from Singapore.

S1.9

SPATIOTEMPORAL STIMULUS PROPERTIES AND VISUAL CONTROL OF EYE GROWTH IN CHICKS

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Ocular growth and refraction are largely regulated locally in the eye via retinal image processing and signaling. This is particularly clear from the effects of form-deprivation and imposing plus-defocus in young chicks. However, the critical image features, retinal neurons and messengers, and cellular targets for action of those messengers in the control of eye growth, remain largely unknown. Amacrine cells are most likely to play key roles in ocular growth control, because they modulate bipolar cell functions in time and space, are tuned to spatiotemporal change, and probably generate control signals that act upon the RPE.

This presentation will build upon three hypotheses:

1. In the absence of visual controls, the eye tends to elongate (default growth). Default elongation is due in part to extrinsic [RPE-independent/vision-insensitive] and in part to intrinsic [RPE-dependent/vision-sensitive] factors.
2. In emmetropic eyes, the retina discriminates good focus and prevents further change. Retinal signals for in-focus are integrated over time, and sufficient time in focus leads to "stop" and the maintenance of emmetropia.
3. In myopic eyes, the retina detects excess plus defocus and prevents further elongation. A net increase in plus-defocus over time increases the activation of glucagon amacrine cells. Glucagon, released as a result of this, causes compensatory plus-defocus effects including arrest of posterior eye enlargement and thickening of the choroid.

Control systems, similar to these in chicks, probably exist also in mammals, but have not yet been documented so well. Results of such experiments could lead to new therapies to prevent myopia, thereby minimizing vision loss and cutting health-care costs.

S1.10

EXPERIMENTAL MYOPIA IN A MUSCARINIC KNOCK-OUT MOUSE

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Purpose: The purpose of this study was to determine if there are differences in the ability to induce form deprivation experimental myopia in a series of mice with specific gene knock-outs for the muscarinic receptors.

Methods: Muscarinic receptor subtypes 2, 4 and 5 homozygous (-/-) mice were backcrossed with B6 wild type (+/+) mice to get heterozygous (+/-) mice for further breeding. Tail biopsies were taken to extract DNA and PCR was carried out using specific primers for genotyping. F5 generation of m2-/-, m4-/-, m5-/- and wt+/+ mice were used for experiments. Contact lenses were placed over the right eyes of 6 knockout and wild type mice from each sub type by gluing on a negative (-10D) contact lens over the eye on post natal day 10. Axial length was measured at 4, 6 weeks and 8 weeks by AC-Master, OLCI (Carl-Zeiss) and at 8 weeks by magnified video image photography. Expression of muscarinic acetylcholine receptor subtypes was detected by western blot and immunohistochemistry.

Results: Axial length at 4 weeks on m2-/-, m4-/-, m5-/- myopic eyes was 2.819 ± 0.09 mm, 3.029 ± 0.03 mm, 3.040 ± 0.03 mm and control eyes were 2.769 ± 0.08 mm, 2.926 ± 0.06 mm, 2.938 ± 0.05 mm respectively. At 6 weeks on m2-/-, m4-/-, m5-/- myopic eyes was 3.273 ± 0.04 mm, 3.332 ± 0.03 mm, 3.337 ± 0.02 mm and control eyes were 3.113 ± 0.07 mm, 3.134 ± 0.03 mm, 3.138 ± 0.03 mm respectively. At 8 weeks m2-/-, m4-/-, m5-/- mice, myopic eyes were 3.546 ± 0.04 mm, 3.732 ± 0.07 mm, 3.737 ± 0.05 mm and control eyes were 3.341 ± 0.02 mm, 3.340 ± 0.01 mm, 3.342 ± 0.01 mm respectively (ANOVA, $n = 6$, $p < 0.05$, Tukey $p = 0.0006$). At 8 weeks, m2+/+, m4+/+, m5+/+ mice, myopic eyes were 3.563 ± 0.08 mm, 3.571 ± 0.09 mm, 3.580 ± 0.06 mm and control eyes were 3.350 ± 0.02 mm, 3.349 ± 0.02 mm, 3.353 ± 0.02 mm respectively (ANOVA, Tukey test, $n = 6$, $p < 0.05$). Western blot and immunohistochemistry showed the expression of all five muscarinic receptor subtypes from m2+/-, m4 +/-, m5 +/- and wt+/+ mice sclera and absence of m2, m4 and m5 from m2-/-, m4-/-, m5-/- respectively.

Conclusions: The results provide initial evidence that the m4 and m5 muscarinic receptors may contribute more than the m2 receptor in terms of scleral growth in experimental myopia. It also confirms the presence of the muscarinic receptor sub types; m1-m5 in mouse sclera.

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Symposium 2
Cornea
Saturday, 3 March 2007

S2.1

IMPACT OF NEW LAMELLAR PROCEDURES ON INDICATIONS, TECHNIQUES AND OUTCOMES OF CORNEAL TRANSPLANTATION

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Targeted lamellar replacement of corneal tissue represents a paradigm shift transforming corneal transplantation today. In the last 5 years, 57% of all published peer reviewed articles on keratoplasty have focused on lamellar surgery, and in 2006, various forms of anterior and posterior lamellar keratoplasty constituted 42% of all 186 keratoplasty procedures performed at the Singapore National Eye Centre. In 2005, US eye banking statistics revealed that 1398 new endothelial keratoplasty procedures (DSEK, DLEK) and 641 anterior LKs were performed in the US.

Data from our Singapore Corneal Transplant Study (SCTS) database of 1715 transplants confirms significantly higher graft survival rates with LK, and comparison visual acuity results of Deep Anterior Lamellar Keratoplasty (DALK) with total corneal stromal replacement are equivalent, if not better, than that of conventional PK surgery, possibly as a result of better graft-host apposition. As a result, DALK is now our procedure of choice for up to 1/3 of all indications for keratoplasty, including ectatic and corneal stromal disease, non-penetrating post-traumatic injuries, post-infectious scarring, and acute infectious keratitis. We have yet to encounter recurrence of infection in 18 consecutive DALK procedures for non-perforated cases of severe, progressive acanthameba, fungal and bacterial keratitis.

The advent of microkeratome-assisted lamellar keratoplasty (Automated Lamellar Therapeutic Keratoplasty, ALTK) affords refractive accuracy in anterior LK surgery for a variety of post-refractive surgery ectasia, keratoconus and post-PRK scarring, but a rapidly emerging role of ALTK is donor preparation for various forms of Endothelial Keratoplasty (EK), including Descemets Stripping Automated Endothelial Keratoplasty (DSAEK), with US eye banks now offering precut donor posterior lamellar tissue.

The recent advent of small incision, sutureless EK surgery as an alternative to conventional PK surgery for endothelial disease represents the most significant change to the field, analogous to the transition from ECCE to phacoemulsification surgery, which previously occurred in cataract surgery. While surgical refinements in EK techniques, and longer-term follow-up with regards to endothelial outcomes remain pertinent, new femtosecond laser-assisted forms of penetrating and lamellar keratoplasty technologies are now being explored.

S2.2

CHRONIC CORNEAL ALLOGRAFT DYSFUNCTION AFTER PENETRATING KERATOPLASTY

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With the clinical application of tissue matching and new immunosuppressive drugs, chronic transplant dysfunction becomes the

predominant cause of long-term graft failure. Chronic corneal allograft dysfunction (CCAD) has been one of the major indications of re-grafting after penetrating keratoplasty. CCAD is characterised as corneal graft displaying a gradual function deterioration months to years after transplantation, with an eventual graft failure (graft opacity). Clinical examination shows that the endothelial cells decline progressively, and the grafts lose endothelial cells at an exponential rate of 7.8% per year within 3 to 5 years after keratoplasty and 4.2% per year within 5 to 10 years. The keratocytes also decrease in the transplanted corneas. Ultrastructural changes such as fibrosis formation and cell atrophy and degeneration can be observed in the failed grafts resulting from CCAD. It has been found that both alloantigen specific factors and non-alloantigen specific factors contribute to the development of chronic transplant dysfunction. The alloantigen specific factors include histoincompatibility, acute rejection episodes, suboptimal immunosuppression, et al. The non-alloantigen specific factors include ischemia, infection, donor quality, et al. Further studies need to be done on the prevention and treatment of CCAD.

S2.3

AVELLINO CORNEAL DYSTROPHY AND ITS ONGOING TREATMENT

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Avellino corneal dystrophy is an autosomal dominant dystrophy with clinical features of both granular and lattice stromal deposits. It progresses with age starting from anterior granular deposits to midstromal lesions with anterior stromal haze. It was known that vision would decrease after LASIK. The several kinds of corneal findings after LASIK will be demonstrated. The figure of the SEM of the flap after LASIK will be displayed. The figures after LASEK will be presented. The minimal degree of the opacity, which would make the surgeon confused with the diagnosis, will be shown. *CR

S2.4

UPDATE ON PTERYGIUM AND OCULAR SURFACE NEOPLASIA

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Recent developments in our understanding of the pathogenesis and management of pterygium and ocular surface neoplasia will be reviewed. *CR

S2.5

INACTIVATING NOTCH1 INDUCES TRANSDIFFERENTIATION OF CORNEAL EPITHELIUM IN EPIDERMIS

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Purpose: To investigate the processes involved in the transdifferentiation of the corneal epithelium in skin-like epidermis.

Methods: We used Notch1 K14 CreLoX mice.

Results: Three months after the inactivation of Notch1 with a CreLoX system loaded to K14, central corneal epithelium became hyperplastic and cornea lost its transparency. This hyperplasia was characterised by increased Ki67 staining, disappearance of K3 staining specific to corneal epithelium, and expression of epidermis-specific differentiation markers such as keratin 1 and loricrin. These data suggested that central corneal epithelium differentiates into a skin-like epidermis in absence of Notch1 gene. Histological analysis of eyelids from Notch1 deficient mice revealed defective meibomian glands, necessary to generate a protective lipid layer on the surface of the cornea. We speculate that the absence of meibomian glands leads to chronic ocular surface irritations and generates an epithelial wound healing process that accelerates the transdifferentiation. We confirmed this hypothesis in suturing lids. In these cases the transdifferentiation did not occur. According to these data, when we wounded the corneal epithelium 3 times in 3 weeks, we accelerated the transdifferentiation process.

In the second part of the project we established a link between Notch1 and specific retinoid-binding proteins (CRBP). If Notch1 is inactivated, cellular retinoid-binding proteins are missing. We concluded that Notch1 inactivation leads to corneal transdifferentiation in epidermis because of the downregulation of specific retinoid-binding proteins. This process creates a vitamin A deficiency in the cornea with a false differentiation.

Conclusions: Our results demonstrate that Notch1 is essential during corneal epithelial repair. Our results place Notch1 as a master gene for controlling the differentiation to obtain a normal corneal epithelium after a wound. Notch1 also regulates CRBP involved in metabolic transformation of the vitamin A in cells. Lack of Notch1 induces a transdifferentiation of the corneal epithelium in a skin-like epidermis during a wound healing process.

S2.6

CLINICAL OUTCOME OF AUTOLOGOUS CULTIVATED LIMBAL EPITHELIUM TRANSPLANTATION FOLLOWED BY PENETRATING KERATOPLASTY

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Purpose: To report the outcome of autologous cultivated limbal Epithelium transplantation (CLET) followed by penetrating keratoplasty (PKP) for ocular surface reconstruction.

Methods: Medical records of twenty-one patients with limbal stem cell deficiency who underwent PKP after CLET were reviewed for recurrence of conjunctivalisation and the outcome of the graft.

Results: Of the 146 eyes with limbal stem cell deficiency treated with cultivated limbal epithelium transplantation 21 underwent PKP at a mean interval of 29.5 weeks (± 11.44) between March 2001 and December 2004. Cumulative survival probability of corneal grafts after PKP was 81.1% at 6 months and 67.6% at 2 years. Mean follow was 81.3 weeks ($SD \pm 51.6$) (18.6 months) after CLET and a mean follow up of 45.5 weeks ($SD \pm 45.9$) (10.4 months) after PKP. The mean duration between the 2 surgeries was 29.5 weeks ($SD \pm 11.4$) (6.7 months). The total follow up was 81.3 weeks ($SD \pm 51.6$) (18.6 months). Recurrence of conjunctivalisation was seen in total of 14.28% (3/21 eyes) and the cumulative survival probability after CLET was 90.5% at the end of 6 months, and 84.0% at 2 years. Rejection after PKP was seen in 4 out of 21 eyes (19%) and the cumulative survival probability of corneal grafts after PKP was 81.1% at 6 months and 67.6% at 2 years. These rejection episodes were independent of the failure of CLET.

Conclusions: Penetrating keratoplasty after cultivated limbal epithelial transplantation proves to be an effective measure for ocular surface reconstruction following limbal stem cell deficiency.

S2.7

LIPID MEMBRANE RAFT INHIBITION PREVENTS PSEUDOMONAS INTERNALISATION IN CORNEAL EPITHELIUM IN VIVO AFTER CONTACT LENS WEAR AND IN VITRO

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Purpose: Corneal epithelium (CE) responds inversely to hypoxia induced by contact lens wear (CTLW) with increased binding and internalisation of *Pseudomonas aeruginosa* (PA) the most frequent cause of CTLW-related microbial keratitis. The purpose of this study was to determine if membrane lipid rafts (MLR) and/or Cystic Fibrosis transmembrane receptor protein (CFTR) mediate this invasive process following CTLW and in vitro.

Methods: MLR formation was evaluated in vivo in rabbit CE with or without CTLW; immortalised human CE cells (h-TERT) were used in vitro. MLR were detected by FITC-labeled -subunit cholera toxin known to bind MLR component GM1 with confocal microscopy and CFTR by immunostaining. Three pathogenic PA strains were tested; internalisation was assessed by gentamicin survival assay and confirmed in vivo and in vitro by pretreatment of CE cells with three cholesterol metabolism inhibitors; cytotoxic effects were excluded by live-dead assays. Specific interaction of PA and MLR was confirmed by FACS analysis.

Results: Normal rabbit CE does not exhibit MLR or CFTR in vivo; these are induced by hypoxic PMMA CTLW, but not by PA exposure alone to all three test strains. PA exposure after PMMA CTLW showed binding to MLR-forming cells in vivo followed by MLR aggregation and PA internalisation for all strains. A similar sequence of MLR formation and PA internalisation was seen in vitro in h-TERT cells. Internalisation of all invasive strains was blocked in a concentration dependent manner by cholesterol metabolism inhibitors ($p < 0.01$) in vitro; cytotoxic effects were excluded by live-dead assays over concentrations studied. Topical Filipin blocked PA internalisation in vivo, FACS analysis showed exposure of two PA test strains to cell lysate significantly increased fluorescence intensity, demonstrating specific binding of PA to MLR-GM1. By contrast, specific peptide blockade of CFTR did not prevent PA internalisation.

Conclusions: These findings demonstrate that CTLW-mediated PA internalisation involves MLR platforms but are not specifically mediated by CFTR. These findings offer a unique new in vivo strategy; for prevention of CTL-related PA infection by blocking MLR formation.

S2.8

INNATE IMMUNITY ON THE OCULAR SURFACE

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The ocular surface epithelium serves a critical function as the defensive front line of the innate immune system. While the detection of microbes is arguably the most important task of the immune system, an exaggerated host defense reaction to endogenous bacterial flora may initiate and perpetuate inflammatory mucosal responses. The ability of cells to

recognise pathogen-associated molecular patterns depends on the expression of a family of Toll-like receptors (TLRs). A healthy ocular surface is not inflammatory, although ocular surface epithelium is in constant contact with commensal bacteria and bacterial products. Interestingly enough, human corneal epithelial cells (HCEC) failed to respond functionally to peptidoglycan and lipopolysaccharide, while they do respond to viral double-stranded RNA to produce pro-inflammatory cytokines through TLR3. Thus, the ocular surface epithelium may possess a distinct innate immune mechanism from that of immunocompetent cells.

S2.9

USE OF BONE MARROW CELLS TO TREAT CORNEAL DISEASES OF GENETIC DEFECTS IN MOUSE

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Corneal stroma consists of heterogeneous cell types including dendritic keratocytes, macrophages and other bone marrow derived cells, e.g., T-cells. In adult mice, keratocan expression is limited to stromal keratocytes, thus its expression is considered as a differentiation marker for keratocytes. Injuries cause cell death of keratocytes via apoptosis and/or necrosis and loss of keratocan expression. Healing of injured corneas is characterised by regeneration of keratocytes that express keratocan. To examine the hypothesis whether bone marrow derived cells are capable of trans-differentiation and contribute to the regeneration of keratocytes in injured corneas. Chimera mice were prepared by reconstitution of bone marrow cells of lethally irradiated keratocan null mice (Kera^{-/-}) by transplanting bone marrow cells from green mice [C57BL/6-TGN(ACTbEGFP)10sb, The Jackson Laboratory]. The chimera Kera^{-/-} mice were subjected to corneal epithelium debridement, which was allowed to heal for six weeks. The keratocan expression in corneas of experimental mice was examined by whole mount electro-immunostaining with anti-KeraC-Alexa555 conjugates using a confocal microscope, western blot analysis and RT-PCR. In naïve uninjured chimera, bone marrow derived green cells appeared in corneal stroma within one month, but they do not express keratocan. In injured corneas healed for six weeks, keratocan was detected. Bone marrow cells isolated from green mice were intrastromally injected into Kera^{-/-} mice to further verify that bone marrow derived cells might undergo differentiation and synthesise keratocan. Keratocan was detected in corneas of Kera^{-/-} mice three weeks after intrastromal injection of that had received bone marrow cells. In part supported by grants: NIH EY010556, EY011845, EY012486; Research to Prevent Blindness, Inc., Ohio Lions Eye Research Foundation.

S2.10

HERPES SIMPLEX VIRUS INFECTIONS: FROM THE OUTSIDE IN

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The purpose of this presentation will be to discuss possible mechanisms by which the neurotropic herpesviruses, which normally infect the cornea, may be able to infect the retina and/or the central nervous system.

S2.11

CHARACTERISATION OF A SELF-ASSEMBLED ARTIFICIAL STROMA

J.D. ZIESKE

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Purpose: The corneal stroma consists of a stack of alternating collagen lamellae, which contain arrays of aligned, evenly spaced, uniform collagen fibrils. This architecture is responsible for the mechanical strength and transparency of the cornea. The goal of our present project is to devise methods to stimulate corneal fibroblasts to secrete an extracellular matrix that mimics corneal stromal matrix. In this report, we discuss our investigation on the characterisation of the intrinsic ability of human corneal fibroblasts to assemble an organised extracellular matrix.

Methods: Human corneal fibroblasts were cultured on polycarbonate transwells and stimulated to secrete matrix with 2-O- α -D-glucopyranosyl-L-ascorbic acid (g-Asc). The resulting constructs were analysed by confocal and differential interference contrast (DIC) imaging at four, eight, and twelve weeks.

Results: Consistent 3-dimensional vertical organisation was detected within the stromal constructs. The constructs consisted of: 1) a dense confluent layer of spindle shaped cells adjacent to the transwell membrane, which exhibited a high degree of alignment; 2) a thick layer of aligned matrix with only a few cells; 3) several layers of randomly organised cells; and 4) a layer of highly organised cells at the surface of the construct. The thickness of the corneal constructs was 36 microns at four weeks and 50 microns at eight weeks. The layer of aligned matrix was 25 microns at both four and eight weeks.

Conclusions: Primary human corneal fibroblasts can be stimulated to assemble constructs with consistent vertical stratification and layers of aligned collagen. These constructs may be useful in developing an artificial stroma.

S2.12

FUNCTIONS OF LEUCINE-RICH REPEAT PROTEOGLYCAN IN THE CORNEA

S. CHAKRAVARTI

Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, UNITED STATES OF AMERICA

Lumican and keratocan are major KS-bearing proteoglycans, while decorin and biglycan are chondroitin sulfate/dermatan sulfate bearing proteoglycans of the cornea. Recent studies are beginning to show that in addition to serving structural functions, these proteoglycans are present in the pericellular matrix from where they regulate various cellular functions including host innate immune defense. We have shown that mice deficient in lumican have less than normal proinflammatory response to bacterial endotoxin lipopolysaccharide (LPS)-induced keratitis and delayed corneal wound healing. We further investigated the signaling pathway affected by lumican-deficiency. Our study suggests that lumican is required for efficient response to LPS via the toll-like-receptor 4 pathway.

Symposium 3
EVER Sponsored Symposium
Saturday, 3 March 2007

S3.1
GENE THERAPY: A WAY TO GO FOR CORNEAL TRANSPLANTATION

U. PLEYER
 Charite, Humboldt University, Berlin, GERMANY

Purpose: Gene therapeutic strategies are currently under development for delivering genes to somatic cells. Since immune mediated corneal graft rejection remains a therapeutic challenge, we have been interested in new strategies to modulate the allograft immune response.

Methods: In a series of experiments we investigated the efficacy and toxicity of nonviral as well as adenoviral vectors to corneal cells. Several approaches to prevent allograft rejection including modulation of the Th1/Th2 cytokine balance, intervention on costimulatory and anti-apoptotic molecules were investigated.

Results: Significant prolongation of corneal graft survival could be obtained following different gene therapeutic strategies. Our results indicate that selection of the appropriate target for intervention is crucial and the combination of complementary approaches might be an interesting option for future developments.

Conclusions: Gene therapy may have great potential in a number of corneal disorders. Further investigations are not only directed to modulate the immune response, but also to improve biological properties of corneal transplants e.g. during organ culture prior to transplantation.

S3.2
A COHORT STUDY OF ENVIRONMENTAL PREDICTORS OF THE DEVELOPMENT OF MYOPIA

S.M. SAW
 Community Occupation and Family Medicine, National University Singapore MD3, Singapore, SINGAPORE

S3.3
THE BEIJING EYE STUDY: CLINICAL IMPLICATIONS FOR RETINA AND GLAUCOMA

J. JONAS
 Department of Ophthalmology, GERMANY

S3.4
BLOOD FLOW CHANGES IN PSEUDOEXFOLIATION SYNDROME AND GLAUCOMA

V. DAYANIR
 Adnan Menderes University, Aydin, TURKEY

Symposium 4
Cataract/Lens
Saturday, 3 March 2007

S4.1
CHRONIC POST-CATARACT SURGERY INFLAMMATION

S.P. CHEE
 Singapore National Eye Centre, Singapore, SINGAPORE

Inflammation following cataract surgery may be infectious or non-infectious. This talk discusses the clinical features that help to distinguish chronic endophthalmitis from other inflammatory syndromes.

S4.2
EYE MODELS FOR EVALUATION OF INTRAOCULAR LENSES

S. NORRBY
 AMO Groningen BV, Groningen, THE NETHERLANDS

Purpose: To analyse alternative bench eye models for evaluation of intraocular lenses.

Methods: The current international standard for evaluation of optical quality of IOLs (ISO 11979-2) prescribes an eye model with an aberration free cornea. Newer aspheric IOLs are not correctly evaluated in such an eye model because real corneas have positive spherical aberration. Several published eye models, recent corneal topography and clinical data were analysed by means of optical ray tracing to find out what amount of spherical aberration a representative eye model should have in the cornea.

Results: Newer corneal topography measurements of the anterior or both corneal surfaces, and pseudophakic eye wavefront measurements indicated an average coefficient of about $0.3 \mu\text{m}$ for a 6 mm pupil as the Zernike spherical aberration term, $Z(4,0)$, in the average population. If the anterior surface of the cornea is described as a conicoid of revolution with $Q = -0.14$ this amount of spherical aberration is obtained ($Q = 0$ for a spheroid and $Q = -1$ for a paraboloid; in between is a prolate ellipsoid). Two alternative eye models are proposed based on this finding. Calculated as well as measured results in terms of MTF curves will be presented. Measured IOL models include AMO 911A, AMO Z9000, Alcon SN60AT, Alcon SN60WF, B&L L161AO and Canon KS-3Ai.

Conclusions: The optical performance of IOL models in the context of an optical bench eye model is greatly influenced by the amount of spherical aberration in the cornea. In order to make justifying comparisons of aspheric IOLs, an eye model with the same level of spherical aberration as the natural cornea must be used. Newer results indicate the cornea has more spherical aberration ($Q = -0.14$) than hitherto generally assumed ($Q = -0.26$). Some reservation must be made for possible differences between ethnic groups. *CR

S4.3
BLUE LIGHT AND OCULAR TUMOUR

M.N. BURNIER
 Montreal General Hospital, Montreal, PQ, CANADA

Purpose: Little is known about the effect of blue light on inducing melanocytic malignant transformation. We chose to investigate the effect

of blue light (475 nm wavelength) on the proliferation rates of uveal melanoma cells. In addition, we tested two different intraocular lenses (IOLs) to determine the possible effects of UV absorbing and blue light filtering IOLs on the changes in proliferation.

Methods: Four human uveal melanoma cell lines (92.1, MKT-BR, OCM-1, SP6.5) were exposed to blue light with and without the presence of UV absorbing and blue light filtering intraocular lenses. Cells covered by aluminum foil were used as a control. The proliferation rate of the cells compared to a control was then assessed using the Sulforhodamine-B proliferation assay.

Results: Cells exposed to blue light showed a statistically significant ($p < 0.05$) increase in proliferation. Those exposed to blue light through a standard UV absorbing intraocular lens showed a smaller increase in proliferation, while those exposed with a blue light filtering intraocular lens showed no increase in proliferation over the control in all four cell lines.

Conclusions: The exposure of cells to blue light led to an increase in proliferation in all cell lines compared to the control. The use of blue light filtering IOLs abolished these increases in proliferation in the four cell lines. These results indicate that blue light filtering IOLs may have a protective effect on proliferation rates of uveal melanoma cells exposed to blue light.

S4.4

SCIENTIFIC PERSPECTIVES FOR PHASES OF PHACOEMULSIFICATION

K. JOHAR

Cellular and molecular biology, Iladevi Cataract and IOL Research Centre, Ahmedabad, INDIA

Phacoemulsification cataract removal surgery involves incision, capsulorhexis, hydro-dissection, lens aspiration and IOL implantation as the major phases. At Iladevi Cataract and IOL Research Centre, we are investigating scientific basis for different phases of phacoemulsification.

The phacoemulsification probe uses ultrasound energy to break cataracts into pieces. The bimanual technique uses this probe without an insulating sleeve (sleeveless) while micro-coaxial technique uses it with a microsleeve (sleeved). Our study in the rabbit eyes showed that sleeved probe phacoemulsification is better than sleeveless probe phacoemulsification in respect of abrasive and thermal damage to the corneal stroma.

Trypan blue staining of anterior capsule is performed to achieve better contrast during capsulorhexis in white mature cataracts. Our study on human eyes showed that trypan blue staining leads to decreased viability of LECs. This study suggests that use of trypan blue during cataract surgery may have harmful effects on other parts of the eye.

In order to separate the lens nucleus from the capsular bag, hydro-dissection is performed. Our study on the fresh cadaver eyes showed that if hydro-dissection is performed along with rotation of nucleus, it leads to removal of LECs and equatorial fibers. Therefore, the rotation of lens nucleus along with hydro-dissection may decrease the incidence of posterior capsular opacification (PCO).

At the end of cataract removal surgery, the stroma of corneal wound is hydrated in order to perform complete sealing of the wound. Our randomised clinical trial on the ingress of trypan blue in the anterior chamber, after hydration or no hydration of corneal stroma, showed that hydration leads to reduced ingress of trypan blue in the anterior chamber. This may reduce the risk of post-operative endophthalmitis.

To evaluate the bimanual and micro-coaxial type of phacoemulsification, we have studied the ingress of trypan blue into the

anterior chamber at the end of surgery. The ingress of trypan blue was higher in the bimanual than micro-coaxial technique.

This study suggests that the micro-coaxial technique is superior to bimanual technique in preventing ingress of ocular surface fluid into the anterior chamber. These scientific perspectives will lead to a better understanding of the surgical techniques and may help us to improve and evolve techniques for better outcomes.

S4.5

COMPARISON OF DIFFERENT FORMULAE FOR CALCULATING IOL POWER IN ASIAN EYES

W.H. CHUA

Refractive Surgery, Singapore National Eye Centre, Singapore, SINGAPORE

To date, there has been no prospective study conducted to evaluate the accuracy of the IOL power calculation formulae commonly in use. It is well established that the frequently used IOL formulas do not show significant differences when used in eyes of average axial length (i.e. between 22 mm and 25 mm) and it is at the extremes of axial lengths where discrepancies arise. The purpose of this study was to compare three IOL power calculation formulae and determine the most appropriate formula for accurate prediction of postoperative refractive error in Asian eyes with axial length less than 22 mm and greater than 25 mm that are undergoing phacoemulsification cataract surgery.

This was a randomised prospective study of patients with axial lengths less than 22 mm and greater than 25 mm undergoing phacoemulsification cataract surgery. Biometry was performed by a single operator using the Zeiss IOLMaster. Eyes that cannot be accurately measured by the IOLMaster e.g., those with advanced cataract, were excluded. Each patient was randomly assigned to have their IOL power calculated using one of three IOL power calculation formulae (SRK-T, Hoffer Q and Holladay 2). Standard phacoemulsification was performed with temporal clear corneal incisions. In all cases, the Alcon MA60 acrylic intraocular lenses were implanted in the capsular bag.

The primary outcome measure of this study was the mean absolute error (MAE), which was calculated by subtracting the intended formula-derived preoperative refractive error from the actual postoperative refractive error.

S4.6

INNOVATIONS AND MODIFICATIONS IN PHACO

T. AKAHOSHI

Mitsui Memorial Hospital, Tokyo, JAPAN

Innovations in the technology and technique open the new paradigm of the surgery and the modification makes it easy for everybody. The goal of cataract surgery, as I define, is to remove the cataract through the smallest incision and to implant a good IOL. It should be performed most efficiently and least invasively, safely as well. In this lecture I will reveal the story behind my innovations.

The technique of nucleofracture named Phaco Prechop, which divides the nucleus prior to the phacoemulsification, reduced the U/S energy and shortened the surgical time remarkably. The Knuckle Tip realised the safer removal of the nucleus as well as the cortex by a single procedure. The Nano and Ultra Sleeves farewellled bimanual phaco and realised the micro coaxial surgery.

New phaco tips for the OZil such as Square Tip enhanced the

efficiency of the phacoemulsification and Staright OZil Tips brought the torsional technology familiar to everybody. New Micro Coaxial I/A tips improved the maneuverability of the cortical removal through the micro incision. Modifications in the irrigating system has made the sub-2 mm coaxial surgery much easier and safer for every surgeon. The Counter Traction Implant Technique using a new injector handpiece realised a 6.0 mm IOL implantation through the same incision. Each invention had its own necessity to be innovated and my present surgery is built on the mount of these small inventions.

S4.7

INTRODUCTION: BIOMETRY SYMPOSIUM AND PRECISE OUTCOMES

R. YEOH

Singapore National Eye Centre, Singapore, SINGAPORE

S4.8

HIGH RESOLUTION IOL POWER CALCULATIONS

W.E. HILL

East Valley Ophthalmology, Mesa, AZ, UNITED STATES OF AMERICA

Over the last decade, the accuracy of intraocular lens power calculations has improved dramatically. With current technology, it is now possible to consistently attain highly accurate postoperative refractive outcomes normal eyes in a manner previously unknown. However, this high degree of accuracy can only be achieved through an in-depth understanding of how each technological piece of the calculation puzzle is utilised to the fullest.

The best attainable mean absolute error based on a review of the literature up to the year 2006 for keratometry, axial length measurement, IOL power calculation, IOL stability, and variations in retinal thickness will be reviewed in conjunction with the typical accuracy of modern intraocular lens manufacturing. By approaching IOL power calculations in a scientific manner, the clinician can now achieve a level of predictability previously unmatched. *CR

Symposium 5

Ocular Epidemiology/International Ophthalmology Saturday, 3 March 2007

S5.1

PATTERNS OF VISUAL IMPAIRMENT AND RELATED CAUSES IN ASIAN AND WESTERN POPULATIONS

T.Y. WONG

Centre Eye Research Australia, University of Melbourne, Melbourne, AUSTRALIA

Up to 20 million people are estimated to be blind in Asia, a figure that is expected to increase as the population ages. In Western populations, the prevalence of visual impairment and its major causes have been well described. In the past decade, several large population-based studies have provided new information on the epidemiology of visual impairment and the major eye diseases in Asia. These include epidemiological studies from China, India, Taiwan, Mongolia, Singapore and Japan. In particular,

the epidemiology of myopia and glaucoma has been well characterised and has shown significant differences in prevalence and risk factors as compared to Western populations. These racial/ethnic differences have provided important insights into the underlying causes of these conditions, as well as the relative public health implications of these conditions in different countries. In contrast, the epidemiology of cataract appears to be similar between Western and Asian populations. There are fewer population-based data on diabetic retinopathy and age-related macular degeneration in Asia, two conditions that are likely to have important racial/ethnic differences. New epidemiological studies in Asia will provide information on these conditions to allow clearer comparison of East-West differences.

S5.2

CLASSIFICATION OF ETHNICITY AND ITS IMPACT IN ASIAN AND NON-ASIAN POPULATIONS

I.G. MORGAN

ARC Centre of Excellence in Vision Science, Australian National University, Canberra, AUSTRALIA

Race or ethnicity can impact on health outcomes through genetic differences in populations, through cultural and social practices or socio-demographic characteristics associated with ethnicity, or through discriminatory practices towards particular racial or ethnic groups. There are genetic differences between populations of different geographical or continental ancestries, although it should be noted that between-group genetic variations account for only around 10% of total genetic variation in human populations, and are minor compared to within-group variations. It is also important to note that between-group variations are more likely to be seen in rare diseases, rather than in those with a high prevalence. In genetic terms, the population of Asia is extremely heterogeneous, with major populations derived from at least three major branches of the human family - Caucasian (South Asian and Middle Eastern), Northern East Asian (eg Mongolian, Korean and Japanese), Southern East Asian (e.g. Malay, Vietnamese, Burmese).

The Chinese population is derived from both the Northern East Asian and Southern East Asian lines. In addition, there is a diversity of cultural and social practices within Asia, and a diversity of stages of economic development between countries of the region. Studies of groups of specific geographical origin living in different physical, social and cultural environments, both within Asia and in migrant communities in other parts of the world, can be particularly informative about the contributions of genetic and physical, cultural and social environmental factors to health outcomes, particularly when detailed information is collected about the many factors that may be involved.

S5.3

RECENT FINDINGS IN THE PATTERN AND RISK FACTORS FOR GLAUCOMA IN POPULATIONS OF ASIAN AND OTHER DESCENT

P.J. FOSTER

Epidemiology, Institute of Ophthalmology, London, UNITED KINGDOM

This presentation will discuss recent findings in the prevalence and risk factors for glaucoma in populations of Asian descent, and compare and contrast these with data from European and African derived populations. *CR

S5.4

PATTERN AND RISK FACTORS FOR DIABETIC RETINOPATHY AND OTHER RETINAL DISEASES IN ASIAN AND OTHER POPULATIONS

P. MITCHELL

Ophthalmology, University of Sydney, Sydney, AUSTRALIA

S5.5

RECENT FINDINGS IN THE PREVALENCE AND PROGRESSION OF MYOPIA AND CHANGES IN EYE GROWTH IN CHILDREN IN POPULATIONS OF ASIAN AND OTHER DESCENT

L. HYMAN

Department of Preventive Medicine, Stony Brook University, Stony Brook, NY, UNITED STATES OF AMERICA

Myopia, an ocular disorder of major public health significance worldwide, is of particular concern in populations of Asian descent where it is a leading cause of low vision and blindness. Recent studies indicate a high prevalence of myopia among children in Asian countries such as Singapore, Taiwan, Hong Kong and Japan with rates as high as 40% for 7-9 year olds in Singapore and 57% for 11-12 year old in Hong Kong and suggest that these rates in these populations are increasing. In contrast, the prevalence of myopia among children in the United States, Australian and other non-Asian countries is less than half to one third of these levels. Similarly, myopia progression and changes in eye growth show similar patterns of more progression and more change in eye growth in Asian populations. The dramatic differences in myopia prevalence and progression between populations of Asian and non-Asian descent are likely to be due to complex and multiple variations in genetic and environmental factors among these groups. These differences will be described and possible explanations will be discussed.

S5.6

RECENT FINDINGS IN THE PREVALENCE OF REFRACTIVE ERROR IN ADULTS IN POPULATIONS OF ASIAN AND OTHER DESCENT

S.M. SAW

Community Occup & Family Med, National Univ Singapore MD3, Singapore, SINGAPORE

Purpose: To compare the prevalence rates of refractive errors in Asian and non-Asian adults.

Methods: The rates of myopia, astigmatism, hyperopia and anisometropia in Asian adults in the Singapore Malay Eye Survey of Malay (SIMES) adults aged 40 to 79 years, the Tanjong Pagar survey (TP) of Chinese adults aged 40 to 79 years and the Sumatra Eye Survey of Indonesian adults aged 21 years and above will be compared with the rates from similarly aged non-Asian adults in the Blue Mountains Eye Survey in Sydney, the Beaver Dam Eye Survey, the LA Latino Study, and the Barbados Eye Study. Comparisons, however, were limited by differing definitions, refraction techniques and age ranges of the population.

Results: The rates of myopia are 38.7% in the TP study, 26.2% in the SIMES study and 26.1% in Sumatra. However, the rates of myopia were lower in the Blue Mountains Eye Study (15%), LA Latino Study (16.8%), and the Barbados Eye Study (21.9%), and similar to the Beaver Dam Eye

Study (26.2%). Similar comparisons for hyperopia, astigmatism and anisometropia will be presented.

Conclusions: The rates of myopia are higher in Asian countries, possibly due to both genes, environment and gene-environment interaction.

S5.7

RECENT FINDINGS IN THE PREVALENCE AND RISK FACTORS FOR AGE-RELATED MACULAR DEGENERATION IN ASIAN POPULATIONS: SIMILARITIES AND DIFFERENCES WITH WHITE POPULATIONSJ.J. WANG¹, T.Y. WONG²*¹Centre for Vision Research, Department of Ophthalmology, University of Sydney, Westmead, NSW, Australia, ²University of Melbourne, Melbourne, AUSTRALIA*

Population-based studies conducted in the western world have long shown an apparent racial difference in the prevalence of age-related macular degeneration (AMD). The prevalence of both early and late AMD has been found to be lower in blacks or Hispanic whites compared to non-Hispanic whites. In the Multi-Ethnic Study of Atherosclerosis, the overall frequency of AMD varies from 2.4% in blacks, 4.2% in Hispanics, 4.6% in Chinese, to 5.4% in whites. Chinese Americans have the highest frequency of exudative AMD (age-sex adjusted odds ratio 4.30, 95% CI, 1.30-14.27, compared to whites).

Until recently, limited data from Asia were available for direct comparison. New studies from Japan and India indicate that the prevalence and incidence of early and late AMD in Japanese and Indian populations appears not to be substantially different from whites in the western world. In Chinese populations, the Beijing Study reported a very low prevalence of both early (1.4%) and late AMD (0.2%) in individuals aged 40+ years.

The few Asian population-based data on AMD risk factor pattern showed that the principle risk factors found in white populations (age, smoking and complement factor H) were also observed in Asians. One clinical study showed that the distribution frequency of complement factor H Tyr402His allele was similar between Indian and white AMD patients.

According to the Hisayama Study, the distribution of individual AMD lesions in Japanese appeared to be predominately soft distinct drusen (8.4%), while soft indistinct drusen (0.5%) and retinal pigmentary abnormalities (3.2%) were less frequently seen. This pattern is different compared to that reported from white populations of similar age (soft distinct drusen 10-16%, soft indistinct drusen 5-8% and pigmentary abnormalities 7-13%).

Limitations in methods across studies may have contributed to the previously reported difference in AMD prevalence between Asian and white populations. Standardised methodology and uniform AMD definitions (e.g., Wisconsin or International AMD classification system) are critical for new studies in Asia to determine whether there are real differences in AMD frequency and risk factor pattern between Asian and western populations.

S5.8

RATES AND CAUSES OF VISUAL IMPAIRMENT IN JAPAN

T. YAMAMOTO

Department of Ophthalmology, Gifu University Graduate School of Medicine, Gifu-shi, JAPAN

The purpose of this presentation is to show the rates and causes of visual impairment in Japan. Data obtained at a population-based cross-sectional

study subjected 3,021 persons are presented. Low vision and blindness were defined as BCVA in the better eye worse than 20/60 to a lower limit of 20/400 and worse than 20/400, respectively, according to the World Health Organization criteria. The overall prevalence of blindness according to the criteria was 0.14%. The primary causes were optic atrophy, myopic macular degeneration, retinitis pigmentosa, and uveitis. The overall prevalence of low vision according to the WHO criteria was 0.39%. The leading causes of low vision were cataract followed by glaucoma, and those of monocular blindness were myopic macular degeneration, glaucoma, and trauma. The prevalence of low vision and blindness in Japanese adults was one of the lowest among those reported.

S5.9

RATES AND CAUSES OF VISUAL IMPAIRMENT IN CHINA

M. HE

Preventive Ophthalmology, Zhongshan Ophthalmic Center, Guangzhou, CHINA

Data detailing prevalence of eye diseases in China have increased considerably over the last 20 years. We attempt to review the data on prevalence and causes of visual impairment, discuss their implications on eye care in China. Consideration is also given to limitations of methodology with the intention of identifying priorities for the future studies.

S5.10

COST OF VISUAL IMPAIRMENT IN AUSTRALIA: IMPLICATIONS FOR OTHER POPULATIONS IN THE ASIA-PACIFIC REGION?

H.R. TAYLOR

Centre for Eye Research Australia, University of Melbourne, East Melbourne, AUSTRALIA

Our studies have shown that vision loss cost Australia a total of AU\$9.85 billion in 2004 and ranks seventh in causes of loss of well-being. An intervention package to address avoidable vision loss would save \$5 for each \$1 spent and give a lifetime savings of AU\$911 million. Although specific for Australia, these data can help guide health care policy debate and the priority given to eye care in other developed economies.

Symposium 6 ARVO Sponsored Symposium Saturday, 3 March 2007

S6.1

IMMUNE ROLE IN AGE-RELATED MACULAR DEGENERATION AND IMPLICATIONS FOR THERAPY

R. NUSSENBLATT

National Eye Institute, Bethesda, MD, UNITED STATES OF AMERICA

A review of the present data will be reviewed dealing with the possible immune mechanisms leading to age-related macular degeneration. From this one can postulate immune therapies that could be possibly used.

S6.2

RETINAL REPAIR AND ITS STEM CELL INVOLVEMENT

M. TAKAHASHI

Laboratory for Retinal Regeneration, Center for Developmental Biology, Riken, Kobe, JAPAN

Regeneration in the mammalian retina is severely limited. Previously we demonstrated that in the adult mammalian retina, Müller glia de-differentiate and produce retinal cells, including photoreceptors, after acute injury in vivo. However, the number of newly generated retinal neurons is very limited.

Here we demonstrate that Wnt/beta-catenin signaling promotes proliferation of Müller glia-derived retinal progenitors and neural regeneration after damage or during degeneration. Wnt3a treatment increases proliferation of de-differentiated Müller glia more than twenty-fold in the photoreceptor damaged explanted retina.

Supplementation with retinoic acid or valproic acid induces differentiation of these cells primarily into photoreceptors. Activation of Wnt signaling by glycogen synthase kinase-3beta inhibitors promotes retinal regeneration, and conversely inhibition of the signaling attenuates regeneration. Notably, this Wnt3a-mediated regeneration of retinal cells also occurs in rd mice, a model of retinal degeneration.

These results provide the first evidence that Wnt/beta-catenin signaling contributes to CNS regeneration after damage. The remarkable promotion of retinal regeneration by Wnt3a suggests that the Wnt/beta-catenin pathway is a promising drug target for regeneration within the adult CNS subsequent to injury or degenerative diseases.

S6.3

NEW DEVELOPMENTS IN UNDERSTANDING THE PATHOGENESIS OF OCULAR VIRAL INFECTIONS

S.S. ATHERTON

Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA, UNITED STATES OF AMERICA

The purpose of this overview presentation will be to review new information relevant to understanding the pathogenesis of virus infections of the eye.

S6.4

NOVEL THERAPEUTIC MODALITIES FOR OCULAR SURFACE DISEASES BY REGENERATIVE MEDICINE

S. KINOSHITA

Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, JAPAN

The cornea is the transparent, main optical element of the eye that focuses light onto the retina, and its surface is covered by a non-keratinised, stratified epithelium that serves to protect the corneal interior from ingress by external agents. It is believed that corneal epithelial stem cells exist in the basal cell layer of the limbal region, the transitional zone between the cornea and conjunctiva on the ocular surface, and that the complete loss of corneal epithelial stem cells results in corneal scarring with vascularisation which severely affects visual function. This devastating diseased state is known as corneal epithelial stem cell deficiency, the treatment of which has remained unsuccessful worldwide until recently.

In efforts to solve this problem, our extensive research has focused on the development of basic understanding and novel therapeutic modalities for corneal epithelial stem cell deficiency, obtaining success in treating these corneas by regenerative medicine. Clearly, ocular surface reconstruction by tissue engineering using somatic stem cells will prove to be the next generation of therapeutic modalities.

Thus, we first investigated the transplantation of cultivated human corneal epithelial stem cells on amniotic membrane for treating corneal epithelial stem cell deficiency. The cultivated sheets were created by co-culture with 3T3 fibroblast, air-lifting, and corneal epithelial stem cell culture on amniotic membrane. These cultivated cells demonstrated the presence of keratin 3 and 12 specific to in vivo corneal epithelium, tight junction related proteins, and a reasonable amount of proliferative activity. Transplantation of cultivated autologous corneal epithelial stem cell sheets in 5 unilaterally affected patients was completely successful in all cases. Cultivated allogenic corneal epithelial sheets in 36 bilaterally affected patients also survived on the corneal surface in all cases, proving quite effective in achieving ocular surface stability. A few cases, however, developed immunological rejection, opportunistic infection, etc.

Second, and in order to solve the above-mentioned problem, we established a system for the transplantation of cultivated autologous oral mucosal epithelial sheets. The histology of the in vitro oral mucosal epithelial sheets was similar to that of in vivo corneal epithelial sheets. Moreover, the cultivated oral mucosal epithelial sheets expressed positive for keratin 3. Since the transplantation of these sheets survived on the ocular surface and resulted in the recovery of corneal transparency, cultivated autologous oral mucosal epithelium may become a viable substitute for diseased corneal epithelium. In fact, the outcome of our clinical trials involving 45 eyes that received this type of surgery has been a resounding success.

As a result of our diligent research in the field of regenerative medicine, both past and present, we have made enormous progress in the basic understanding and development of new therapeutic modalities such as the transplantation of cultivated mucosal epithelial stem cell sheets. We firmly believe that our efforts will lead to the cure of all devastating ocular surface disease in the near future. *CR

Symposium 7 Glaucoma Sunday, 4 March 2007

S7.1

CENTRAL CORNEAL THICKNESS & ITS ASSOCIATION WITH AGE, GENDER AND IOP - THE CHENNAI GLAUCOMA STUDY

L. VIJAYA

Ophthalmology-Glaucoma, Sankara Nethralaya, Chennai Tamil Nadu, INDIA

Purpose: To report the characteristics of central corneal thickness (CCT) and the association with age, gender, intraocular pressure (IOP) across the rural and urban populations.

Methods: The Chennai Glaucoma Study (CGS) is a prevalence study of adults aged 40 years and above in rural and urban populations of the southern Indian State of Tamil Nadu. As a part of comprehensive eye examination, CCT and IOP measurements were obtained. CCT was measured using DGH 550 ultrasonic pachymeter prior to any contact procedure and pupillary dilatation. Average of ten readings were recorded in microns. IOP was measured using a Goldmann applanation tonometer.

Only the right eye measurements were used for analysis.

Results: The mean CCT in rural population ($502.4 \pm 32.8 \mu\text{m}$) was significantly lower ($p=0.0001$) than the urban population ($520.2 \pm 32.4 \mu\text{m}$). Generally in both populations males had thicker corneas than females ($p=0.0001$; Rural - $507.1 \pm 33.7 \mu\text{m}$ vs. $498.5 \pm 31.5 \mu\text{m}$; Urban - $524.4 \pm 32.6 \mu\text{m}$ vs. $516.9 \pm 31.8 \mu\text{m}$). The gender difference was significant only up to 70 years of age. In both populations CCT was positively correlated with IOP and diabetes and had negative correlation with age and myopia. In the rural population, eyes with pseudoexfoliation had thinner corneas ($p=0.001$).

Conclusions: CCT in this South Indian urban population was significantly thicker than the rural population.

S7.2

T CELL-BASED VACCINATION FOR GLAUCOMA AS AN ADD-ON THERAPY FOR ANTI-HYPERTENSIVE DRUGS

M. SCHWARTZ

Neurobiology, Weizmann Inst of Science, Rehovot, ISRAEL

Over the last decade it has become widely accepted that glaucoma be viewed as a slowly progressive neurodegenerative disease in which neurons (retinal ganglion cells and their axons) that are still healthy are under threat of destruction as a result of the toxic conditions engendered by degenerating neurons that have already fallen victim to the disease. This means that at any given time, intervention that rescues the surviving neurons will slow down progression of the disease. This view of glaucoma prompted scientists to compare glaucoma with other neurodegenerative diseases in terms of mediators participating in disease progression, beyond the primary risk factors, and in terms of the mechanisms taking place, in an attempt to attenuate this spread of damage. Recognition that degeneration may progress in glaucoma by factors emanating from the damaged neuronal tissue irrespective of the primary risk factors have led us to search for self-compounds that are responsible for the spread of degeneration and for screening for pharmacological or physiological ways for halting the progression. Pharmacological approaches include the use of drugs that can neutralize mediators of toxicity (e.g., glutamate antagonists), or that can block their production (e.g., inhibitors of nitric oxide synthesis), or that can up-regulate survival genes and thereby make neurons more resistant to the threatening conditions. Our physiological approach involves the recognition of the immune system as the body's defense mechanism that requires boosting in a well-controlled way and development of a therapeutic vaccination as a way of boosting this physiological defense in a safe way. *CR

S7.3

AQUEOUS SHUNT DEVICES IN GLAUCOMA - CURRENT EVIDENCE AND FUTURE CHALLENGES

K. BARTON

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S7.4

A COMPARISON OF ADHERENCE TO GLAUCOMA MEDICAL THERAPY FOR PATIENTS ON ONE MEDICATION COMPARED TO TWO DIFFERENT MEDICATIONS USING A COMPUTERIZED MEDICATIONS EVENT MONITORING SYSTEM (MEMS) DEVICE

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Purpose: The importance of maintaining constant lowered intraocular pressure (IOP) in glaucoma treatment is borne out by the AGIS study. Thus, treatment adherence to ocular hypotensive therapy is key in preventing glaucomatous visual field loss. However, data on time-stamped use of ocular hypotensive therapy is sparse, especially with today's therapies. We evaluated the use of ocular hypotensive therapy in a population of patients in a private practice using MEMS devices.

Methods: Enrolled were 62 patients, half currently using prostaglandin (PG) monotherapy (1 Rx), and half currently using PGs and a second bottle of ocular hypotensive medication (2 Rx). All provided informed consent and were repeatedly told that their medication use was being monitored. Subjects were examined at 30 and 60 days, but no adherence feedback was provided.

Results: At entry, the 2 Rx group had slightly more severe glaucoma than the 1 Rx group (visual fields, cup-disc ratio). Overall, adherence with prescribed PG doses in both groups was excellent. In the 1 Rx group, 97% of patients had 5 or less dosing errors (missed and exceeded daily doses). This was similar to the patient adherence with PG doses of 90% in the 2 Rx group. However, patient adherence with the second medication dropped to 73% of patients having 5 or less dosing errors. Overall, patient adherence in the 2 Rx group was 63%. The timing of those doses was also variable and far from ideal. More than one-quarter of the inter-dose intervals were more than 2 hours late, compared with less than 10% of the prostaglandin dosing. Theoretical coverage (percent of time within the nominal dosing interval) was $85.6 \pm 12.6\%$, with 23% (7/31) of patients having less than 80% coverage. This compared with an average of 97% coverage for the prostaglandin. Most patients using b.i.d. or q.d. dosing (89%, 25/28) had a least one inter-dosing drug intervals for the second drug less than 10 hours (nominal b.i.d dosing is 12 hours). Approximately one-quarter of doses of α -adrenoceptor antagonists dosing intervals were less than 10 hours.

Conclusions: Time-stamped adherence data is was very useful in determining patients' behavior vis-à-vis dosing of ocular hypotensive medications. Even in a population in a private office who were aware of monitoring, adherence was an issue, particularly with patients using more than one bottle of glaucoma medication.

S7.5

LOCAL PENETRATION OF TOPICALLY INSTILLED DRUGS TO THE RETROBULBAR SPACE AND ITS CLINICAL IMPLICATION

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Several previous studies demonstrated that topically instilled anti-glaucoma drugs favorably affected posterior fundus or retrobulbar

circulation, which suggested that topically instilled drugs penetrated to the retrobulbar space and acted on the blood vessels supplying the optic nerve head (ONH) or posterior choroid. However, it has been difficult to measure the drug concentration in the retrobulbar space. Using the method of autoradiography, we could demonstrate that topically instilled iganidipine, a water-soluble DHP-derived calcium channel blocker, or nipradilol, a clinically used ocular hypotensive alpha-beta blocker, rapidly reached the retrobulbar space 30 minutes after the instillation by periorcular diffusion at a concentration of about 0.01 μM , which is sufficiently high enough for these drugs to exert vaso-dilative effects.

In accordance with the results of these autoradiographic studies, unilaterally instilled iganidipine significantly increased the ONH circulation in rabbits and monkeys only in the treated side without affecting intraocular pressure (IOP) or blood pressure, and unilateral instillation of nipradilol increased the ONH circulation only in the treated side without being correlated with IOP-lowering effect in normal humans. Similar findings were also obtained for topical latanoprost or unoprostone in normal humans.

Our results indicated that we can modify blood flow through vessels in the retrobulbar space and consequently the ONH or posterior choroid circulation, which may have clinical implications in treating eyes with ocular disorders associated with impaired circulation.

S7.6

NOVEL APPLICATIONS OF MICROPULSE LASER FOR GLAUCOMA MANAGEMENT

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S7.7

TREATMENT OF PRIMARY ANGLE CLOSURE GLAUCOMA BY SELECTIVE LASER TRABECULOPLASTY

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Purpose: To investigate the effects of Selective Laser Trabeculoplasty (SLT) as a treatment for Primary Angle Closer glaucoma (PACG).

Methods: All the visible pigmented trabecular meshwork of 45 eyes of 33 patients with PACG, a patent peripheral iridotomy and a clear view of at least 90 degrees of the meshwork was treated by SLT.

Results: The average baseline IOP was 24.1 ± 2.1 mmHg. In the 42 eyes that reached one month follow up, the average IOP was 19.6 ± 2.7 mmHg (19% reduction). At three months, the average IOP in 26 eyes was 19.3 ± 3.0 (19% reduction). Fifteen eyes reached 6 months follow up with an average IOP of 19.3 ± 1.8 mmHg (20% reduction). There was a reduction of 2 mmHg or more in 90% of the patients at one month, 85% at three months and 93% at six months. There were no significant complications.

Conclusions: When a sufficient extent of the angle is visible, SLT seems to be an safe, effective and simple method of reducing the IOP in eyes with PACG and a patent iridotomy. *CR

S7.8

ROCK INHIBITOR FOR THE TREATMENT OF GLAUCOMA

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A series of basic and clinical studies have been conducted to elucidate the intraocular pressure (IOP)-lowering effects and its associated characteristics of selective Rho-associated protein kinase (ROCK) inhibitors. Our results showed that administration of selective ROCK inhibitors resulted in a significant decrease in IOP in a dose-dependent manner. An increase in the outflow facility was observed in the treated rabbit eyes. Similar IOP-reducing effects were also found in monkey eyes. Pharmacokinetic analysis showed peak concentration at 2 hours in aqueous humor and at 4 hours in choroid/retina and iris/ciliary body. Also, autoradiogram demonstrated high radioactivities in eyelid, conjunctiva, cornea and iris/ciliary body. Administration of selective ROCK inhibitor also resulted in significant increase in relative blood flow at optic nerve head. In our studies using axotomy models, selective ROCK inhibitor induced larger number of regenerating axons revealed by retrograde labeling with fluorescence dye. From our results, we conclude that selective ROCK inhibitors may be a useful therapeutic modality for the treatment of glaucoma. At present, we are conducting clinical trials for elucidating the safety and usefulness of this compound for the treatment of glaucoma. *CR

S7.9

NEW INSIGHTS INTO CELL MATRIX CONTRACTION

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The process involved in tissue repair and remodeling play a part in most blinding diseases and is also vital for other processes such as development. Over the years, there have been different theories for the cell mediated contraction of tissue. Recent techniques, particularly in imaging, have given us new insights into the mechanisms of tissue remodeling and methods to control this process. This includes real time matrix and cellular imaging which will be demonstrated, as will the effects of various modulating strategies which may have therapeutic implications. *CR

S7.10

REGULATION OF EXTRACELLULAR MATRIX TURNOVER IN THE TRABECULAR MESHWORK

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S7.11

MORPHOLOGICAL CHANGES IN GLAUCOMATOUS EYES

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Eyes with primary open angle glaucoma (POAG), pseudoexfoliation glaucoma (PEXG) and steroid induced glaucoma all show an increase in

extracellular material (ECM) in the trabecular meshwork (TM). Even if the kind of ECM differs in the different glaucoma diseases, there is a correlation between the amount of ECM in the TM and axon loss in the optic nerve. In eyes with POAG there is a correlation between the so called sheath derived plaques (SD-plaques) in the subendothelial region of Schlemm's canal and axon loss, in eyes with PEXG a correlation between PEX material and axon loss. In eyes with POAG in the postlamina region of the optic nerve, the connective tissue septae are thickened, and there is an increase in type IV and VI collagen. In addition, the number of capillaries within the septae is decreased. In POAG eyes there is also a decrease in capillary density. This is not the case in eyes with PEXG. The standard deviation in eyes with POAG is larger than in PEXG, presumably due to subgroups within the POAG diseases. In about 50% of POAG eyes, TGF₂ is elevated in the aqueous humor. Treatment of human TM cells in monolayer cultures or perfused human anterior eye segments and human astrocytes with TGF₂ in concentrations comparable to those present in glaucomatous eyes indicate that both cell types can be stimulated to increase the expression of those ECM components seen in POAG eyes. These findings show that TGF₂ could be one factor involved in the pathogenesis of a subgroup of POAGs.

S7.12

PROSTAGLANDIN PHARMACOLOGY-LESSONS FROM THE MOUSE

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Background: The recent development of techniques that permit accurate measurement of IOP and aqueous humor dynamics in the mouse eye provides new avenues for investigating the molecular pharmacology of glaucoma medications.

Methods: The effect topical prostaglandins (PG) on IOP, aqueous humor dynamics and MMP expression was determined in FP receptor knockout and littermate wildtype mice.

Results: Single applications of topical PG reduced IOP in wildtype mice. IOP lowering was associated with a significant increase in outflow facility. No IOP lowering was observed in the FP knockout mouse. Repeat applications of PG led to increased MMP expression at the RNA level in wildtype but not FP receptor knockout mice.

Conclusions: Acute IOP lowering and MMP upregulation in response to topical PG therapy are critically dependent on an intact FP receptor.

S7.13

ENDOTHELIN AND EXPERIMENTAL OPTIC NEUROPATHY

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This work will review the importance of endothelin-1 and the expression of the endothelin B receptor in experimental optic nerve damage. *CR

S7.14

PREVALENCE STUDY OF PRIMARY GLAUCOMA IN 2006 IN SHUNYI DISTRICT OF BEIJING CITY

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Purpose: To estimate the prevalence of primary glaucoma in a population-based study and derive the estimate for the number of adults suffering glaucoma in China.

Methods: The venue of the Study was Shunyi District of Beijing. Sample Size was calculated to estimate anticipated 2% prevalence of primary glaucoma in 50 years old or above within error bound of 25% with 95% confidence. A random sample was obtained through cluster sampling of villages. The examinees received the basic eye examination and examinations to rule out glaucoma. Family and personal history of glaucoma was inquired. Peripheral anterior chamber depth was measured by Von Herick method. IOP was measured by Perkins handheld applanation tonometer. Gonioscopy by Goldmann lens and UBM in bright and dark situations were performed in necessary.

Results: The prevalence of glaucoma is 3.64%, among them, PACG is 1.68%, POAG is 1.77%, other glaucoma is 0.19%. 61.1% of glaucoma patients have vision impairment.

Conclusions: Glaucoma is a serious eye disease leading to blindness according to the prevalence and the visual function of glaucoma patients.

S7.15

ROLE OF LENS EXTRACTION IN CACG

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Purpose: To define the relative roles of lens extraction and trabeculectomy in chronic angle closure glaucoma (CACG).

Methods: Two randomized controlled trials on the treatment of CACG are currently in progress in Hong Kong. The first trial randomizes eyes with CACG and co-existing cataract into receiving either phacoemulsification or combined phaco-trabeculectomy. The second trial randomizes eyes with CACG but no cataract into receiving either phacoemulsification or trabeculectomy. Intraocular pressure control, additional surgical interventions, and surgical complications are the main outcome measures.

Results: In eyes with CACG and co-existing cataract, combined phaco-trabeculectomy is associated with a lower requirement for glaucoma medications up to 18 months after surgery. However, more additional surgical interventions, in particular interventions to maintain filtration, and also more surgical complications, occur in the combined phaco-trabeculectomy group.

Conclusions: Data from these 2 randomized controlled trials will eventually move us one step closer to defining the relative roles of lens extraction and trabeculectomy in CACG patients.

S7.16

IS IRIDOTRABECULAR CONTACT A DISEASE THAT SHOULD BE TREATED

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Iridotrabecular contact (ITC) was recently agreed by a panel of experts (AIGS Consensus Committee on Angle-Closure Glaucoma) to be a pathological phenomenon that requires treatment. Hence, it is regarded as the defining characteristic of angle-closure glaucoma. This presentation examines the evidence for this presumption, discusses the implications for management of cases of suspected angle-closure, and aims to identify areas requiring further research.

S7.17

SCREENING FOR ANGLE CLOSURE IN SINGAPORE

T. AUNG

*Glaucoma, Singapore National Eye Ctr, Singapore, SINGAPORE***Symposium 8
Retina (I)
Sunday, 4 March 2007**

S8.1

THE NATURAL HISTORY AND PROGNOSIS OF NEOVASCULAR AGE-RELATED MACULAR DEGENERATION

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Purpose: To describe the natural history and progression of visual loss in eyes with untreated neovascular age-related macular degeneration (AMD).

Methods: A systematic review of the literature from 1980 to August 2005 was performed. Studies reporting disease progression outcomes for untreated patients with neovascular AMD were included. Outcome measures were summarized using simple counts and means. Random effects meta-analyses were conducted and tests of heterogeneity were performed where appropriate. Main outcomes analyzed were changes in visual acuity loss, development of co-morbidities and fellow eye involvement.

Results: 53 primary studies were included, with a total of 4362 untreated neovascular AMD patients. Nearly half of the studies (28) were randomized clinical trials. The quality of the studies was high with over 80% providing level I or II evidence. Mean baseline visual acuity (VA) among study patients was 0.64 LogMar (~20/87 Snellen). The mean visual acuity change in LogMar progressed from a change of 0.1 (1 line lost) at 3 months to a change of 0.3 (2.7 lines lost) after 12 months and 0.4 (4 lines lost) after 24 months. The proportion of patients who developed severe vision loss (>6 lines) from baseline increased from 21.3% at 6 months to 41.9% by 3 years. The proportion of patients with worse VA than LogMAR 1.0 (20/200 Snellen) increased from 19.7% at baseline to 75.7% by 3 years. Neovascular AMD developed in the fellow eye in 12.2% of patients by 12 months and in 26.8% by 4 years. Meta-analyses of vision outcome by subtype of neovascular AMD was not possible.

Conclusions: A doubling of the visual angle of presenting visual acuity may be expected to occur in the year after initial presentation in eyes

with untreated neovascular AMD. Data are scarce on the development of co-morbidities over time. No conclusions can be drawn on the differences in rates of disease progression by neovascular AMD subtype. The diversity of reporting formats, paucity of long-term natural history data, and heterogeneity among the reported clinical studies impose limits to the clear understanding of long term prognosis for visual function in neovascular AMD. *CR

S8.2

YOU ARE WHAT YOU EAT: NEW AND OLD NUTRITIONAL RELATIONSHIPS WITH AGE-RELATED MACULAR DEGENERATION

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S8.3

RISK FACTORS OF AGE-RELATED MACULAR DEGENERATION IN INDIA

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Purpose: The age-related macular degeneration risk factors were studied in two different studies. The factors studied were smoking, systemic diseases, and Complement factor-H (CFH), Toll-like Receptor 4 (TLR4), and APOE gene polymorphisms.

Methods: The above associations were studied in two different studies and population. The smoking and systemic factors association was studied in a population based study, the Andhra Pradesh Eye Disease Study (APEDS) involving 10,000 individuals in pre selected clusters in urban and rural Andhra Pradesh in south India. The second was a hospital based study that screened 100 AMD patients and 120 normal controls. Statistical analysis included Odd's ratios, univariate and multivariate analysis and calculation of allele, genotype and estimated haplotype frequencies.

Results: The age-gender prevalence of AMD was 1.82% (95% confidence interval 1.35% -2.25%). There was 1.05 odds of increasing AMD with increasing age. Smoking was associated with AMD (odds 2.8) and the population attributable risk (PAR) was 10-14% for smoking and 10% for hypertension. A significant association was noted with the Tyr402His (CFH) variant amongst AMD cases ($p=2.77 \times 10^{-6}$). Individuals homozygous for the minor allele "CC" had a significantly higher risk ($p<0.0001$) of AMD (OR=22.2; 95%CI, 19.6-24.8) than those carrying a single copy of the "C" allele (OR=1.4; 95%CI, 0.78 -2.58), even after adjusting for age, gender and diabetes. Distributions of genotype frequencies for APOE polymorphisms in patients were not significantly different from the controls ($p=0.76$). The $_2$ allele was slightly higher in the cases, while the $_4$ allele was higher in controls; the carriers of $_4$ allele had a reduced risk ($p=0.03$) of AMD (OR=0.42, 95%CI, 0.19-0.91). TLR4 did not exhibit any association with AMD.

Conclusions: The prevalence of AMD in south India is no different than that reported in other populations. Cessation of smoking and reduction of hypertension could reduce the risk of AMD. CFH polymorphism Tyr402His appears indicative of AMD pathogenesis. Diabetes, age and gender in presence of the homozygous "CC" genotype in CFH carry an increased risk of AMD. Hence this polymorphism could be used as a potential marker for predictive testing across continents.

S8.4

PREVENTIVE STRATEGIES FOR AGE-RELATED MACULAR DEGENERATION

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Recent advances in our understanding of age-related macular degeneration (AMD) have prompted a need to reassess our approach to fighting blindness from this sight-threatening condition. Traditionally, physicians have played a key role in the diagnosis and treatment of AMD patients and have referred those who are afflicted with irreversible visual impairment and blindness for visual rehabilitation. More recently, new insights into the natural history and risk factors of AMD as well as recent evidence from animal and clinical studies have suggested strategies for possible primary and secondary prevention of AMD.

Primary prevention is preventing a disease from occurring in the first place. Any primary preventive strategy will have to raise the awareness of AMD and its modifiable risk factors so that at-risk behaviors can be changed to reduce the risk of AMD development or progression. For example, it has now been firmly established that cigarette smoking is positively associated with AMD development and progression in a dose-dependent fashion, and smoking cessation reduces the risk of developing AMD to that of non-smokers after 20 years. Anti-smoking campaigns to encourage smoking cessation and discourage non-smokers from starting the habit are therefore potentially helpful to reduce AMD development and progression. A diet rich in green leafy vegetables, which contain antioxidants and macular pigment, has also been found to reduce the risk of AMD. Unfortunately, the awareness of AMD and its risk factors among the general public is currently low, and more efforts are necessary to raise the awareness of AMD and its modifiable risk factors.

Secondary prevention is preventing a disease from getting worse after the onset of the condition. A major multi-center randomized controlled trial has shown that for patients with moderate AMD or AMD in one eye, supplementation with a combination of antioxidants (vitamin C, vitamin E and beta-carotene) and zinc can reduce the risk of progression to advanced AMD. It has been recommended that physicians should screen persons 55 years and older with regular dilated fundus examination to determine their risk of developing advanced AMD. Those at risk should consider nutritional supplementation to reduce this risk. The use of Amsler grid or preferential hyperacuity perimetry to monitor for metamorphopsia suggestive of choroidal neovascularisation so that treatment can be instituted early will also help to reduce morbidity from wet AMD.

In conclusion, the increasing impact of AMD, coupled with the limited therapy available for its treatment, has led many investigators to search for factors that could be modified to prevent the onset or alter the natural course of AMD. The identification and modification of risk factors has the potential for greater public health impact on the morbidity from AMD than the few treatment modalities at hand. There is currently sufficient evidence to include a preventive strategy in our fight against blindness from AMD.

S8.5

OCULAR ANGIOGENESIS TREATMENT - ASIAN PERSPECTIVE

C.L. ANG

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Understanding the pathogenic pathway for ocular neovascularization has led to the discovery of new methods for treatment of choroidal

neovascularization (CNV) and other retinal neovascular diseases. Inhibition of vascular endothelial growth factor (VEGF), has demonstrated that the neovascularization process can be retarded or reversed. The development of various VEGF inhibitors has found uses in the treatment of choroidal neovascularization, and some novel uses like proliferative diabetic retinopathy and neovascular glaucoma. We present our experiences in an Asian population.

S8.6
SYMMETRY OF AGE-RELATED MACULAR DISEASE

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Symmetry of phenotype between eyes of an individual with respect to changes in early AMD including drusen type, distribution and fluorescent characteristics on fluorescein angiography implying that these characteristics reflect risk factors including environment and genetic pressures. Confirmation that genetics factors are important is illustrated by concordance of these characteristics between siblings being greater than would be seen by chance alone and the greater concordance between monozygotic than dizygotic twins. Concordance of late complications exists but is less pronounced.

Conclusions: Segregation of patients according to phenotype should aid genetic research in AMD.

S8.7
HYPOXIA AND THE BLOOD/ RETINAL AND BLOOD/ BRAIN BARRIERS

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Retinal hypoxia has been demonstrated in early diabetic retinopathy, experimental retinal vein occlusion as well as exposures to high altitude. Since hypoxia leads to vascular leakage, we aimed to examine the disruption of blood retinal barrier (BRB) in hypoxic retina. Adult Wistar rats were subjected to hypoxia and the mRNA and protein expression of vascular endothelial growth factor (VEGF) and nitric oxide (NO) were carried out. VEGF is known to increase the permeability whereas NO is involved in dilatation of the blood vessels in response to hypoxia. The permeability of the blood vessels was assessed by injecting rhodamine isothiocyanate (RhIC) and horseradish peroxidase (HRP) intraperitoneally or intravenously after hypoxic exposure. The leakage of both tracers was then followed in the retina. In addition, the expression of aquaporin 4 (AQ4), a water transporting protein, was investigated in the retina as it has been recently reported that that AQ4 participates in edema formation. Along with the above, ultrastructural changes in the Müller cells and astrocytes were examined as these cells have been reported to participate in the maintenance of the BRB. Significant increase in the mRNA and protein expression of VEGF, NO and AQ4 along with leakage of RhIC and HRP was observed in the hypoxic retina. Astrocytes and Müller cell processes contained HRP reaction products. Administration of melatonin in hypoxic rats proved to be beneficial in maintaining the integrity of BRB in hypoxic conditions as it suppressed the VEGF concentration and NO production in the retina and reduced leakage of RhIC.

S8.8
THE CONTRIBUTION OF PHOTORECEPTOR METABOLISM TO OCULAR ANGIOGENESIS

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Two hours of hypoxia in the rat results in a breakdown of the inner blood retinal barrier and an accumulation of tracer dye (ruthenium isothiocyanate) and of angiogenic factors (VEGF, IGF, eNOS, iNOS, nNOS) in the inner and outer segments of the retinal photoreceptors. In the pig, retinal capillary ischemia induced by embolisation with fluorescent microspheres causes a similar accumulation of angiogenic factors in the photoreceptor inner and outer segments but not in the photoreceptor cell bodies. The photoreceptor inner segments with their high concentration of mitochondria have the highest metabolic demands that may not be met in hypoxic conditions. The photoreceptors and particularly their outer segments appear to act as a sink for the accumulation of angiogenic factors in the hypoxic retina. This is of relevance to the etiology of intraocular angiogenesis and to the mode of action of panretinal photocoagulation in the treatment of neovascularisation.

S8.9
PATHOGENIC MECHANISM OF COMPLEMENT FACTOR H (CFH) AND A₄₂-MEDIATED INFLAMMATORY SIGNALING IN ALZHEIMER'S DISEASE (AD) AND AGE-RELATED MACULAR DEGENERATION (AMD)

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Purpose: Alzheimer's disease (AD) and age-related macular degeneration (AMD) are respectively, the most common causes of senile dementia and central vision impairment and blindness in our aging population. Deficiencies in complement factor H (CFH; an anti-inflammatory, complement protein) and down-regulation of soluble amyloid precursor protein alpha (sAPP_α; a monomeric, neuroprotective protein), coupled to up-regulation of amyloid-beta (A₄₂) peptide, are characteristic of both AD and AMD. While A₄₂-peptides are abundant in both AD amyloid and amyloid-like drusen of AMD, CFH and sAPP_α presence is lowered. Elevated cholesterol, a known risk factor for the development of both AD and AMD, may contribute to these pathogenic effects. These studies were undertaken to further our understanding of the molecular mechanisms involving CFH, sAPP_α, A₄₂ peptides and β -secretase activities to inflammatory signaling and cellular degeneration.

Methods: Cell culture including control and stressed (5% hypoxia or 20 ng/ml IL-1 α) retinal pigment epithelial (RPE) cells, human brain microvascular endothelial cells (hBMEC), retinal choroidal endothelial (RF/6A) cells, human neural (HN) cells in primary culture, AD and age-matched control temporal lobe neocortex and primary visual cortex, AMD-affected and control human retina. Techniques used were confocal microscopy, DNA array analysis (Affymetrix and Oligo GEArray), RT-PCR, Northern and Western immunoassay, ELISA, bioinformatics and statistical analysis.

Results: Secretase-mediated cleavage of beta-amyloid precursor protein (β -APP) generates a series of neurotrophic or neurotoxic amyloid peptides while depleting plasma membranes of β -APP. Pathogenic stress factors divert production of sAPP_α peptides to amyloidogenic A₄₂-peptide

species in human neurons and also in RPE, hBMEC and RF/6A cells. In vitro, cholesterol and 24S-HC were found to intensify this pathogenic response.

Conclusions: APP holoprotein depletion, decreased CFH and sAPP abundance and increases in α -secretase-mediated A₄₂-peptide abundance may contribute to common pathogenic mechanisms leading to age-related, inflammation-mediated degeneration not only in AD brain, but also in human retinal disease.

S8.10

COMPLEMENT FACTOR H AND POLYPOIDAL CHOROIDOPATHY

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S8.11

NATURALLY OCCURRING ANTI-ANGIOGENESIS FACTORS

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The biological control of angiogenesis is beginning to be elucidated, with a result that several anti-angiogenesis therapies are now undergoing clinical evaluation for retinal diseases such as neovascular age-related macular degeneration (AMD) and proliferative diabetic retinopathy (PDR). These therapeutic approaches are typically aimed at neutralizing pro-angiogenic molecules like vascular endothelial growth factor (VEGF). However, a number of naturally occurring anti-angiogenesis factors appear in the retina and these factors also are being investigated as possible pharmacological avenues.

One of the first naturally occurring anti-angiogenesis factors to be discovered was the matricellular protein thrombospondin (now known as thrombospondin 1 or TSP1). Since this discovery, various other naturally occurring anti-angiogenesis factors have been found such as the closely related molecule thrombospondin 2 (TSP2). Other molecules with apparent anti-angiogenic activity include the proteolytic derivatives endostatin and angiostatin (from collagen XVIII and plasminogen respectively), the adamalysins ADAMTS 1 & 8 (A Disintegrin And a Metalloproteinase with ThromboSpondin motifs), pigment epithelium-derived factor (PEDF), and the recently discovered small leucine-rich repeat proteoglycan (SLRP) opticin. Interestingly, a splice variant of VEGF also possesses anti-angiogenic properties: the variant VEGF(A)_{165b} inhibits VEGF(A)₁₆₅-mediated vascular endothelial cell proliferation and migration. Several of the above molecules are produced and/or are present in and around the retina. For example, retinal pigment epithelial cells are capable of synthesizing TSP1, TSP2, ADAMTS-1 and ADAMTS-8 while opticin accumulates particularly at the vitreoretinal interface. Furthermore, there is mounting evidence that local reduction of TSP1, at least, favors the development of new blood vessels in retinal or chorioretinal pathology. Such observations suggest that therapies based on TSP1 or other naturally occurring anti-angiogenic molecules should be of use in conditions like AMD and PDR.

S8.12

A NOVEL ANTI-ANGIOGENIC STRATEGY SUPERIOR TO AND COOPERATIVE WITH VEGF - A INHIBITION

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Purpose: The purpose of this study was to study the effect of inhibiting the expression of RTP801, a novel HIF-1 α -responsive gene in the laser injury induced model of choroidal neovascularization (CNV).

Methods: CNV was induced by laser injury in C57BL/6J, RTP801^{-/-} and ^{+/+} mice, and volumes measured 14 days later by confocal evaluation. Two short interfering RNAs against RTP801 (RTP801i), inactive anti-RTP801 siRNA (negative control), PBS, VEGF-A165 aptamer (Macugen), anti-VEGF-A neutralizing antibody (Ab) or mouse IgG were injected into the vitreous on the day of injury and 7 days after the injury. Real-time RT-PCR analysis of gene expression profiles in the neural retina and RPE/choroid was performed before and after laser injury. Flow cytometry was used to quantify choroid infiltrating macrophages and neutrophils.

Results: RTP801^{-/-} mice displayed 30% reduction of CNV volume compared with RTP801^{+/+} mice (p=0.003). RTP801i suppressed gene expression in vivo both in RPE and in sensory retina. Both RTP801i significantly (up to 75%) reduced CNV volume in a dose-dependent manner and to a significantly greater degree than Macugen (p=0.015) or anti-VEGF-A neutralizing Ab (p=0.016). RTP801i cooperated with anti-VEGF-A Ab and with Macugen in reducing both CNV volume and leakage. RTP801i significantly induced mRNA of PEDF in neural retina and reduced VEGF-A164, MIP2, MCP-1 and PKC β 2 mRNAs in RPE/choroid. RTP801i decreased maximal neutrophil and macrophage infiltration into the choroid to a significantly greater extent than either anti-VEGF-A Ab or Macugen.

Conclusions: RTP801i is a novel strategy to inhibit CNV in age-related macular degeneration that displays efficacy both as monotherapy and in combination with anti-VEGF-A agents. Its therapeutic effect may result from suppressing inflammation and angiogenic signaling as well as from promoting anti-angiogenic cytokines. *CR

Symposium 8

Retina (II)

Sunday, 4 March 2007

S8.13

MULTIMODAL IMAGING OF THE RETINA USING A COMBINED SYSTEM FOR SLO, OCT AND MFERG

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Structural and functional imaging techniques such as multifocal ERG and optical coherence tomography are establishing themselves as powerful methods for the assessment of a wide range of retinal disorders. Here we present a high resolution multimodal imaging technique using combined optical coherence tomography (OCT), scanning laser ophthalmoscopy (SLO) and multifocal ERG. The OCT/SLO is a relatively new technique that provides high-resolution and high definition images of the surface anatomy of the fundus using a combination of confocal imaging principles of SLO as well as high resolution tomographic images of subsurface tissue structure using OCT. We present a modification of the system by including an organic light emitting diode micro display within the optics of the optical coherence tomography system to provide simultaneous micro mFERG and

OCT/SLO imaging of the macula. The display allows a maximum luminance of 820cd/m² and can be driven at rates of up to 85Hz with a fast rise time of 1msec. This new technique should provide improved resolution and greater accuracy in the assessment of retinal disorders without compromising the capabilities and flexibility of either technology.

S8.14

POSTERIOR HYALOID IN AGE RELATED MACULAR DEGENERATION - AN US- OCT STUDY

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Purpose: Age related macular degeneration (AMD) is a major cause of legal blindness in elderly people. There are multiple theories of the etiology of AMD including senescence, ischemia, oxidative stress and genetic factors. There is also increasing knowledge of the events causing AMD. The role of the posterior hyaloid in the pathogenesis of neovascular AMD is not yet examined. It was the aim of this study to compare the behavior of the posterior hyaloid in neovascular and non neovascular AMD.

Methods: Eyes of patients over 55 years were assigned to one of three subgroups (neovascular AMD: group 1, non neovascular AMD: group 2, healthy macula: control group, group 3). Besides clinical examinations OCT scans were performed including 6 radial lines through the fovea, additional lines through the upper and lower arcade and radial lines through the optic disc as well as B-scan ultrasound.

Results: 50 eyes with neovascular AMD and 57 with "dry" AMD and 56 eyes of the control group out of 163 eyes enrolled could be included. 33.6% of the patients were male, 66.4% were female. The mean age was 74 years (range: 55-89 years). There was equal distribution of gender and age in all subgroups. 34.0% of the eyes in group 1 and 71.9% of the eyes in group 2 and 60.7% of group 3 had a complete detachment of the posterior hyaloid in ultrasound. A partially detached posterior hyaloid with a tight adhesion in the centre surrounded by a detached vitreous surface was detected in the OCT in 38% of group 1 and 7% of group 2 and 10% of group 3.

Conclusions: There was a significantly higher incidence of complete vitreous detachment in eyes with "dry" AMD. There was a significantly higher incidence of a central adhesion surrounded by a flat detachment of the posterior hyaloid in neovascular AMD in the OCT. Pathogenetic factors of neovascular AMD might cause these tight adhesions. However, the attached posterior vitreous might be a possible pathogenetic factor by itself. Vitreoretinal traction or the more intense exposure to cytokines or free radicals in the vitreous gel could be etiologic factors.

S8.15

GENE TRANSFER IN THE TREATMENT OF OCULAR ANGIOGENESIS

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S8.16

INTRAVITREAL TRIAMCINOLONE VERSUS BEVACIZUMAB FOR TREATMENT OF INTRAOCULAR EDEMATOUS AND NEOVASCULAR DISEASES

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The advantages and disadvantages of intravitreal triamcinolone acetonide versus bevacizumab for the treatment of intraocular edematous and neovascular diseases such as exudative age-related macular degeneration, proliferative diabetic retinopathy, diabetic macular edema, retinal vein occlusions, and uveitis will be discussed, with respect to the anti-edematous effect, anti-angiogenic effect, cataractogenesis and increase in intraocular pressure. *CR

S8.17

TRIAMCINOLONE IN DIABETIC MACULAR EDEMA

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S8.18

REVERSAL OF VISUAL LOSS IN A PROGRESSIVE INHERITED MACULOPATHY FOLLOWING AUTOLOGOUS FULL THICKNESS CHOROIDAL - RPE TRANSPLANTATION

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Purpose: Inherited macular dystrophies result in irreversible loss of central vision. RPE degeneration often accompanies the retinal changes and it is not always clear whether the primary degeneration occurs in the neuroretina or the RPE. The current treatment was based on the observation that central vision remained good for decades but then suddenly dropped over months. This may represent a period of time in which the neuroretina is still viable but is dysfunctional secondary to loss of RPE support. As such a window of opportunity exists to salvage function from the existing photoreceptor pool before irreversible loss of function occurs. Thus, RPE transplantation at this point may restore recently lost retinal function. We describe a case of progressive inherited macular dystrophy with reversal of vision loss following full thickness autologous choroidal-RPE transplantation.

Methods: Patient: 53 years; caucasian; male; progressive macular degeneration over several years. Acuity at presentation was 20/30 bilaterally and declined to 20/120 in the year preceding surgery. Vision declined in the left eye 6 months prior to the right with eventual loss of reading ability. Surgery: The technique employed was a modification of that described by van Meurs (2003).

Results: Surgery: Uncomplicated full thickness autologous choroidal-RPE transplantation was performed (right eye). The graft remains viable and perfused at 12 months follow up. The patient demonstrates excellent foveal fixation. Reading speed recovered from 0 wpm preoperatively to 57 wpm at logMAR 0.6 postoperatively. Multifocal ERG demonstrated improved function of the retina over the graft site as compared to preoperative levels with no change in areas of retina that had not been grafted.

Microperimetry demonstrated areas of normal retinal sensitivity within the graft site.

Conclusions: We report a successful transplantation of full thickness choroid-RPE in a case of recent vision loss in the second eye of a patient with progressive inherited macular dystrophy. Imaging demonstrated a viable perfused graft with significant restoration of retinal function, partial restoration of reading ability and stabilization of retinal degeneration. This case demonstrates the potential for recovery of neurosensory function following RPE transplantation. It may be possible to salvage function further if it is performed earlier with a larger pool of viable photoreceptors and with an improved quality of RPE. This case demonstrates for the first time a treatment for macular dystrophy in a currently untreatable group of conditions.

S8.18

ASSESSMENT OF READING BEHAVIOR WITH AN INFRARED EYETRACKER FOLLOWING 360 DEGREE MACULAR TRANSLOCATION FOR AGE RELATED MACULAR DEGENERATION

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Purpose: Difficulty in reading is common in central visual loss from macular disease and is linked to reduced quality of life. Translocation (MT360) has been

used to restore reading in wet age related macular degeneration (AMD). In this study we aim to show the potential of MT360 in restoration of normal vision and reading behaviour. MT360 is an invasive and involved procedure and documenting the restoration of close to normal function is valuable. We observe the 6 best outcome cases from a series of 23 patients treated with MT360. We investigate the influence of MT360 as a treatment (new foveal location, muscle surgery, torsion) on reading behaviour (number, direction, and velocity of saccades) and fixation characteristics (preferred retinal loci (PRL) and stability of fixation). Outcome measures from the MT360 cohort were compared to age matched normals and patients with untreated dry + wet AMD. The retinal sensitivity at the new foveal location was examined using microperimetry to establish the threshold visual function.

Methods: 6 patients (aged 61-74) underwent the following assessment: ETDRS Acuity / Reading Acuity and Speed, Critical Print Size / Contrast Sensitivity / Eyetracker Assessment of Saccadic Behaviour / Fixation Stability / Microperimetry.

Results: 6 patients showed a significant improvement in all aspects of visual function compared to preMT360. The MT360 cohort showed better performance in all parameters compared to the dry + wet AMD controls but poorer performance than the normal group. Although not all parameters reached significance the common trend was for performance to advance: normal > MT360 > dry > wet. Of note, reading speed, fixation quality, and number of PRL of the MT360 cohort were found not to be different from the normal controls. The saccadic behaviour of the MT360 cohort displayed a greater number of horizontal saccades, with longer latency and reduced velocity as compared with all other groups. Microperimetry showed that patients 1, 3 + 4 recovered normal foveal sensitivity and displayed the best visual outcomes yet displayed the worst preoperative visual function.

Conclusions: This study suggests MT360 can significantly restore visual function beyond that of untreated AMD. This recovery approximates closely normal function and matches it in reading speed and quality of fixation.

Despite this eyetracker analysis indicates the effect of this complex surgery, in particular the extraocular muscle surgery, negatively influences the saccadic behaviour when reading. Finally microperimetry suggests a healthy preoperative neurosensory retina is required to rescue function and not necessarily good preoperative vision.

S8.19

EFFECTS OF ADVANCED GLYCATION ENDPRODUCTS AND OXIDATIVE STRESS ON RAT RETINAL ORGAN CULTURE AND NEUROPROTECTION BY BRIMONIDINE

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Purpose: Many diseases of the eye lead to neurodegeneration, especially of the retinal ganglion cells (RGCs). Two common stressors of neurons are the formation of reactive oxygen species (ROS) and advanced glycation endproducts. These mechanisms may be involved in degeneration of RGCs in glaucoma and are linked to the risk of diabetic neuropathy, diabetic retinopathy, age-related macular degeneration. The present study was undertaken to elucidate the effects of glyoxal and hydrogen peroxide (H₂O₂) on rat retinal organ culture and to test the neuroprotectant effects of the alpha-2-adrenergic agonist brimonidine.

Methods: Retinae were treated with medium (control), glyoxal, H₂O₂ or glyoxal + brimonidine. For intracellular pH measurements, retinae were incubated with BCECF-AM prior to measurement of the emission ratio at two wavelengths. For semiquantitative analysis of the mitochondrial membrane potential (mmp), cells were incubated with JC-1 and changes were investigated with fluorescence microscopy. For detection of ROS retinae were incubated with carboxy-H₂DCFDA and the ROS-content observed with confocal laser scan microscopy. The ultrastructure of the mitochondrial double membrane in the multipolar ganglion cell layer near the cell nucleus was investigated by transmission electron microscopy.

Results: In our post vivo model we found a manifest damage of the RGCs after treatment with glyoxal and H₂O₂. All monitored parameters changed so as to indicate the occurrence of cell stress (ROS increase, pH and mmp decrease, signs of ultrastructural damage in mitochondria). The additional application of Brimonidine significantly inhibited all these parameter changes.

Conclusions: Our results show that the rat retinal whole mount model is an ideal model to investigate early intracellular damage. Further, these results are helpful in understanding the effects of neuroprotective drugs and how best to apply them in the treatment of glaucoma. The mechanism of the neuroprotective effect of brimonidine is currently unknown - however, time courses in our cell stress parameters point to mitochondria as possible sites of action.

S8.20

ASSOCIATION BETWEEN HIGH GLUCOSE-INDUCED OVEREXPRESSION OF EXTRACELLULAR MATRIX COMPONENTS AND INTEGRIN EXPRESSION IN MICROVASCULAR ENDOTHELIAL CELLS

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Purpose: To determine whether downregulation of high glucose-induced fibronectin (FN) and collagen type IV (Col) overexpression will be

accompanied by a corresponding reduction in expression of integrin subunits alpha-5 and alpha-3, that specifically bind to FN or Col, respectively.

Methods: To determine whether modulation of FN or Col expression affects alpha-5 or alpha-3 integrin subunit expression, rat microvascular endothelial cells (RMECs) were grown in normal (5 mM) or high (30 mM) glucose condition for 9 days and transfected with FN-antisense oligonucleotides (AS-oligos) or Col-AS oligos. Three days post-transfection, cells were harvested and protein analyzed by Western blot for FN, Col, alpha-5 and alpha-3 integrin expression. In parallel, cells plated on cover slips and similarly transfected with FN-AS or Col-AS oligos, were examined for FN, Col, alpha-5 and alpha-3 integrin immunoreactivity by fluorescence microscopy. Cells transfected with random oligos were used as control.

Results: Western blot analysis indicated increased Col, alpha-3 integrin, FN, and alpha-5 integrin expression in cells grown in HG medium compared to cells grown in N medium ($135 \pm 12\%$ of control, $p < 0.05$; $131 \pm 14\%$ of control, $p < 0.05$; $205 \pm 61\%$ of control, $p < 0.05$; and $215 \pm 48\%$ of control, respectively). In cells transfected with AS-Col oligos, both Col and alpha-3 integrin expression was significantly reduced to near normal levels compared to cells grown in HG condition. Similarly, in HG cells transfected with AS-FN oligos, both FN and alpha-5 integrin expression was significantly reduced. Cells transfected with random oligos showed no effect on Col, alpha-3 integrin, FN, alpha-5 integrin expression. Using immunofluorescence microscopy we observed a similar trend that indicated increased Col, alpha-3 integrin, FN, and alpha-5 integrin immunostaining in cells grown in HG medium compared to cells grown in N medium. Interestingly, when Col and FN overexpression was reduced in cells grown in HG medium, alpha-3 and alpha-5 integrin expression was also reduced, respectively. The cells grown in HG medium and transfected with random oligos showed no effect.

Conclusions: The findings indicate that high glucose-induced Col and FN overexpression regulates corresponding integrin expression in microvascular endothelial cells. It appears that the antisense-oligo strategy is useful in not only downregulating Col and FN overexpression but may have the added benefit of reducing integrin overexpression associated with endothelial cell dysfunction in diabetes.

S8.21

COATS DISEASE - AN INDIAN PERSPECTIVE

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Purpose: To describe the patient profile, clinical features, treatment and outcome patterns in 308 eyes with Coats' disease in a tertiary care centre over the last decade.

Methods: Patients diagnosed of Coats' disease between Jan 1996 and Jan 2006 were included. Eyes with a secondary cause for exudation were excluded. Clinical staging of Coats' disease as proposed by Shields, et al was followed. Patient profile, presenting features, treatment and outcome were analyzed. Optimal structural outcome was defined as cessation of telangiectatic activity with preservation of globe shape, no rubeosis iridis and normal intraocular pressure. Favorable structural outcome was defined as an attached retina along with an optimal structural outcome. Functional success was defined as improved vision, better than counting fingers.

Results: 280 patients (308 eyes) were diagnosed of Coats' disease over the last decade. Mean age at presentation was 16.5 yrs (Range 4 months to 80 years). Decreased vision (55.5%), unilateral affection (90%) and

male preponderance (82.8%) were the chief presenting features. Anterior segment involvement was seen in 67 (21.8%) eyes. Telangiectasia was seen in 302 (99%) eyes while exudation was noted in 274 (89%) eyes. Retinal detachment was seen in 153 (50%) eyes of which 98 (32%) eyes had a total retinal detachment, at presentation. Four quadrant disease was seen in 207 (67.2%) eyes. Visual acuity was $< 6/60$ in 249 (80.9%) eyes. Of the 145 (47.1%) eyes which underwent treatment, laser photocoagulation was the commonest (33.3% eyes). 109 of 176 treated eyes (61.93%) had a favorable anatomical outcome while 207 of 280 eyes (74%) had an optimal structural outcome. 17 (5.3%) eyes were enucleated. Complications following treatment included phthisis bulbi in 22 eyes (7%), neovascular glaucoma in 16 eyes (5%), epiretinal membrane and rubeosis iridis in 13 eyes (4.4%) each.

Conclusions: On an average, Indian patients with Coats disease present over 5 years later than their western counterparts. Male predominance (82.8% v/s 76%) is even more pronounced. More than half (55.5% v/s 43%) patients present with decreased vision. A large majority (80.9% v/s 76%) had vision worse than 6/60 at presentation. Macular telangiectasia is seen five times (5.5% v/s 1%) more commonly. Extensive, four quadrant exudation is also seen more frequently (67.2% v/s 55%). However, exudative retinal detachment is less extensive and less frequent. Unusual presentations such as pain, vitreous hemorrhage (4.3%) and a higher incidence of anterior segment involvement (21.8%) are distinctive to Indian eyes.

S8.22

STARGARDT DISEASE: ETIOLOGY AND TREATMENT

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Summary of current developments in treatment of Stargardt disease.

Symposium 9 Refractive Surgery Sunday, 4 March 2007

S9.1

ROLE OF BASEMENT MEMBRANE IN CORNEAL WOUND REPAIR

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One of the intriguing questions regarding corneal wound repair is how the epithelium and stromal cells interact during the repair process. It has been known for several years that even a simple wound to the corneal epithelium stimulates the subjacent stromal cells to undergo apoptosis, which subsequently leads to the stimulation of proliferation of stromal cells adjacent to the area of cell death. The acellular zone is then repopulated. Interestingly, when the basement membrane is intact the repopulating cells do not differentiate into myofibroblasts. However, when the basement membrane is removed, the stromal cells adjacent to the wound healing epithelium differentiate into myofibroblasts leading to haze and even scarring. In this presentation, I will discuss results from several wound models that indicate the importance of the basement membrane in directing the wound response.

S9.2

SCREENING OF LASIK CANDIDATES - WHO IS THE KERATOCONUS SUSPECT?

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Introduction: More than 50% of corneal ectasia cases after LASIK surgery are attributed to unrecognized preoperative forme fruste keratoconus (FFKC). Hence exclusion from LASIK is critical in patients with FFKC.

Methods: The role of Orbscan II anterior segment analyzer and Zywave II aberrometer (Zyoptix Diagnostic Workstation, Bausch & Lomb) in the screening for keratoconus is discussed and the results of a one year observational prospective study conducted at the Singapore National Eye Centre is reported. One hundred and sixteen patients with keratoconus were enrolled and 179 eyes were evaluated using the Orbscan II topographer with 70 control eyes. Wavefront measurements were measured with the Zywave II aberrometer (6mm dilated pupil): 35 eyes with keratoconus, 38 eyes with keratoconus suspect and 166 normal control eyes.

Results: Compared to the control group, the keratoconus group showed significantly different values in all indices ($p < 0.001$); the keratoconus suspect group showed significantly different values in the following indices ($p < 0.01$): maximum posterior elevation (46 μ m/26 μ m), 3mm irregularity (2.44D/1.05D), 5mm irregularity (2.61D/1.38D) and thinnest corneal pachymetry (504 μ m/554 μ m). The mean of total higher order root mean square (RMS) values (3rd to 5th) was 1.73 \pm 0.71 μ m (0.49-3.32 μ m) in eyes with keratoconus, 0.94 \pm 0.66 μ m (0.25-2.68 μ m) in eyes with keratoconus suspect, and 0.49 \pm 0.16 μ m (0.17-0.94 μ m) in normal myopia eyes.

Conclusions: The Orbscan II system and Zywave II aberrometer provide useful information in the evaluation of keratoconus morphology and differentiate keratoconus suspect cases from normal eyes. They serve as important screening tools in refractive surgery.

S9.3

CLINICAL COMPARISON BETWEEN SURFACE & STROMAL ABLATIONS

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S9.4

ASPHERIC LASIK: RESULTS OF A MULTICENTRE TRIAL

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*CR

S9.5

COMPARISON OF TORIC IMPLANTABLE CONTACT LENS AND WAVEFRONT-GUIDED LASIK FOR HIGH MYOPIC ASTIGMATISM

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Purpose: To compare the postoperative visual function after Toric ICL™ (Staar Surgical) implantation and after wavefront guided laser in situ keratomileusis (WFG-LASIK) in eyes with high myopic astigmatism.

Methods: We investigated fourteen eyes of 10 patients undergoing Toric ICL implantation (Staar Surgical) and 49 eyes of 29 patients undergoing WFG-LASIK (Technolas217z, Bausch&Lomb) for the correction of high myopic astigmatism (over -6.0 diopters [D]). Ocular higher-order aberrations (HOAs) and contrast sensitivity function were measured by Hartmann-Shack aberrometry (KR-9000, Topcon) and contrast sensitivity unit (VCTS-6500, Vistech) before and 3 months after surgery, respectively. From the contrast sensitivity, the area under the log contrast sensitivity function (AULCSF) was calculated.

Results: For a 4-mm pupil, ocular HOAs were 0.12 \pm 0.03 μ m preoperatively and 0.13 \pm 0.03 μ m postoperatively ($P < 0.05$, Wilcoxon sign rank test) in eyes undergoing ICL implantation. However, in eyes undergoing WFG-LASIK, the corresponding figures were 0.12 \pm 0.05 μ m preoperatively and 0.29 \pm 0.09 μ m postoperatively ($P < 0.001$). The postoperative AULCSF was significantly increased after Toric ICL implantation ($P < 0.05$), whereas, after WFG-LASIK, it was significantly decreased ($P < 0.05$).

Conclusions: Ocular HOAs were not significantly changed after ICL implantation, but they were significantly increased after wfg-LASIK. Moreover, contrast sensitivity was significantly improved after Toric ICL implantation, whereas it was deteriorated after WFG-LASIK in eyes with high myopic astigmatism. Toric ICL is superior to WFG-LASIK in visual performance in the correction of high myopic astigmatism.

S9.6

IMPLANTABLE CONTACT LENSES - CURRENT PROGRESSD.F. SWEENEY¹, K.M. MCLEAN²*¹Vision CRC, Sydney, NSW, 1466, Australia, ²University of New South Wales, Sydney, AUSTRALIA*

The cornea is responsible for 70% of the eye's refractive power and this can be adjusted by altering the anterior or posterior radius of curvature or by changing the index of refraction of the cornea itself. Laser surgical techniques such as photorefractive keratectomy (PRK) and laser in-situ keratomileusis (LASIK) achieve refractive correction by ablation of intrastromal corneal tissue. However, these techniques are non-reversible and may have other complications. Refractive error can also be corrected by altering the corneal curvature via the surgical insertion of a suitable synthetic material within the corneal tissue. This may involve implantation of a lens within the corneal stroma in a LASIK style flap (corneal inlay) or superficially beneath the corneal epithelium (corneal onlay). Our group is developing synthetic corneal implants (onlay and inlay) as permanent but reversible means of vision correction. Design principles for these applications include the matching of the mechanical properties of the implant material with those of the ocular environment, biostability, biocompatibility and ensuring sufficient permeability to maintain corneal health. In addition in the onlay application the lens is required to allow re-

epithelialisation. Progress in developing a family of biostable perfluoropolyether (PFPE) polymers for these applications is discussed and the biological response in *in vivo* models including unsighted humans will be described.

S9.7

MYDRIATIC CHANGES IN VAULTING AND ANTERIOR CHAMBER DEPTH IN POSTERIOR CHAMBER PHAKIC IOL IMPLANTED EYES

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Purpose: In phakic IOL (ICL™: Version 4, STAAR Surgical Company) implantation, ICL vaulting (distance from ICL to anterior surface of crystalline lens) is highly important when we predict the rate of ICL-induced cataract, however, mydriatic changes have not been elucidated. We investigated changes in ICL vaulting and anterior chamber depth before and after mydriasis in ICL implanted eyes.

Methods: Eleven eyes of 7 patients undergoing ICL implantation between August 2002 and March 2005 in Kitasato University Hospital were included in this study. Mean age was 40.3 ± 10.3 (mean \pm SD, range: 29 to 58) years old, mean preoperative manifest refraction (spherical equivalent) was -15.0 ± 5.6 (range: -22.8 to -4.0) diopters, and mean follow-up period was 24.3 ± 10.1 months. Before and after mydriasis under mydriatic cycloplegic agent (Mydrin-P: 0.5%; tropicamide plus 0.5% phenylephrine hydrochloride), anterior segment structure was measured with Scheimpflug imaging system (Pentacam™: OCULUS) for three times, and vaulting and anterior chamber depth were calculated from the digitally captured images.

Results: ICL vaulting was significantly increased from 216.7 ± 114.3 μ m (before mydriasis) to 411.7 ± 142.9 μ m (after mydriasis) ($p < 0.01$, Wilcoxon's signed-ranks test). Anterior chamber depth was also significantly increased from 3033.0 ± 267.1 μ m (before mydriasis) to 3149.5 ± 290.6 μ m (after mydriasis) ($p < 0.01$).

Conclusions: Mydriasis induced a significant increase in ICL vaulting and anterior chamber depth in ICL implanted eyes.

S9.8

IMAGE GUIDED FEMTOSECOND-LASER CORNEA SURGERY

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In recent years, it has been demonstrated, that specific aspects of laser vision correction procedures can take advantage of unique light-tissue interaction processes that occur with femtosecond laser pulses. Various ophthalmic-surgical applications, such as e.g. laser flap-cutting for LASIK, lamellar surgery for cornea transplants and non-invasive intrastromal refractive surgery, have been demonstrated. The cutting process can be described by the model of optical laser induced optical breakdown (LIOB). Ultrashort laser pulses induce minimal side-effects due to its low required pulse energies, thus generating extremely small gas bubbles and reduced shock-wave phenomena. The surgical procedures can be observed by confocal microscopy and optical coherence tomography allowing for closed loop, image-guided operation. *CR

S9.9

APOPTOTIC VS. NECROTIC STROMAL CELL DEATH IN THE GENERATION OF INFLAMMATION AFTER LASIK WITH THE FEMTOSECOND LASER AND THE MICROKERATOME

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The levels of apoptotic vs. necrotic stromal cell death is a critical determinant of the level of inflammation generated after PRK. The femtosecond laser directly generates necrosis of stromal cells at the interface in comparison to microkeratome-generated cell death that is primarily apoptosis. Care must be taken in analyzing the results of the TUNEL assay that may erroneously identify necrosis as apoptosis. The level of inflammation with the femtosecond laser is easily controlled by using the lowest level of energy possible in producing the lamellar and side cuts.

S9.10

A RANDOMIZED CONTROLLED TRIAL EVALUATING THE EFFICACY OF NEUROVISION TECHNOLOGY IN LOW MYOPIA

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Purpose: NeuroVision's NVC vision correction technology is a non-invasive, patient-specific treatment based on visual stimulation and facilitation of neural connections responsible for vision. It involves the use of an internet-based computer generated visual training regime using sets of patient specific stimuli based on Gabor patches, to sharpen Visual Acuity (VA) and Contrast Sensitivity (CSF). This RCT evaluates the efficacy of NVC technology in the enhancement of unaided vision in adult low myopes in the Singapore Armed Forces.

Methods: Preliminary, unmasked interim statistical analysis of end of treatment study efficacy and safety are reported. 101 adults (77 male and 24 female) with low myopia (mean cycloplegic spherical equivalent of -1.08 D (range -0.5 D to -1.5 D)) and baseline unaided VA of 0.1 LogMAR or worse in both eyes) and with a mean age of 34 years (range 16 to 55 years) were recruited in a randomized double masked controlled trial and divided in two groups, one receiving NVC treatment, and the other receiving placebo NVC treatment. Investigations included manifest and cycloplegic refraction, LogMAR unaided VA (UAVA) and sinusoidal grating CSF (Sine Wave Contrast Sensitivity charts), before and after the treatment. Results reported here are part of an interim subgroup analysis (subjects with baseline UAVA of at least 0.2 LogMAR or worse in both eyes) performed at 3/4 of the study completion on all patients to date who have completed treatment. Success criterion was improvement in UAVA of 2 lines and more. Analysis was performed by an independent third party, and all study patients and trial investigators remain masked at this stage.

Results: UAVA improvement (LogMAR) improved by -0.178 in Group A vs. 0.023 in Group B. 29.6% of the subjects in Group A achieved the success criterion of 2 LogMAR lines of improvement in UAVA in both eyes vs. 0% in group B ($p = 0.020$). 64.8% of the subjects in Group A achieved the similar success criterion in at least one eye vs. 7.7% in group B ($p < 0.0005$). Mean manifest refractive error was not significantly changed after treatment in both groups, and no side-effects were encountered during treatment.

Conclusions: Interim analysis of this RCT evaluating NVC treatment in low myopes confirms that statistically significant differences in UAVA and CSF between masked treatment groups have been achieved at this stage. Almost 30% of study subjects analyzed improved UAVA by 2 lines in both eyes, and almost 2/3 similarly improved in at least one eye, in group A. These differences are also clinically significant. Results of this preliminary interim analysis suggest that final completion of the RCT is likely to provide definitive evidence of efficacy and safety of NV treatment in improving visual acuity and contrast sensitivity function in adult low myopes. *CR

S9.11

AN OPTICAL COHERENCE TOMOGRAPHY-BASED INTRAOCULAR LENS POWER FORMULA

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Purpose: To develop an intraocular lens (IOL) formula that is not biased by prior laser vision correction (LVC).

Methods: Twenty-seven eyes from 27 patients who underwent uneventful cataract surgery from 4/27/2005 to 5/10/2006 at Doheny Eye Institute were enrolled in the prospective observational study. All patients received Alcon AcrySof™ SN60AT (24 eyes) or SA60AT lenses (3 eyes). IOL-Master (Zeiss) was used to measure anterior corneal curvature and eye axial length. A high-speed (2000 Hz) optical coherence tomography (OCT) system was used to measure central corneal thickness, posterior corneal curvature, anterior chamber depth and crystalline lens thickness preoperatively. We developed an IOL formula that uses the OCT and IOL-Master measurements to predict refractive outcome.

Results: The prediction error for postoperative manifest refraction spherical equivalent was 0.04 ± 0.44 D for the OCT-based IOL formula and -0.35 ± 0.42 D for the SRK/T formula. Twenty-one eyes (78%) were within 0.5D for OCT formula compared to 18 eyes (67%) for SRK/T. Hoffer Q and Holladay formulae provided similar results and the differences between formulae were not statistically significant.

Conclusions: For normal cataract patients, the OCT-based IOL formula was equivalent to the current theoretic formulae in accuracy. Because OCT directly measures posterior corneal power, it may be more accurate post-LVC. A trial on post-LVC cataract surgery is in progress. *CR

Symposium 10

Neuro-Ophthalmology/Orbit

Sunday, 4 March 2007

S10.1

MECHANISMS IN AUTOIMMUNITY TO NEURORETINA

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The eye, despite (and perhaps partly because of) its immune privileged status, is vulnerable to autoimmune attack. In the USA, autoimmune uveitis is estimated to cause about 10% of the cases of severe visual handicap. Animal models of experimental autoimmune uveitis (EAU) are providing a platform to study basic mechanisms and develop clinical approaches to

this blinding condition. This presentation will critically examine current concepts regarding tolerance to immunologically privileged retinal antigens and evidence concerning specific checkpoints in the pathogenesis of the disease. We will discuss how knowledge gleaned from studies in animal models can point to novel therapeutic targets. *CR

S10.2

THE ROLE OF T CELLS IN OPTIC NEUROPATHIES: A COMPROMISE BETWEEN NEED, AND THE RISK OF AUTOIMMUNITY

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Autoimmunity was once understood as an attack by the body against its own tissues. Some years ago our group showed, however, that autoimmune T cells directed against self-antigens residing in the optic nerve or eye can protect against neurodegenerative conditions there. Specificity of the circulating T cells was required for their homing to, and activation at the site of injury. The activated T cells provided cytokines and growth factors that instruct resident microglia to support cell survival and regrowth.

In seeking mechanisms regulating autoimmune T-cell activity without causing disease, we found that naturally occurring CD4+CD25+ T cells (Treg), the regulatory cells previously thought to act only as suppressors of autoimmunity (thereby preventing autoimmune disease), rigorously control autoimmune cells and thus enable 'protective autoimmunity' to exist. Their suppression, while preventing autoimmune disease, is a barrier to the protective autoimmunity needed to prevent neurodegeneration, e. g. after optic nerve insult. Thus, a physiological compromise can have pathological consequences.

Strengthening of the physiological mechanism by boosting a well-controlled autoimmunity or further weakening Treg might spawn novel treatments for optic neuropathies. An optimal treatment would benefit both inflammatory and non-inflammatory neurodegenerative diseases. Carefully controlled use of weak agonists of self-antigens is a promising strategy for protecting neural tissues and attenuating inflammation. *CR

S10.3

TISSUE ENGINEERING FOR RETINAL REPAIR

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Stem and progenitor cells can be combined with polymer substrates to generate tissue equivalents in culture. The replacement of retinal tissue lost to disease or trauma using retinal progenitor cells (RPCs) delivered on polymer scaffolds and transplanted into the sub-retinal space of the damaged retina is a promising therapeutic strategy.

Micromachining-based, ultra-thin PMMA poly (methyl methacrylate) scaffolds may provide a suitable cytoarchitectural environment for tissue engineering and transplantation to the diseased eye. Here, adhesion of RPCs to polymer, as well as migration and differentiation in the host retina were compared for PMMA scaffolds (6 μ m thickness) with either smooth or porous (11 μ m diameter) surface topography. RPCs were cultured under identical conditions on smooth or porous laminin-coated polymer scaffolds and transplanted into the subretinal space of C57bl/6 mice. RPCs could be cultured on both scaffolds with similar results, although transplantation

with non-porous scaffolds showed limited RPC retention. Porous scaffolds demonstrated enhanced RPC adherence during transplantation and allowed for greater process outgrowth and cell migration into the host retinal layers. Integrated cells expressed the mature neuronal marker neurofilament-200 (nf-200), the glial marker glial fibrillary acidic protein (GFAP) and the retinal-specific marker recoverin. No host foreign body response was seen.

In conclusion, ultra-thin film PMMA scaffolds micromachined to contain through pores retain adherent RPCs to a considerably greater extent than unmachined versions during the transplantation process and can serve as a biocompatible substrate for cell delivery in vivo. *CR

S10.4

VISION RESTORATION THERAPY

K.Y. GOH

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Introduction: Visual field defects arising from post-geniculate lesions in the brain are considered by many clinicians untreatable because the well organized visual pathways of the brain are regarded as hard-wired and hence loss of visual function deemed irreversible. However, recent studies in humans have indicated that vision restoration therapy increases the size of intact visual field even many years after brain damage.

Methods: Case review of 8 patients who had post-geniculate visual loss started vision restoration therapy between September 2005 and March 2006. There were 7 males and 1 female. Age ranged from 20 years to 66 years (mean = 46.9 years; median=50 years).

3 had visual field defects from strokes; the remaining 5 had surgery related or arterio-venous malformation hemorrhage related lesions.

All patients successfully completed 6 months of twice daily visual rehabilitative therapy for 6 days every week.

At the end of the period of therapy their visual field size were analyzed based on the number of stimuli detected on a High Resolution Perimetry (HRP) screen before and after therapy. Fixation control was assessed by the degree of fixation loss.

Results: The average percentage stimulus detection change was tracked for all patients after each module of therapy. This showed a progressive increase as therapy progressed: 2.48% (first month), 4.96% (second month), 6.78% (third month), 11.45% (fourth month), 14.90% (fifth month) and 18.62% (sixth month). The patients were also questioned on their subjective improvement in their daily lives. 3 patients did not show an increase of stimuli detection of more than 15%.

Conclusions: Vision restoration therapy may be a useful form of visual rehabilitation for patients with post-geniculate visual field loss although our early results do not show a uniform improvement in all patients. The physiologic basis of this improvement may lie in the process of neuroplasticity which causes an increase of receptive field sizes of neurons near the lesion area and strengthening of long range horizontal connections in V1 which usually exhibit subthreshold activity.

S10.5

OCULAR MOTOR NERVE PALSIES IN SINGAPORE

S. TOW

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Purpose: To evaluate the epidemiology of ocular motor cranial nerve palsies seen in 4 public sector hospitals in Singapore.

Design: Prospective multi-center cohort study.

Methods: All patients with new-onset ocular motor nerve palsies seen in 4 public sector hospitals over a 22-month period (September 2002 and June 2004) were identified. Demographic details, presenting symptoms, relevant neuro-ophthalmic findings, concurrent systemic disorders, and the progress of the cranial nerve palsy were recorded using a standardized protocol. The data were entered in a national database.

Results: One hundred and seventy-one patients were recruited during the study period. Sixty-one percent were males. Two patients had two separate episodes of ocular motor nerve palsy. Of the 173 cases, the 6th cranial nerve was the most commonly affected (43%) followed by the 4th, 3rd and multiple cranial nerve palsies (22%, 20% and 15% respectively). Ischemia was the commonest etiology identified for isolated ocular motor nerve palsies, seen in 49% of cases. Twenty-seven (16%) patients presenting with an oculomotor nerve palsy were found to have at least one previously undiagnosed vascular risk factor. Neoplasm was the commonest etiology when more than one ocular motor nerve was involved. Resolution of the nerve palsy varied according to etiology. Ninety-five percent of patients with ischemic ocular motor nerve palsies had complete resolution, which occurred at an average of 3.7 months.

Conclusions: The most frequently affected cranial nerve was the 6th, in keeping with published reports¹⁻³. The commonest etiology was ischemia, differing from previous reports in which the largest etiologic group was undetermined for all ocular motor nerves, followed by ischemia, trauma and neoplasm for the 3rd, 4th and 6th cranial nerves respectively³. These differences may reflect an etiologic profile specific to Asians. In 16% of patients presenting with an oculomotor nerve palsy, a new diagnosis of at least one vascular risk factor per patient was made.

S10.6

SURGICAL REPAIR OF TESSIER NUMBER 10 CLEFT

X. FAN

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Background: Tessier number 10 cleft is rare. Little has been published about their surgery management. The soft-tissue abnormalities include coloboma of middle third of upper eyelid, absent of lateral eyebrow, temporal hairline moving down, epibulbar cysts, symblepharon and amblyopia. There may be an upper "central" orbital cleft.

Methods: Five cases of Tessier no. 10 cleft referred to the Department of Ophthalmology of Shanghai the Ninth People's Hospital between January of 1998 and January of 2006 underwent a standard ophthalmological assessment. All cases were examined by three-dimensional computed tomography. Surgery were divided into four stages: eyebrow reconstruction using temporal hairline transposition flap, eye enucleation and HA implantation, eyelid reconstruction with a hard palate mucosa-lined pedicled myocutaneous flap, and wearing ocular prosthesis.

Results: All cases were females and unilateral. They all manifested typical soft-tissue abnormalities, but CT showed no bony cleft. In all cases, follow-up after one year showed complete recovery of lid function. Both closure of the reconstructed eyelid and its position were satisfactory. The artificial eye looked and moved almost as naturally as a normal eye. The reconstructed eyebrow was symmetry with the opposite side.

Conclusions: The four-stage method is useful and effective in repairing Tessier no. 10 cleft. Early and appropriate surgical intervention is necessary for good visual and functional outcome. Key words: Tessier no. 10 cleft, cleft surgery.

S10.7

THYROID ASSOCIATED ORBITOPATHY: UNDERSTANDING THE SCIENCE UNDERPINS THE TREATMENT

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Thyroid related ophthalmopathy refers to a constellation of orbital signs and symptoms, characterized by periocular soft tissue swelling and inflammation, lid retraction, proptosis, motility disturbance and optic neuropathy, usually associated with typical imaging findings and autoimmune thyroid disease. Overall incidence is 16/100,000 in women and 3/100,000 in men. There are many hypotheses as to the underlying pathogenesis, with research directed towards the TSH receptor and IGF-1R and various muscle autoantibody candidates. Humoral and cell mediated immunity influences the process with T-cell recruitment being influenced by a range of cytokines, adhesion molecules and chemo-attractants, resulting in fibroblast stimulation, adipogenesis and glycosaminoglycane secretion. There are also important environmental (smoking), genetic and racial factors that remain to be understood. Finally, in man, various site specific factors relating to orbital fibroblast behavior, the confines of the bony orbit, local pressure effects, venous outflow disturbance and the proximity to the maxillary and ethmoid sinuses play a role. The influence of these factors on the disease process and its treatment will be discussed.

S10.8

CLINICAL AND PATHOLOGIC FEATURES OF EXPLANTED POROUS ORBITAL IMPLANTS

P. DOLMAN

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Methods: The charts and histology were reviewed on 21 patients requiring explantation of porous orbital implants. Clinical data recorded included age, gender, primary surgery, and type of implant. Histological data included zones of necrosis, absent tissue ingrowth, and bacterial infection.

Results: The spheres included 16 Medpor, 4 hydroxyapatite, and 1 bioceramic. The primary surgeries were 15 enucleations, 3 eviscerations and 3 secondary implants. Histology showed zones of central or anterior necrosis in 17, areas of absent tissue ingrowth in 14, mixed inflammation in 20, and dead or viable bacterial colonies in 15. Most bacterial colonies were Gram-positive cocci in clusters or chains.

Discussion: Exposure of porous implants has been reported to occur in 5-15% of cases. While these can usually be safely repaired, a small minority may continue to expand and develop signs of infection, necessitating removal. This paper is the largest review of such explanted spheres including all porous materials currently used. It suggests that anterior exposure allows bacterial colonization. Poor tissue ingrowth and secondary zones of necrosis may limit the penetrance of topical or systemic antibiotic therapy. Enucleations appear more likely to lead to implant infection and removal, since they represent 75% of this series, while eviscerations account for over 75% of our primary globe removal surgery.

Conclusions: In cases of porous orbital implant exposure, antibiotic therapy via oral, intravenous, or even direct implant injection, is recommended to control infection prior to attempting surgical repair of the underlying defect.

Symposium 11**Genetics****Monday, 5 March 2007**

S11.1

TALES OF THE UNEXPECTED; DOMINANT RETINITIS PIGMENTOSA MUTATIONS IN PRE-MRNA SPLICING FACTORS

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Retinitis pigmentosa (RP) is caused by mutations in a variety of proteins, most of which have known functions in the retina. However, one of the most perplexing findings of recent retinal genetics research was the discovery of mutations causing dominant RP in four ubiquitously expressed splicing factors. Three of these, PRPF8, PRPF31 and PRPF3, are known components of the spliceosome, a macromolecular structure responsible for precise excision of introns from pre-mRNA. The fourth, PAP-1/RP9, was thought to be a transcriptional regulator. However, after RP-causing mutations were identified, it was shown that PAP-1 protein localizes in nuclear splicing speckles, interacts with PRPF3 and another splicing factor, U2AF35, and alters the pattern of splicing in a minigene assay, suggesting that this too is a splicing factor.

In this talk I will overview work which led up to the initial report of these mutations and subsequent studies which have attempted to determine how mutations in these essential cellular housekeeping proteins lead to retinal degeneration in the absence of any other defects. I will also present new data showing differences in splicing efficiency between cell lines from splicing factor RP patients and controls. These observations tend to confirm that this form of RP does indeed result from aberrant splicing of retinal transcripts, and argues against an alternative hypothesis that RP in these patients might result from a defect in some as yet undiscovered alternative function of these proteins.

S11.2

CXORF2 CANDIDATE GENE SCREENING AND X-LINKED MYOPIAT.L. YOUNG¹, R. METLAPALLY¹, A. SHAY², S. ZUCHNER², Y.J. LI²*¹Duke University Eye Center, Durham, NC, United States of America, ²Duke University Center for Human Genetics, Durham, NC, UNITED STATES OF AMERICA*

Purpose: CXorf2 is a nested gene within the red and green opsin cone pigment gene tandem array on chromosome Xq28. In the literature, only 3 copies of CXorf2 have been reported within the opsin gene array. We have previously mapped a gene locus on Xq28 for X-linked myopia with cone dysfunction and protanopia in a large Minnesota family. We screened CXorf2 for genomic DNA sequence mutations and copy number variations using real-time PCR in this pedigree.

Methods: Primer pairs were designed to amplify the 5 exons of CXorf2 including intron /exon boundaries. PCRs were performed and amplified products were sequenced using standard techniques. Normal and affected individual DNA sequences were compared alongside the known reference sequence (UCSC genome browser) for CXorf2.

To examine the copy number variation of the CXorf2 gene, gene expression assays (Assays-by-Design™) consisting of a mix of unlabeled PCR primers and TaqMan® MGB probe (FAM™ dye-labeled) were designed to

quantitate the pattern of expression at the genomic DNA level. A similar opsin gene assay was designed to validate the sensitivity of the experiment, as the opsin copy numbers were previously determined in the current pedigree. A GAPDH assay served as an endogenous reference. All real-time PCR reactions were performed with Taqman® Universal PCR Master Mix on the ABI7900HT Fast Real-time PCR System. Data were analyzed using the Comparative CT method, where the amount of target normalized to an endogenous reference and relative to a calibrator (known individual value) is calculated. Results were plotted as the copy number present in each individual with respect to the assigned number of copies in the calibrator.

Results: No coding sequence variants were found. The copy number for the opsin genes observed in affected males (4), control males (3), and a carrier female (7) confirmed previous testing. The CXorf2 gene targeted assay on affected male individuals expressed up to 5 copies of CXorf2.

Conclusions: The targeted opsin gene assay confirms that employing real-time custom gene expression assays is a reliable way to study copy number variation. Copy number variations have been proposed to play a role in disease inheritance and susceptibility due to their ability to affect genic dosage. Further evaluation of CXorf2 copy number variation is underway.

S11.3

FAMILY STUDY OF MIDD DUE TO A3243 MUTATIONS

A.C. BIRD

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A survey of 7 probands harbouring the A3243G mitochondrial and 36 maternal family members was undertaken with respect disease manifestations of disease. In none of the relatives was the diagnosis made. Blood samples were taken to seek evidence of diabetes, determine renal function, and to screen maternal relatives for the A3243G mt mutation. A comprehensive audiological evaluation was performed on 4 probands and 19 audiological asymptomatic maternal relatives. The presence or absence of additional disease characteristics previously reported to be associated with MIDD or other mitochondrial disorders was ascertained by use of a medical questionnaire. Marked inter- and intra-familial variation associated with the A3243G mt mutation with respect to retinal appearance, hearing loss, diabetes and other systemic features. Age-adjusted hearing loss and macular disturbance were the most frequent findings in our mutation positive subjects, whereas diabetes was the least frequent finding in our sample. Patients with progressive hearing loss merit ophthalmological assessment, in order to detect retinal abnormalities consistent with MIDD. Conversely, patients with macular features in keeping with MIDD should have audiological testing, even in the absence of diabetes, thereby helping to establish the diagnosis of MIDD and assist in providing accurate advice on prognosis and genetic counseling.

S11.4

VARIATIONS IN THE MYOC GENE IN INDIAN PATIENTS WITH POAG AND IN VITRO AND IN VIVO STUDY ON THE SECRETION OF GLY367ARG MUTANT MYOCILIN PROTEIN

P. SUNDARESAN

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Glaucoma is a group of ocular disorders characterized by a specific pattern of Optic nerve and visual field defects, if untreated leads to permanent

loss of vision. Primary congenital Glaucoma (PCG) has been associated with CYP1B1 gene and Primary Open Angle glaucoma (POAG) has been associated with MYOC, OPTN and OPTC genes.

We studied all known candidate genes variants in Indian POAG patients and the result will be presented. Mutations in the Myocilin gene (MYOC) leading to a perturbed outflow of aqueous in the trabecular meshwork (TM) has been associated with the pathophysiology of glaucoma. This study examines the expression of normal and mutant Myocilin (Gly367Arg) in cultured TM cells. Normal and mutant MYOC cDNA constructs were used to transfect the TM cells. In order to confirm the method of transfection, RT-PCR was carried out. Further, confocal microscopic analysis was used to determine the cellular localization of Myocilin protein. The extracellular nature of Myocilin in the culture supernatant and cell lysates of the transfected cells was analyzed by western blot. Myocilin protein in the aqueous humor of POAG patients, including patients with Gly367Arg was quantified by dot blot. Molecular Modeling and Dynamics for the mutant was demonstrated with the native Myocilin model using GROMACS. Gly367Arg mutation causes accumulation of Myocilin protein within TM cells with extensively reduced secretion, while wild type Myocilin was characterized by intracellular localization and extracellular secretion. The secreted Myocilin in the aqueous humor of patients with Gly367Arg mutation correlated with the in vitro findings, confirming the disease-causing glaucomatous phenotype. Further, Gly367Arg mutation occurs in a hydrophobic region causing aggregation, leading to burial of a charged residue resulting in the conformational change to accommodate the mutation.

We suggest that Gly367Arg is a potential mutation causing malfunction of TM cells either by dominant negative effect or gain of function of mutant Myocilin. The structural model indicates the aggregation of Myocilin protein confirming the pathogenic significance of Gly367Arg.

S11.5

SLC4A11 GENE INVOLVEMENT IN RECESSIVE CONGENITAL HEREDITARY ENDOTHELIAL DYSTROPHY (CHED2) AND FUCHS ENDOTHELIAL DYSTROPHY (FECD)

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The gene SLC4A11, which encodes BTR1 (Bicarbonate transporter related protein-1), is a member of the SLC4 bicarbonate transporter family. It was recently identified as the gene underlying the recessive form of Congenital Hereditary Endothelial Dystrophy (CHED2). Mutations in SLC4A11 have been identified in all CHED2 families analyzed to date suggesting that CHED2 is clinically and genetically homogeneous. Although rare, both homozygous and compound heterozygous mutations have been identified in this gene in both Asian and Caucasian individuals afflicted with CHED. Characterization of mutant proteins have revealed that mutations cause aberrant post translation modification and reduced cellular accumulation of this membrane bound protein. Furthermore mutations cause blockage to the plasma membrane targeting, thereby excluding this protein from its site of action. Therefore disease is caused by functional loss of the protein. As CHED and FECD have similar pathological features and may share a common mechanism of disease SLC4A11 gene involvement in Chinese and Indian FECD patients was also investigated. This identified several heterozygous mutations which when characterized showed mutant protein behavior similar to that observed with CHED2 mutations. Preliminary data thus suggest that SLC4A11 mutations may also play a role in the pathogenesis FECD.

S11.6

VERTEBRATE EYE DEVELOPMENTAL DEFECTS ARE CAUSED BY LOSS OF FUNCTION OF THE BCL-6 CO-REPRESSOR, BCOR

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S11.7

GENETIC TESTING: A NEW ERA IN MANAGEMENT OF EYE DISEASES

R. ALLIKMETS

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Overview of genetic testing of retinal diseases

S11.8

APPLICATION OF GEOMICS IN AN OPHTHALMIC GENETIC CLINIC IN INDIA

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Introduction: The genetic clinic in our hospital has been providing services since 1994 and of late we have been applying genomic knowledge and techniques in our counseling process.

Methods: Any patient attending our ophthalmic clinic with a genetic disease is referred to our clinic on the advice of the clinical consultant or on patient request. While counseling, patients are explained clearly on the nature of the genetic eye disease and the family is offered genetic tests that are available in the department which are chromosomal or molecular genetic investigations. Microsatellite haplotyping and mutational screening of genes are the routine tests used.

Results: On an average 1000 patients are counseled each year. Haplotyping data in retinoblastoma (13q14), microcoria (13q31-q32), congenital glaucoma (2p21), retinitis pigmentosa and Nance-Horan syndrome (Xp22.13) families were used in predictive diagnosis and counseling. Data from mutational screening of RB1, RHO, MYOC, CYP1B1 and PRPF31 genes were used for counseling in several families.

Conclusions: Current genomic knowledge and skill were applied during genetic counseling of our patients with ophthalmic inherited diseases and it helped us to improvise our counseling sessions. To the best of our knowledge very few centers in our country provide such service in human genomics.

S11.9

UPDATE IN THE GENOMIC MAPPING FOR EYE DISEASE GENES

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Advent of high throughput technologies, availability of the genome information from the Human Genome and Hapmap Projects, and powerful data analysis programs have led to identification of large number of chromosomal loci for eye diseases. Consequently some disease causative

or susceptibility genes have been discovered. We search for glaucoma genes by whole genome scanning, family linkage analysis, haplotype analysis and recombination mapping, and utilizing MLINK, ILINK and LINKMAP programs. We have narrowed and established regions in chromosomes 5q22.1-q32 and 15q22-24 as independent loci for juvenile-onset POAG. Using similar approaches we have also defined novel loci for high myopia and retinitis pigmentosa. Identification of novel genes in these regions is underway. Recently we completed a whole genome SNP linkage disequilibrium analysis in an association study and identified the HTRA1 serine protease gene for exudative age-related macular degeneration. We also constructed the first early fetal human eye cDNA library via expression sequence tag and have characterized 5,534 non-redundant clusters. Among the expressed genes in our library, 48% (2,680/5,534) did not share with current NEIBank human eye cDNA data. Our integration approach of genome-wide search, molecular investigation, and bioinformatics lead to discoveries of novel genes and gaining new insights into gene function both in eye diseases and during normal development of the eye.

S11.10

A GENOME-WIDE SCREEN FOR POAG: EVIDENCE FOR THE ROLE OF RACE IN GENETIC LINKAGE RESULTSM.A. HAUSER¹, J.L. WIGGS², K.R. LAROQUE-ABRAMSON¹, J.L. HAINES³, M.A. PERICAK-VANCE¹, R.R. ALLINGHAM¹*¹Duke University, Durham, NC, United States of America, ²Harvard Medical School, Boston, MA, United States of America, ³Vanderbilt University, Nashville, TN, UNITED STATES OF AMERICA*

Purpose: POAG is a complex inherited disorder. Here we report a major SNP-based genome-wide screen on a large POAG family dataset.

Methods: A genome-wide screen was performed on 142 multiplex POAG families each containing 2 or more affected family members. The marker panel consisted of 5067 SNPs (Linkage Panel IV) and was performed utilizing the Illumina Bead Station platform. Two point heterogeneity lod scores were calculated. Allegro software was used to calculate parametric multipoint HLOD scores. For multipoint analyses a single SNP was selected from each bin of SNPs in linkage disequilibrium ($r^2 > 0.16$). Genotype data were analyzed in toto and after stratification by Caucasian and African-American race.

Results: The genome-wide linkage screen was conducted on our expanded multiplex POAG family dataset containing 142 families (including 87 Caucasian, 37 African-American families). The genome-wide screen produced approximately 2.7 million genotypes for analysis. Analyses were performed on the total dataset. For multipoint analyses the Caucasian and African-American family subsets were also analyzed. Peak 2-point MLOD scores for chromosomes 3, 6, 14, and 15 were 4.4, 2.6, 4.4 and 3.5, respectively. Peak lod scores for these loci on 3, 6, 14 and 15 using parametric and non-parametric multipoint analyses for all analyses for these loci were 2.6, 1.7, 2.1, and 2.6, respectively. Additional potential loci were found by multipoint analysis on chromosomes 12 and 17. Peak lod scores for these loci were 2.2 and 2.0, respectively. Multipoint linkage scores for the loci on chromosome 3, 14 and 15 loci were primarily obtained from the African-American subset. The lod score for the locus on chromosome 12 locus was derived from the Caucasian subset. Contributions by both racial subsets contributed to the lod scores on chromosomes 6 and 17.

Conclusions: We have performed the most definitive genome-wide linkage screen using over 5000 markers in one of the largest multiplex POAG

family datasets described to date. We have identified novel loci for POAG. For the first time we report chromosomal loci for POAG that appear to be derived primarily from either Caucasians and African-American family subsets. These findings suggest that different genetic loci may explain in part variations in the prevalence and phenotype of POAG among races described in population-based studies.

S11.11
MOLECULAR GENETIC STUDIES ON GLAUCOMAS IN INDIA

D. BALASUBRAMANIAN

Director of Research, L V Prasad Eye Institute, Hyderabad, INDIA

The talk will concentrate on the molecular genetics of primary congenital glaucoma (PCG), see in children across several locations in India. The gene CYP1B1, associated with PCG, has been screened and over 50 mutations in the gene have been detected. The most common mutation seen is R368H, accounting for about 35% of the cases studied. SNP and haplo analysis has also been done and the possibility of risk or predisposition has been suggested. In silico bioinformatic analysis of the protein CYP1B1 has been done and the effects of several mutations on the structural properties of the protein have been surmised. Current efforts are towards expressing the wild type and the R368H mutant proteins and comparing their structural and functional differences.

S11.12
COMPREHENSIVE ANALYSIS OF MICRO RNA PROFILE IN RETINOBLASTOMA

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MicroRNAs (miRNAs) are a family of small, non-coding RNAs that regulate gene expression in a sequence-specific manner and are differentially expressed in different tissues. It also contributes to cancer development and progression. We studied the miRNA profile among retinoblastoma cell lines (WERI-Rb1, Y79) and retinoblastoma tissues using the TaqMan MicroRNA Assays. Apart from identified those miRNAs there were well characterized association with cancer, such as miR-92. We identified 7 miRNAs that were differentially up-regulated in retinoblastoma cell lines (2-60 folds) comparing to other cancer cell lines. We further determined the genome-wide DNA copy number abnormalities in retinoblastoma cell lines using the Agilent high-resolution Microarray-based Comparative Genomic Hybridization arrays (244K format; with a resolution of approximately 6.5kb/probe across the human genome). Importantly, a subset of miRNAs showed genome copy changes correlate with up-regulated miRNA expression. Our findings support the notion that copy number alterations of miRNAs were prevalent in cancer including retinoblastoma and may account partly for the miRNA deregulation reported in tumors. In addition, those differentially up or down regulated miRNAs identified and may contribute to the pathogenesis of retinoblastoma. . Currently, we are using the RNAi knock down approach to identify their potential target gene(s) of those up-regulated miRNAs. Keywords: MicroRNA, Retinoblastoma

Symposium 12
Immunology, Inflammation and Microbiology
Monday, 5 March 2007

S12.1
MACULAR EDEMA — MECHANISMS AND TREATMENT

B.C. CHENG

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S12.2
ROLE OF INFLAMMATION IN AGE RELATED MACULAR DEGENERATION

J. AMBATI

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Age related macular degeneration (AMD) affects tens of millions worldwide and its prevalence approaches that of cancer in industrialised nations. Growing evidence suggests that perturbations in the immune system leading to inflammatory consequences underlie both the development of AMD and the angiogenic switch that transforms the disease from an atrophic to a neovascular state. This presentation will discuss recent advances in the genetic basis of AMD in the context of in vivo evidence of complement activation as the triggering event in the transformation of the disease from one characterised by drusen to the vision-threatening state of choroidal neovascularisation. Potential targets for intervention at different states of AMD will be highlighted. *CR

S12.3
THE ROLE OF CYTOKINES AND CHEMOKINES IN UVEITIS

P.J. MCCLUSKEY

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S12.4
TOLL-LIKE RECEPTOR

D. WAKEFIELD

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Innate immunity is the first line of host defense that is responsible for the immediate and rapid immune response to microbial challenge and has been considered, until recently, to be rather non-specific. In contrast, adaptive immunity is delayed, requiring clonal expansion, is antigen-specific, mediated by antigen receptors on B and T lymphocytes, and is characterised by immunologic memory. TLRs have recently emerged as a key component of the innate immune system that detects microbial infection and triggers inflammation and antimicrobial host defense responses. In addition, TLRs are a critical link between innate and adaptive immunity by controlling the activation of the adaptive immune response. TLRs enable the host immune system to recognise and respond to microbes by their "signature" or molecular pattern, triggering the earliest immune responses that leads to inflammation. This review provides an overview of TLR research of specific relevance to the eye and provides a

perspective on the implications of these findings for better understanding ocular immunity and the immunopathogenesis of inflammatory eye disease.

S12.5

EXPERIMENTAL UVEITIS — IMMUNOPATHOGENESIS

J.M. LIVERSIDGE

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Autoimmune uveoretinitis is considered to be a CD4 T cell driven cellular immune response to retinal or other ocular antigen. As epitope spreading is a feature of disease pathogenesis, antigen based therapies to induce tolerance to eliciting antigen are problematical. To control aggressive ocular inflammation therefore, we have focused on the dual role of myeloid monocyte and dendritic cells in mediating both tissue damage and disease resolution. In this presentation I will discuss this dual role, and how the specialised ocular microenvironment may influence ocular immune function in vivo.

S12.6

MITOCHONDRIAL OXIDATIVE STRESS AND INFLAMMATION

N.A. RAO

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Purpose: The animal model of Experimental Autoimmune Uveitis (EAU) is found useful in understanding the mechanisms of photoreceptor damage in intraocular inflammations and uveitis. Recent studies on EAU revealed oxidative stress leading to the photoreceptor damage and this takes place prior to macrophage infiltration. In the current study, the mechanism of the oxidative stress is investigated.

Methods: Prior to macrophage infiltration in the EAU retina, the eyes from iNOS knock out and wild type animals were processed to detect nitric oxide derived reactive species in the retina, cytokines that induce iNOS, translocation of iNOS in to mitochondria and mitochondrial membrane oxidative damage.

Results: In the wild type animals there was selective upregulation of T-cell infiltration markers and iNOS inducing cytokines followed by mitochondrial translocation of this protein with localised mitochondrial oxidative stress. Such oxidative stress was absent in the knock out mice.

Conclusions: The results indicate that photoreceptor mitochondrial oxidative stress could be initiated by activated T-cell infiltration in the retina and their cytokines may trigger the iNOS upregulation and translocation. The resulting lipid peroxidation products and their chemotactic properties amplify the inflammation by recruiting the macrophages and other inflammatory cells.

S12.7

MOLECULAR PHENOTYPE OF THE RETINAL VASCULAR ENDOTHELIUM

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Molecular heterogeneity of the vascular endothelium throughout the body has recently been appreciated, largely as a result of global cell profiling strategies. Our work has focused on defining the specific molecular phenotype of the retinal vascular endothelial cell in comparison to the choroidal vascular endothelial cell. Although immediately adjacent, the retina and choroid are involved in very different diseases; of particular interest is the role of the retinal vascular endothelium in posterior uveitis, a vision-threatening inflammatory or infectious disease of the posterior segment of the eye, based on the unique molecular phenotype of this endothelium. We have investigated the gene expression profiles of cultured human retinal and choroidal vascular endothelial cells both in unstimulated and stimulated (by infection with *T. gondii* tachyzoites or incubation with lipopolysaccharide) states using Affimetrix GeneChip oligonucleotide arrays; despite strong correlation between retinal and choroidal endothelial gene expression profiles, approximately 10% of 8747 gene transcripts are differentially expressed prior to any stimulation. Using quantitative real-time RT-PCR, we have confirmed array data suggesting that human retinal endothelial cells express relatively higher levels of a number of gene transcripts relevant to the immune response, including ICAM-1, VCAM-1 and E-selectin. In addition, we have used public software (PAINT with TRANSFAC Pro) to identify common cis-regulatory motifs within the promoter region of genes that are differentially expressed in retinal or choroidal endothelial cells. In a pilot study, we have used 2-dimensional difference gel electrophoresis to compare protein preparations isolated from human retinal and choroidal vascular endothelial cells; with this technique we have identified approximately 30 proteins that are differentially abundant. In summary, our data indicate significant differences in the transcriptomes and proteomes of vascular endothelial cells of retinal versus choroidal origin. These findings are consistent with the concept of ocular vascular endothelial cell heterogeneity. Our findings may direct the development of new treatments for posterior uveitis that target tissue-specific interactions between the retinal endothelium and an infiltrating cell or invading microbe.

S12.8

PRIMARY INTRAOCULAR LYMPHOMA

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S12.9

ROLE OF POLYMERASE CHAIN REACTION IN INTRAOCULAR UVEITIS

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Intraocular infections, particularly, of viral origin show characteristic clinical appearances to enable an ophthalmologist to make accurate diagnosis. However, some of these including infectious endophthalmitis, viral retinal inflammations and uveitis may show atypical clinical presentations with

fundus picture leading to a diagnostic dilemma in the etiology of the disease process. The recent nucleic acid-based molecular biological tests are showing promising results of value as diagnostics. Polymerase chain reaction (PCR) is the best studied and most widely used test with a turn around time of around 8 to 12 hours depending on the method of DNA extraction or conversion of RNA into cDNA in case of reverse transcription PCR and further the thermal profile. The collection of aqueous humor (AH) is a simple and safe office procedure, while that of vitreous fluid and lens materials require surgical procedures. Typically, PCR as a diagnostic test is required when the patient presents with a clinical diagnosis for which several infectious agents are known to be associated with the disease or shows typical clinical appearance of a disease due to a specific infective agent but does not respond to the appropriate therapy or requires a confirmation of etiology when a surgical procedure is contemplated. Ocular biopsy material from any of its lesion or inflammatory exudates can be used as primary clinical specimen for a diagnostic PCR test. Typically, AH or vitreous biopsy specimen is preferred either as a diagnostic aspirate. Many studies have proven that by PCR-based techniques, bacterial DNA can be detected in 100% of intraocular specimens from typically clinical bacterial endophthalmitis. Since size of the intraocular clinical specimens is small, PCR may be the test of choice to detect the common herpes viruses associated with retinitis and choroiditis & atypical toxoplasmosis. Less-time consuming and cost effective multiplex PCRs have been developed to detect genomes of more than one infectious agent. Quantitation of the viral load of herpes viruses in clinical specimens by real-time PCRs are being employed. The basic skills required to perform PCR are similar to that required of a good microbiology technician.

S12.10

EMERGING INFECTIOUS UVEITIS

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Viral infections in the eye may present as acute or chronic inflammation. The association of Rubella virus and Fuch's Uveitis Syndrome has recently been reported in Europe. In a review of patients presenting with hypertensive anterior uveitis, 18 of 48 eyes (37.5%) and 5 of 16 eyes (31.2%) with a clinical picture similar to Posner Schlossman Syndrome and Fuch's Uveitis Syndrome respectively, had Cytomegalovirus detected in the aqueous on Tetraplex polymerase chain reaction.

S12.11

VOGT-KOYANAGI-HARADA DISEASE

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Vogt-Koyanagi-Harada disease (VKH) is generally a rare multisystemic autoimmune disease characterised by a bilateral granulomatous panuveitis that can have variable involvement of the central nervous, auditory and integumentary systems. The diagnosis of VKH is difficult to make in the acute stage because of the lack of extracocular manifestation at the onset of disease. However fluorescein angiography at the acute stage appears to be a sensitive and helpful test for diagnosis of VKH. Lumbar puncture is reserved for atypical cases. VKH disease may manifest with varying degrees of severity at onset of disease. There is controversy with regard to the duration of therapy and the point at which immunomodulatory agents should be added. High dose systemic

corticosteroids in the early stages is advocated to reduce complications and subsequent recurrences of ocular inflammation. Neither corticosteroid monotherapy nor combined steroid and immunosuppressive regimens appear to positively affect the overall visual outcome of chronic VKH. However, aggressive therapy to prevent the development of chronic changes should be the goal, as the frequency of ocular complications increases with increased disease recurrence and duration.

S12.12

BEHCET'S DISEASE

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S12.13

OCULAR LESIONS DUE TO HUMAN IMMUNODEFICIENCY VIRUS IN THE DEVELOPING COUNTRIES

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India has rapidly become an epicenter of a major epidemic in recent years — the acquired immunodeficiency syndrome (AIDS). Most patients (76%) were in the 20 to 40 year age group. Heterosexual exposure to commercial sex workers was the most common risk factor for HIV infection. Cytomegalovirus retinitis and HIV retinopathy were the most common HIV associated ophthalmic lesions. Pulmonary tuberculosis and oropharyngeal candidiasis was the most commonly associated systemic infections. Ocular involvement was seen in 40 percent of patients. Ocular involvement was most common in children who contracted the disease through perinatal transmission. No case of ocular Kaposi's sarcoma was seen. Ocular tuberculosis was seen in 15 (1.96%) out of 766 consecutive patients of HIV/AIDS. Our study showed the spectrum of ocular lesions associated with HIV infection in India is different from that seen elsewhere in the world.

S12.14

HLA-B27 UVEITIS IN CHINESE — AN ANALYSIS OF 504 PATIENTSY.M. CHUNG¹, C.P. TSAI¹, W.M. HSU²¹*Uveitis Service, Department of Ophthalmology, National Yang-Ming University School of Medicine and Taipei Veterans General Hospital, Taipei, Taiwan;*²*Department of Ophthalmology, National Yang-Ming University School of Medicine and Taipei Veterans General Hospital, Taipei, TAIWAN*

Purpose: HLA-B27 uveitis is the most common type of endogenous uveitis in Taiwan. The clinical features consist of a unilateral nongranulomatous anterior uveitis of sudden onset and limited duration. It appears to be both genetic and environmental contributions to the development of the episode. One of the environmental factors is a seasonal variation. On the other hand, HLA-B27 antigen is also highly linked to the spondyloarthropathies, a group of disorders including ankylosing spondylitis, reactive arthritis, psoriatic arthritis, enteropathic arthritis, and undifferentiated spondyloarthropathies. The main objectives in this study were to assess the clinical features of HLA-B27 uveitis in Chinese patients.

Methods: A retrospective study of 504 consecutive patients seen at the uveitis clinic in Taipei Veterans General Hospital during a period of 18 years between 1987 and 2004. All patients manifested a typical acute anterior uveitis and with positive HLA-B27 antigen. Rheumatologist was consulted simultaneously if spondyloarthropathy was highly suspected.

Results: Three hundred and thirty five patients (66.5%) were male and 169 patients (33.5%) were female, with male to female ratio of 2.0. The median age at the time of the first attack of uveitis was 36 years, with 37 in male and 34 in female. ($p>0.05$) The mean age at the time of the first attack of uveitis was 37.4 ± 13.2 years, with 38.8 ± 14.4 in the male and 34.5 ± 9.8 in the female. ($p>0.05$) Of the 504 patients, the monthly distribution of 1106 attacks showed a significant monthly variation with the high frequency in January and February ($p<0.01$) and low frequency in August ($p<0.01$). Spondyloarthropathy was seen at the last visit in 386 patients (76.6%). Ankylosing spondylitis was the most common one as 55.4%, undifferentiated spondyloarthropathy in 38.6%, Reiter syndrome in 2.8%, psoriatic arthropathy in 2.6% and inflammatory bowel disease in 0.5%. Comparison between patients with and without spondyloarthropathy revealed the earlier onset of uveitis ($p<0.05$) and higher attacks of uveitis ($p<0.025$) in the association group.

Conclusions: A male predominance, a relative young age, a prominent seasonal variation and high association of spondyloarthropathy were found in Chinese patients with HLA-B27 uveitis.

S12.15

AETIOPATHOGENESIS OF UVEITIS ASSOCIATED WITH LEPTOSPIROSIS IN HUMANS

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Purpose: Leptospirosis is a widespread zoonosis caused by the spirochaete, *Leptospira* sp. In humans, several months after recovery from acute systemic illness, 10-40% of patients develop uveitis, which is characterised by a specific combination of clinical features. The objective of this study is to confirm the leptospiral etiology of uveitis and to understand the underlying pathogenic mechanism.

Methods: Paired serum and aqueous humor samples were collected from clinically diagnosed leptospiral uveitis patients. The aqueous humor samples were tested for the presence of leptospire by culture and PCR. The serum samples were tested for anti-leptospiral LPS IgM antibodies by microscopic agglutination test (MAT) and ELISA. Further, the aqueous humor and serum samples were analysed for the nature of infiltrating cells, profile of cytokines by Cytometric Bead Array and the causative factor for the inflammatory response by dot blot. Behcet's uveitis, phacolytic uveitis, age-related cataract patients and healthy persons were included as controls.

Results: Though it was not possible to isolate *Leptospira* sp., 75% of aqueous humor samples from leptospiral uveitis patients were positive in PCR for G1/G2 primers. None of the controls were positive. 77% of leptospiral uveitis patients were seropositive either by MAT or ELISA. A selective infiltration of neutrophils as well as a significant increase in the levels of protein, cytokines — IL-12p70, TNF, IL-6, IL-8 and IL-10 was observed in aqueous humor of leptospiral uveitis patients. Phacolytic uveitis was characterised by a high proportion of "activated" macrophages and increased levels of inflammatory cytokines IL-6 and IL-8 in aqueous humor, while in Behcet's uveitis, a predominant infiltration of neutrophils and increased levels of IFN- γ was observed. A significant level of *L. icterohaemorrhagiae* LPS was observed in aqueous humor of leptospiral

uveitis patients compared to controls.

Conclusions: The leptospiral etiology in patients clinically diagnosed as leptospiral uveitis was established based on serology and PCR. Leptospiral LPS was identified to be specific for serodiagnosis. Accumulation of leptospiral LPS in the anterior chamber of leptospiral uveitis patients is quite possibly responsible for predominant infiltration of neutrophils and local production of inflammatory cytokines involved in the development of uveitis. Hence suppression of inflammation will have significant implication in the treatment of leptospiral uveitis.

Symposium 13 Stem Cell Biology/Ocular Surface Monday, 5 March 2007

S13.1

TRANSPLANTATION OF RETINAL STEM CELLS

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Work in several laboratories has shown that retinal progenitor cells (RPCs) can be isolated from the retina of mice, rats, cows, and humans. Work by our group has established that murine RPCs (isolated from GFP+ transgenic donors) can be grafted into the subretinal space of mature, rd mice. The grafted RPCs differentiate into photoreceptor cells expressing rod and cone markers, and recipient mice show evidence of improved visual performance. While work in rodents provides an important and necessary step in the development of a treatment for retinal degenerations, several aspects of the mouse eye stand in the way of progress towards clinical applicability. These include the small size of the eye, the small vitreal cavity, and the low cone/rod ratio. The substantial progress in stem cell biology and transgenic pig technology has provided the impetus for expansion of the scope of this work from the mouse to the pig. I will present recent data on the use of porcine RPCs transplanted to the pig retina. Ongoing studies are evaluating RPC integration and differentiation, and I will discuss novel approaches at retinal repair using tissue engineering strategies.

S13.2

CULTURING PROGENITOR CELLS FROM THE MAMMALIAN RETINA

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Multipotent progenitor cells have been isolated from the brain and retina, expanded in culture, and transplanted to the central nervous system (CNS). There is now evidence that these cells can be grown from a number of mammalian species including mouse, rat, pig, cat, and human, as well as other species. These cells display a number of characteristic markers regardless of species of origin, while some markers appear to be differentially expressed between species. There are also detectable differences in marker expression by progenitors from retina versus brain. Furthermore, within a given culture there is heterogeneity of marker expression. The underlying basis for these various differences ranges from obvious to obscure. In addition, co-culturing cells on biodegradable

polymer scaffolds can also influence the expression of markers by these cells and the same is true for transplantation. Following transplantation to the subretinal space, CNS progenitor cells engraft in the diseased retina of mature recipients and express photoreceptor markers such as rhodopsin. *CR

S13.3

INDUCTION OF PHOTORECEPTORS FROM EMBRYONIC STEM CELLS

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Retinal cell transplantation is expected as one of the future treatments of the obstinate retinal diseases. However, the problem of short supply of the donor cells is apparent even if it works well. The embryonic stem (ES) cell is one solution of the problem.

We have established a feeder- and serum-independent culture method that induces directed differentiation of primate ES cells (human and non-human) into mature retinal cells.

The SFEB (Serum-free Floating culture of Embryoid Body-like aggregates) method combined with long term application of Dkk-1 and Lefty-A (SFEB/DL) efficiently induces differentiation of monkey and human ES cells into Rx+ or Mitf+ retinal progenitors, which were consistent with the characteristics of eye primordia during development.

Some of these progenitor cells differentiate into RPE with hexagonal shapes, pigment granules, apical microvilli, basal membranes, and tight junctions. Further treatment with some factors promotes the progenitors to differentiate into rhodopsin+/recoverin+ photoreceptors efficiently. The next step of preparation of donor cells is to select the required cells out of undifferentiated cells that may cause tumours.

S13.4

TRANSPLANTATION OF BDNF SECRETING MESENCHYMAL STEM CELLS PRESERVES VISUAL FUNCTION IN CHRONIC HYPERTENSIVE RAT EYES

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A serious problem with developing useful therapies for treatment of CNS disorders, including blinding ocular diseases, is in developing effective methods that prevent further degeneration and also facilitate recovery of function. Stem cell transplantation offers a novel and extremely exciting possibility to circumvent these limitations. Mesenchymal stem cells (MSCs) are an attractive source of cells for transplantation and neuroprotection as they are easily isolated, can be engineered to express exogenous genes, and are a potential source of cells for autologous transplants. In this study rat MSCs were engineered as vehicles for delivery of brain-derived neurotrophic factor (BDNF) following transplantation to chronic hypertensive rat eyes. Chronic ocular hypertension (COH) was induced in Brown Norway rats by laser cauterisation of the trabecular meshwork. Lentiviral constructs were used to transduce MSCs to produce BDNF. As a control, a second group of MSCs were transduced with a green fluorescent protein (GFP) lentiviral construct. The increased expression and secretion of BDNF from transduced MSCs was verified using ELISA, and bioactivity of the BDNF was assessed using a neurite outgrowth assay

from rat dorsal root ganglia cultures. Two days post induction of COH, either BDNF secreting MSCs (BDNF-MSCs), or control GFP expressing MSCs (GFP-MSCs) were injected intravitreally into damaged eyes. Tonometry was used to measure intraocular pressure at multiple time points post COH induction. Analysis of retinal function was performed using electroretinogram (ERG) recordings at days 20 and 40 post induction. Following the final recording session, retinal tissue was prepared for immunohistological analysis. Our results indicate that eyes transplanted with BDNF-MSCs displayed significant preservation of visual function when compared to the control group of animals treated with the control GFP-MSCs. The BDNF-MSC treated eyes also appear to display a greater level of morphological preservation of the retinas and optic nerves as compared to their counterparts treated with GFP-MSCs. These results suggest the transplantation of genetically modified MSCs may be a very useful strategy for treatment of retinal disease.

S13.5

IMMUNE RESPONSE FOLLOWING TRANSPLANTATION OF NEURAL STEM CELLS DERIVED FROM BRAIN AND AMNION

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Neural stem and progenitor cells isolated from various tissue have been shown to be potentially useful for retinal regeneration. However, studies of this type have shed little light on the immune responses following transplantation. Risk of immune rejection must be examined before successful clinical application of allogeneic or xenogenic stem cell transplantation can be achieved. We have therefore investigated the immunogenic and antigenic properties of neural progenitor cells from the CNS and amnion, using heterotopic transplantation models.

Our results indicate that allogeneic CNS progenitor cells survive at least 4 weeks in a heterotopic non-immune-privileged site, during which time they neither sensitise the host, nor express detectable levels of MHC class I or II. This in vivo data is in accord with flow cytometric results showing that CNS progenitor cells do not express MHC class I or class II, either at baseline or upon differentiation in 10% serum. Exposure to IFN-gamma, however, reversibly up-regulates expression of these key transplantation antigens. These results show CNS progenitor cells as possessing inherent immune privilege. Since CNS progenitor cell allografts were rejected following specific sensitisation of the host, CNS progenitor cells are able to display alloantigens, albeit not in an immunogenic form. Amniotic cells have recently appeared to offer the potential for differentiation into various cells, such as neuronal cells, hepatocytes and bone marrow cells. Amnion mesenchymal cells (AMCs) have been shown to display the phenotype for neural progenitors and have the ability to differentiate into neuronal cells in vitro. Cultured AMCs express MHC class I, but not class II, but these cells express both MHC class I and class II in host eyes after subretinal transplantation. AMCs are infiltrated with T cells and are acutely rejected in eyes of immunocompetent hosts. Anti-donor-specific IgG in host sera increases. Conversely, subretinally transplanted AMCs in immunodeficient hosts survive for 24 weeks with no evidence of inflammation, and express retinal markers. These results indicate that AMCs display antigenicity and immunogenicity and are vulnerable when transplanted into the subretinal space, and that these cells obtain long-term survival and possess the potential to differentiate into retinal cells when hosts immune response is suppressed. Human amnion is easy to obtain, and has been already been broadly used for ocular surface applications clinically. These findings indicate advantages as a source of

stem cells from an ethical perspective, particularly with respect to potential clinical applications.

S13.6

SUCCESSFUL TRANSPORT AND IN VITRO EXPANSION OF CORNEAL ENDOTHELIAL PRECURSOR CELLS, USING A NOVEL THERMOGELATION POLYMER (TGP)

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Purpose: To assess the efficacy of a novel TGP for transportation, at varying temperatures, of corneal endothelial cell layers and for expansion and characterisation of the precursor cells in vitro.

Methods: Endothelial cell layers, harvested from cadaver corneal buttons in, a hospital (Tiruchirappalli, India), were transported more than 320 km (200 miles) to a central laboratory (Chennai, India). The transportation was done at varying temperatures, ranging from 22° C to 30° C, taking an average of 10 hrs, and using two methods of preservation. In one method (Group I), specimens were suspended in a basal culture medium (Dulbecco's minimal essential medium containing BFGF+EGF + B27+ Collagenase type1); in the other method (Group II), specimens were embedded in TGP above which the same basal culture medium was added. Upon arrival at the laboratory, the specimens were trypsinised and grown for two weeks as sphere suspension cultures, Gr. I in the basal culture medium and Gr.II in TGP topped with basal culture medium. Viable cells in both groups were counted immediately upon receipt and thereafter at regular intervals during the expansion, and were characterised by RT-PCR.

Results: Immediately after transport, Gr II yielded a higher number of viable cells than did Gr.I. During expansion, Gr. II exhibited a predominance of single cells forming spheres whereas Gr I exhibited aggregations of developing spheres. Spheres from both the groups tested positive for the neuronal marker B-3 tubulin and negative for cytokeratins K3 and K12.

Conclusions: TGP is superior to basal culture medium in maintaining the viability of the cells of the corneal endothelial layer during transportation at varying temperatures. Post-transportation, such viable cells can be grown as sphere forming assays in TGP, with a better yield of definite endothelial precursor cells. *CR

S13.7

THE ECTOPIC CLUSTER OF CORNEAL EPITHELIUM IN THE CONJUCTIVA

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The ocular surface is covered by two biologically distinct epithelia; the corneal and the conjunctival epithelium. Although expression of the keratin12 is generally thought as a hallmark of cornea-type differentiation, the cluster of keratin12 positive cells are also present in approximately 1% of human bulbar conjunctival epithelium. The gene expression patterns of these cells were extremely similar to those of corneal epithelial cells.

In addition, p63 and ABCG2 were expressed in the basal cells beneath the keratin12-positive cells. Thus, one can presume that there are ectopically-residing, self-maintaining corneal epithelial stem cell and its cluster in the human conjunctival epithelium.

S13.8

CK3 AND CK12 ARE EXPRESSED BY ADULT CONJUNCTIVAL EPITHELIAL CELLS PLACED IN CORNEAL STROMA

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Purpose: To determine the effect on the cytokeratin profile of conjunctival epithelial cells placed in the rabbit corneal stroma.

Methods: Conjunctival explants were removed from the fornix of adult New Zealand white rabbits and cultured for 7 days to subconfluence. Cells were removed for use as autologous implants into the central corneal stroma. After 3, 7 and 14 days, the rabbits were killed and the cornea used for histology, immunohistochemistry or the cytokeratins K3/K12, K13, K4 as well as collagen IV and laminin as well as real-time PCR.

Results: The subconfluent cells at 7 days were strongly positive for CK14 and some cells for CK4 as well. However, CK12 a marker for differentiated corneal epithelial cells was always negative. At 3 days of stromal implantation the conjunctival cells expressed CK4/13 uniformly. However, by 7 days after stromal implantation a number of cells expressed CK3/12, CK4 expression remained strong but CK13 was reduced. The cells were surrounded by layer of positive staining for collagen IV and laminin. After 7-14 days of implantation the cells began to reveal stratified organisation and most cells showed CK3/12 staining. The basement membrane stained for collagen IV and laminin. Real-time PCR confirmed the presence of CK 4 and CK 12.

Conclusions: The results suggest that the corneal stroma can influence gene expression and keratin synthesis of implanted cultured conjunctival epithelial cells encouraging them to exhibit more corneal epithelial-like properties. Despite their origins from different stem cell populations it may be possible to interchange conjunctival and corneal cells for therapeutic tissue replacement procedures.

S13.9

REGULATION OF LIMBAL EPITHELIAL STEM CELLS

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The basal layer of the limbal and central corneal epithelium is enriched in stem cells and transient amplifying (TA) cells, respectively. This physical separation of stem and TA cells makes the limbal/corneal epithelium an exceptionally suitable system for isolating basal cells enriched in these two proliferative populations. In an effort to obtain epithelial stem cells and TA cells in their more "natural" quiescent state, we used laser capture microdissection (LCM), to isolate resting limbal and corneal basal cells from frozen sections. The LCM system that we used enabled us to isolate these cell populations directly with minimal tissue processing, thereby improving the yield and quality of RNA. Transcriptional profiling of the RNA isolated from these two populations revealed a set of ~100 genes that were differentially expressed in limbal versus corneal epithelial basal cells. The expression of some of these genes was validated using semiquantitative RT-PCR. The expression profile data were further

validated by the preferential LacZ and immunohistochemical detection of one of the limbal-enriched genes, epiregulin, in a subset of limbal epithelial basal cells. Epiregulin, is a member of the epidermal growth factor family, and has been shown to promote both in vivo and in vitro wound closure in epidermal keratinocytes. Its preferential location in limbal basal cells may affect the increased proliferative response of these cells following wounding.

S13.10

THE LIMBAL STEM CELL NICHE AND ITS RECOVERY

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Purpose: Corneal epithelial stem cells are known to be localised to the basal layer of the limbal epithelium. However the mechanisms regarding the maintenance of these stem cells in their specialised niche remain poorly understood. Patients with limbal stem cell deficiencies can be treated with allogeneic limbal transplantation. In this study, we report the mechanisms regarding stemness in limbal stem cell niche, and an alternative replacement strategy for damaged corneal epithelium involving a tissue-engineered epithelial cell sheet comprising only the patient's autologous oral mucosal epithelial cells.

Methods: N-cadherin is a member of the classical cadherin family and has previously been demonstrated to be expressed by hematopoietic stem cell niche. The expression patterns of N-cadherin in limbal and corneal epithelium were investigated by immunofluorescence staining. For the clinical investigation, tissue-engineered epithelial cell sheets were fabricated ex vivo by culturing oral mucosal cells on temperature-responsive cell culture surfaces for two weeks. After surgically removing conjunctival fibrovascular tissues from corneal sites, cultured autologous cell sheets harvested using a simple reduced-temperature treatment were transplanted directly to patients' denuded corneal surfaces.

Results: Immunofluorescence staining revealed that N-cadherin was expressed in limbal basal epithelium, but not in any epithelial layers of the central cornea. In the clinical investigation, complete re-epithelialisation of patients' corneal surfaces occurred within one week. Corneal transparency was restored and postoperative visual acuity improved.

Conclusions: N-cadherin may be a critical cell-to-cell adhesion molecule in the limbal stem cell niche. Tissue-engineered epithelial cell sheets fabricated ex vivo from autologous oral mucosal epithelial cells are effective for reconstructing the corneal surface and restoring vision in patients with bilateral total limbal stem cell deficiencies. *CR

S13.11

AUTOLOGOUS CULTIVATED LIMBAL EPITHELIAL STEM CELL TRANSPLANTATION: CLINICAL OUTCOME

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From March 2001- June 2006, 400 cultivated limbal epithelium transplantation procedures for limbal stem cell deficiency (LSCD) have been performed at LVPEI. A retrospective study for demographics, primary etiology, previous surgeries, and preoperative and postoperative best corrected visual acuity, the type of cultivated limbal epithelial transplantation, complications and final outcome was performed on all

consecutive patients following CLET with a minimum follow-up time of one year.

Diagnostic criteria for Limbal Stem Cell Deficiency (LSCD):

- Symptoms of decreased vision, redness, watering, photophobia and recurrent attacks of pain.
- Triad of signs: conjunctivalisation, neovascularisation and chronic inflammation.
- Stippled appearance of conjunctivalised cornea with loss of palisades of Vogt.
- Recurrent and persistent epithelial defects.
- Superficial vascularisation, scarring, thick fibrovascular pannus, ulceration, melting and perforation.

Inclusion criteria: Patients with

1. Conjunctivalisation.
2. Absence or partial loss of limbal palisades of Vogts.
3. Persistent or recurrent corneal epithelial defect with persistent inflammation.

Exclusion criteria:

Follow-up duration less than 6 weeks.

Limbal Biopsy

The limbal tissue was harvested from patients undergoing limbal biopsy for cultivated limbal transplantation after the diagnosis of LSCD from healthy contralateral eye or a healthy area of ipsilateral eye. For harvesting the limbal tissue (biopsy), under local or general anesthesia, the conjunctiva of the eye was incised 3 mm behind the limbus at 12 o'clock position and dissected towards limbus and into the clear cornea up to 1mm, using # 15 blade on Bard Parker handle. Depth of dissection was superficial and followed corneal insertion of limbal epithelium. The conjunctiva was then excised at limbus just behind pigmented line (Palisades of Vogts) and limbal tissue with 1mm clear corneal tissue was excised. The limbal tissue was then collected in the Human Corneal Epithelial medium in an ependorff and sent to the laboratory for culturing on the human amniotic membrane (HAM).

Cultivated Limbal Epithelial Transplantation (CLET)

During CLET, the fibrovascular pannus covering the ocular surface was excised from the cornea and sent for histopathological evaluation. A drop of epinephrine (1:1000) was instilled in the conjunctiva prior to pannus excision to achieve homeostasis. After release of the symblephara and adequate homeostasis with cautery, the HAM with the cultivated limbal epithelial monolayer of cells with epithelial side up was spread over the defect. The graft was then secured to the limbal side by interrupted, circumferential 10-0 nylon sutures as well as to the surrounding conjunctival edge by interrupted 8-0 polyglactin sutures.

Following CLET, all patients were treated with 1% prednisolone acetate eye drops and 0.3% ciprofloxacin hydrochloride eye drops. 0.3% Ciprofloxacin hydrochloride eye drops were continued if an epithelial defect continued or as long as bandage contact lens was used. The patients were seen on postoperative day 1, week 1, week 2, week 5, and monthly thereafter. Each examination included a complete history, noting down of new ocular or systemic symptoms, a complete evaluation of recipient as well as donor sites, and any sign of neovascularisation or surface instability. Best corrected visual acuity (BCVA) was measured at each visit on a standard Snellen Visual Acuity Chart. The ambulatory visual acuity was defined as BCVA of more than or equal to 20/200 and non ambulatory visual acuity of less than 20/200. Sutures were removed when indicated (loose or vascularised). Epithelialised sutures were left in place indefinitely.

Criteria for successful transplantation

The success of the procedure was defined by:

Stable ocular surface based on absence of recurrent breakdown of the

corneal epithelium, increased permeability to fluorescein, or conjunctivalisation and subjective improvement in the symptoms of the patient. Minimum follow-up of 6 weeks.

Failure of the procedure was defined by:

Conjunctivalisation, recurrent epithelial breakdown, or persistent epithelial defect.

Results: We included 200 patients who had one year follow up in June 2006 following CLET. Patients with less than six weeks follow were excluded. Thus 143 eyes of 138 patients who underwent CLET during April 2001 - June 2006 were included in the analysis.

The demographic details of the patients, type of cultures and etiology of LSCD are analysed. Of these 96 (69.5%) eyes had total LSCD (3600) associated with loss of palisades where the limbal biopsy was taken from the contralateral eye and 21 (15%) eyes showed partial LSCD (900 - 3300), where limbal biopsy was taken from clinically normal area. On clinical examination, 36 (26%) eyes showed severe conjunctival damage including symblephara (16).

45 (32%) eyes underwent PKP following CLET for visual purpose at a mean follow up time of 8.3 ± 5.9 months (range, 1-26 months). Among these 34 eyes (76%) showed survival of graft while 11 eyes (24%) showed failed graft due to various reasons.

Univariate analysis of factors affecting final outcome of the transplantation did not demonstrate any statistically significant effect of the type of transplantation, duration of symptoms, and etiology of LSCD or previous surgical intervention on the outcome of cultivated limbal epithelium transplantation.

S13.12

CULTIVATED CONJUNCTIVAL AND ORAL EPITHELIAL TRANSPLANTATION FOR THE TREATMENT OF SEVERE CORNEA AND OCULAR SURFACE DISEASE

L.P.K. ANG

Ophthalmology, Singapore National Eye Centre, Singapore, SINGAPORE

The destruction of the ocular surface is a major debilitating cause of ocular morbidity and blindness in the world. Severe ocular surface disease arising from conditions such as Stevens-Johnson syndrome and chemical injury are devastating conditions that may result in limbal stem cell deficiency, corneal scarring and severe visual loss. Management of these complex cases remains a major clinical challenge.

Damage to the conjunctiva may also arise from various diseases, such as pterygia and conjunctival tumours. Treatment of these disorders involves surgical excision of the diseased area resulting in an epithelial defect that may be left to heal on its own, be patched up with an autologous conjunctival graft or with an amniotic membrane. The use of bioengineered conjunctival equivalents represents a novel approach to reconstruct the ocular surface, without causing iatrogenic injury from harvesting large autografts. It is particularly useful in situations where the normal conjunctiva is deficient either from disease or scarring.

Another promising new treatment modality is the use of autologous cultivated oral epithelial transplantation for the treatment of severe cornea and ocular surface disease. Utilizing the patient's own oral tissue overcomes the problems related to allogeneic transplantation, and is particularly useful for treating severe bilateral conditions. We have recently also demonstrated the effective use of autologous serum-derived cultivated oral transplantation for the treatment of severe limbal stem cell deficiency, bringing us one step closer towards developing safer, xenobiotic-free autologous bioengineered tissues for clinical transplantation.

S13.13

IDENTIFICATION, CHARACTERISATION, ENRICHMENT AND EX VIVO EXPANSION OF HUMAN CORNEAL EPITHELIAL STEM CELLS

V. MUTHUKARUPPAN¹, P. ARPITHA¹, N.V. PRAJNA², M. SRINIVASAN³

¹Aravind Medical Research Foundation, Madurai, India; ²Aravind Eye hospital, Madurai, India; ³Aravind Eye Hospital, Madurai, INDIA

Background and Objectives: We have earlier demonstrated that a distinct group of small cells in the limbal epithelium could be identified on the basis of two-parameters: high levels of nuclear protein p63 combined with greater N/C ratio (IOVS, 2005). The objective of the present study is to confirm that the two-parameters in combination, serve as a marker for corneal epithelial stem cells.

Methods: Limbal fragments were subjected to trypsin followed by Dispase II to obtain two populations of epithelial cells, one from suprabasal / superficial layers and another from basal layer alone. The cytospin smears were double-immunostained for p63 and any one of the stem cell related markers and analysed using quantitative confocal microscopy. The limbal explant cultures were pulse labeled with BrdU for 5 days followed by chase for 3-weeks. Two-parameter analysis was used to quantify the number of stem cells in the ex vivo expanded limbal epithelium.

Results: That high levels of expression of p63 combined with greater N/C ratio define human corneal epithelial stem cells is confirmed by the following evidences. (1) There is a five-fold enrichment of such cells isolated from the limbal basal layer. (2) They are positive for the stem cell related markers acidic cytokeratins, keratin 5 and negative for keratin 3, connexin43, 14-3-3sigma and Ki67. (3) They are absent in peripheral cornea (4). They possess label retaining slow-cycling property. Further, we have shown that about 5% of cells are positive for the above markers in ex vivo expanded limbal epithelium. However, such cells were absent in the epithelial sheet from peripheral corneal cultures.

Significance of the Study: The two-parameter analysis serves as a precise marker for corneal epithelial SC. This method will be useful to evaluate the SC content of the graftable epithelial sheet after ex vivo expansion in defined culture condition and to characterise the factors of SC 'niche'.

Symposium 14 Ocular Imaging Monday, 5 March 2007

S14.1

RECENT DEVELOPMENTS IN SCANNING LASER TOMOGRAPHY: APPLICATIONS IN GLAUCOMA

B.C. CHAUHAN

Department of Ophthalmology, Dalhousie University, Halifax, Ontario, CANADA

This presentation will review the recent developments in scanning laser tomography to allow detection of the earliest changes in the optic nerve head in glaucoma. *CR

S14.2
QUALITY ASSESSMENT IN OCULAR IMAGING

H. ISHIKAWA

Eye and Ear Institute, Ophthalmology/Glaucoma, University of Pittsburgh Medical Center, Pittsburgh, PA, UNITED STATES OF AMERICA

Ocular imaging modalities are becoming part of standard clinical care. However, the importance of image quality assessment has been largely overlooked. Since image quality affects outcome measurements directly, not only discriminating poor quality images as invalid scans but also compensating quality effect on the measurements is necessary especially when following patients longitudinally.

This presentation will cover quality assessment issues with optical coherence tomography (OCT), Heidelberg retina tomography (HRT), and scanning laser polarimetry (SLP or GDx).

S14.3
CORRELATION BETWEEN OPTICAL COHERENCE TOMOGRAPHY IMAGING AND RED-FREE RETINAL NERVE FIBRE LAYER PHOTOGRAPHY

K. PARK

Ophthalmology, Seoul National University College of Medicine, KOREA

The normal and glaucomatous features of RNFL photograph and Stratus OCT results will be presented and the correlation between two techniques will be discussed.

S14.4
PERIPAPILLARY RETINAL NERVE FIBER LAYER THICKNESS VARIATIONS WITH MYOPIA

S.T. HOH

Singapore National Eye Centre, Singapore, SINGAPORE

Purpose: To determine the relationship between peripapillary retinal nerve fiber layer (RNFL) thickness and myopia using optical coherence tomography (OCT).

Methods: One hundred thirty-two young males with myopia (spherical equivalent [SE], -0.50 to -14.25 diopters) underwent ophthalmic examination of one randomly selected eye. Optical coherence tomography (OCT-1, version 4.1) was performed by a single operator using circular scans concentric with the optic disc with scan diameters of 3.40 mm, 4.50 mm, and 1.75 x vertical disc diameter (VDD). For each scan diameter, mean peripapillary RNFL thickness was calculated. Statistical analysis comprised repeated-measurements analysis and Pearson correlation.

Results: Mean peripapillary RNFL thickness did not correlate with SE for the 3.40 mm ($r = -0.11$, $p = 0.22$), 4.50 mm ($r = -0.103$, $p = 0.24$), or 1.75 x VDD ($r = -0.08$, $p = 0.36$) OCT scan diameters. Neither did mean peripapillary RNFL thickness correlate with axial length for the 3.40-mm ($r = -0.04$, $p = 0.62$), 4.50-mm ($r = 0.03$, $p = 0.75$), or 1.75 x VDD ($r = -0.02$, $p = 0.78$) scan diameters. Mean peripapillary RNFL thicknesses for the 3.40 mm, 4.50 mm, and 1.75 x VDD scans were 101.1 ± 8.2 microm (95% confidence interval [CI], 99.4-102.8), 78.9 ± 8.2 microm (95% CI, 77.5-80.3), and 97.5 ± 10.9 microm (95% CI, 95.6-99.4), respectively.

Conclusions: Mean peripapillary RNFL thickness did not vary with myopic SE or axial length for any OCT scan diameter investigated. Retinal NFL

thickness measurements may be a useful parameter to assess and monitor glaucoma damage in myopic subjects.

S14.5
IMAGING OF THE RETINAL VASCULATURE AND ITS USE IN CARDIOVASCULAR DISEASE PREDICTION

T.Y. WONG

Centre Eye Research Australia, University of Melbourne, Melbourne, AUSTRALIA

The retinal vasculature can be viewed and imaged non-invasively, offering a unique perspective of the systemic circulation in vivo. Studying pathological changes of retinal blood vessels may be useful for understanding the etiology of various cardiovascular diseases. Recent population-based studies show that a range of retinal vascular characteristics, including retinal arteriolar narrowing, arteriovenous nicking, and isolated retinal hemorrhages, microaneurysms and cotton wool spots, can be reliably measured from photographs. New computer-based imaging methods allow measurement of retinal arteriolar and venular caliber. These retinal vascular changes are associated with concurrent, past and future blood pressure, systemic markers of inflammation, and predict the risk of subclinical and clinical stroke, cognitive decline, coronary heart disease, heart failure, renal disease and cardiovascular mortality, independent of hypertension, diabetes, cigarette smoking and other risk factors. These data suggest that an objective assessment of retinal vascular changes may provide additional information for cardiovascular risk stratification.

S14.6
VERY HIGH-SPEED FOURIER-DOMAIN OPTICAL COHERENCE TOMOGRAPHY FOR GLAUCOMA AND RETINAL DISEASES

D. HUANG

Doheny Eye Institute, University of Southern California, Los Angeles, CA, UNITED STATES OF AMERICA

Purpose: To improve the diagnosis of glaucoma and retinal diseases using Fourier-domain optical coherence tomography (FD-OCT) technology.

Methods: A FD-OCT system (RTVue, Optovue Inc., Fremont, CA) with a speed of 26,000 axial scans/sec and axial resolution of 5 micron is used to scan the macula, circumpapillary nerve fiber layer (NFL) and optic disc. A conventional time-domain (TD) OCT system (400 axial scans/sec, 10 micron resolution, Stratus OCT, Carl Zeiss Meditec, Inc.) is also used for comparison. Human subjects with perimetric glaucoma, age-related macular degeneration (AMD) and retinal dystrophies are compared with normal control.

Results: For glaucoma diagnosis, the AROC (area under receiver operating curve) for FD-OCT macular inner retinal thickness was higher than Stratus macular retinal thickness, while NFL and disc parameters were not significantly different in terms of diagnostic performance. FD-OCT showed details of drusen, choroidal neovascular membrane and photoreceptor degeneration that was not clearly visible in TD-OCT.

Conclusions: FD-OCT technology provides a 65-fold improvement in speed over the conventional Stratus TD-OCT system. This allows scanning over a wider region, at higher sampling density and over a short period of time. The resulting improvement in diagnostic performance is particularly significant in the macular region, where the loss of inner retinal layers in glaucoma and changes in the outer retina in AMD and dystrophies could

be detected with higher sensitivity. *CR

Support: NIH grant R01 EY013516, P30 EY03040

S14.7

QUANTITATIVE ANALYSIS OF THE ANTERIOR SEGMENT USING ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY

S.D. SMITH

Cole Eye Institute, Cleveland Clinic Foundation, Cleveland, OH, UNITED STATES OF AMERICA

Anterior segment optical coherence tomography (AS-OCT) allows rapid, non-contact, high-resolution imaging of the anterior segment. Images obtained with this technology offer the possibility to quantify the anatomic relationships of anterior segment structures including the iris, cornea and the scleral spur. Such measurements have been found to correlate with gonioscopic findings, the clinical standard for defining the presence of angle closure. Data will be presented that demonstrate these correlations and illustrate the potential usefulness of AS-OCT as a clinical tool for the detection of angle closure. *CR

S14.8

MODERN APPLICATIONS OF ANTERIOR CHAMBER ANALYSIS IN OPHTHALMOLOGY

K. KASHIWAGI

Ophthalmology-Medicine Faculty, University of Yamanashi, Tamaho, JAPAN

In this presentation, I will present some modern applications of anterior chamber depth measurement using scanning peripheral anterior chamber depth analyser (SPAC). My topics are as followed: Anterior chamber depth could indicate the IOP change after lens extraction. The IOP fluctuation may have a good relationship with peripheral anterior chamber depth even in eyes without occludable angle. Peripheral anterior chamber depth may be a good indicator of blunt monocular trauma and anterior rotation of ciliary body due to uveitis. I will present other applications of SPAC. In conclusion, examining anterior chamber depth is very useful in some fields of ophthalmology in addition to identifying narrow eyes. *CR

S14.9

COMPARISON OF ANGLE ASSESSMENT WITH THE HEIDELBERG ANTERIOR SEGMENT OCT AND THE SCANNING PERIPHERAL ANTERIOR CHAMBER ANALYSER

H.T. WONG

Department of Ophthalmology, Tan Tock Seng Hospital, Singapore, SINGAPORE

S14.10

MULTIPHOTON IMAGING OF THE HUMAN EYE

J.F. BILLE

Physics, University of Heidelberg, Heidelberg, GERMANY

We applied two-photon laser scanning microscopic imaging techniques to investigate the ultrastructures of human cornea and retina tissues with

submicron resolution. Second harmonic generation (SHG) imaging was employed to characterise the ultrastructures of collagen fibrils in the cornea, sclera and lamina cribrosa. Two-photon excited autofluorescence (TPEF) imaging was utilised to resolve the morphology and spectrum of individual lipofuscin granules in retina pigment epithelial (RPE) cells. Based on our previous knowledge of nonlinear microscopy we are currently building a two-photon scanning laser ophthalmoscope. In first preclinical studies, the safety of the method was demonstrated. *CR

S14.11

OPHTHALMOSCOPIC IMAGING OF THE OPTIC NERVE HEAD IN HEALTH AND DISEASE

J.B. JONAS

Ophthalmology, Universitäts-Augenklinik, Mannheim, GERMANY

Glaucomatous optic neuropathy is classified by morphologic changes in the intrapapillary and parapapillary region of the optic nerve head and the retinal nerve fiber layer. These changes can be evaluated using descriptive optic nerve head variables which are size and shape of the optic disc; size, shape and pallor of the neuroretinal rim; size of the optic cup in relation to the area of the disc; configuration and depth of the optic cup; cup-to-disc diameter ratio and cup-to-disc area ratio; position of the exit of the central retinal vessel trunk on the lamina cribrosa surface; presence and location of splinter-shaped hemorrhages; occurrence, size, configuration and location of parapapillary chorioretinal atrophy; diffuse and/or focal decrease of the diameter of the retinal arterioles; and visibility of the retinal nerve fiber layer. Assessment of these variables is useful for the early detection of glaucomatous optic nerve damage, to follow-up patients with glaucoma, to differentiate various types of the chronic open-angle glaucomas, and to get hints for the pathogenesis of glaucomatous optic nerve fiber loss.

Symposia 15 Paediatrics Monday, 5 March 2007

S15.1

OVERVIEW OF RETINOPATHY OF PREMATURE

F.A. BILLSON

Ophthalmology, Wollahra, AUSTRALIA

S15.2

RETINOPATHY OF PREMATURE IN DEVELOPING WORLD

S. JALALI

Retina Vitreous Services, L V Prasad Eye Institute, Hyderabad, INDIA

Blindness due to Retinopathy of Prematurity (ROP) is an emerging epidemic in India and other middle income group countries. We have developed a model screening program for prevention of ROP Blindness in the Twin cities of Hyderabad and Secunderabad in South India since 1999. We have also trained 30 ophthalmologists in ROP screening and treatment protocol. Sixteen centers were equipped to carry out ROP management at the district level cities. The presentation will highlight our experience

with setting up ROP blindness prevention programs in India and will also integrate information of similar programs in other middle income group countries.

S15.3

RETINOPATHY OF PREMATURITY: NEW DEVELOPMENTS

W.V. GOOD

Smith-Kettlewell Eye Research, San Francisco, CA, UNITED STATES OF AMERICA

Retinopathy of Prematurity: New Findings and Advances

This lecture will review new findings from retinopathy of prematurity clinical trials, and basic science literature. Findings from the Early Treatment for Retinopathy of Prematurity Study will be emphasised. Associated findings, including new information published on myopia, surgery for retinal detachment, and strabismus in ROP will be discussed. Future research directions will also be explored.

S15.4

RETINOPATHY OF PREMATURITY: TREATMENT OF STAGE 4 AND 5 RETINOPATHY OF PREMATURITY

S. CARDEN

Department of Ophthalmology, Royal Children's Hospital, Melbourne, Victoria, AUSTRALIA

While all efforts are made to prevent stages 4 and 5 from developing, the ophthalmologist will inevitably be confronted with infants' eyes at high risk for low vision. The surgical management of stage 4 diseases is debated, with reports of favorable outcomes in some series, and not favorable in others. At stake in any decision to operate for stage 4 is whether surgery per se will evoke further damage, and whether the eye's vision has already been irrevocably blinded (as in stage 4B). Stage 5 management is seldom noted to be of benefit, although promising surgical advances are under investigation.

S15.5

NEUROPHYSIOLOGICAL FINDINGS IN CHILDREN WITH CEREBRAL VISUAL IMPAIRMENT (CVI)

W.V. GOOD

Smith-Kettlewell Eye Research, San Francisco, CA, UNITED STATES OF AMERICA

Neurophysiology Findings in Cortical Visual Impairment

Cortical visual impairment (CVI) is now the leading cause of low vision in children. Residual visual functioning in this condition can be explored in a number of domains, using electrophysiological techniques. Contrast sensitivity, grating and vernier acuity thresholds and signal amplitudes can be measured in this condition, and higher levels of vision can also be measured in some children. This lecture will describe findings in children with CVI, and will also review the role that steady-state electrophysiology can play in defining an appropriate rehabilitation program.

S15.6

DEVELOPMENT OF RETINAL FUNCTION IN NORMAL AND RETINOPATHY OF PREMATURITY SUBJECTS

A.B. FULTON

Ophthalmology, Childrens Hospital and Harvard Medical School, Boston, MA, UNITED STATES OF AMERICA

Purpose: Investigate the development of rod and cone photoreceptor and post-receptor function in healthy term born subjects and those born preterm. Retinopathy of prematurity (ROP) has its onset at preterm ages when the photoreceptors and neural retina are quite immature.

Methods: Electroretinographic responses to full-field and multi-focal stimulation were recorded. Models of photo-transduction yielded rod and cone photoreceptor response parameters for the retina as a whole; post-receptor response parameters were also derived. Cone driven responses of the posterior retina to multi-focal stimulation were analysed. Psychophysical thresholds in parafoveal and peripheral retina were measured to track regional development of retinal sensitivity. The data from term born subjects (n = 376) and those born preterm (n = 151) were compared. The subjects with a history of preterm birth were stratified by severity of ROP: none, mild, severe. Those with severe ROP required laser ablation of the peripheral avascular retina.

Results: For full-field stimuli, rod photoreceptor and post-receptor response parameters have developmental courses indistinguishable from the logistic growth curve that summarises the developmental increase in the visual pigment, rhodopsin; values reach 50% of adults' at approximately 11 weeks post-term. In those born preterm, rod and rod driven activity was significantly compromised in subjects with ROP even if the ROP had been mild. In those without ROP, rod and rod driven activity was normal for corrected age. In those with severe ROP, the response deficits were not correlated with area of retina ablated. Remarkably, the rod response deficits persisted into early adulthood. Normal cone photoreceptor and cone driven post-receptor parameters, derived from the responses to full-field stimuli, mature earlier than the rod parameters. The full-field cone activity is less affected by ROP than are the rod responses. In the normally late maturing posterior retina, the parafoveal rods have a more protracted course of development in ROP than in control subjects. The cone mediated responses of the posterior retina to multi-focal stimulation show significant deficits that remain detectable in older children and young adults with a history of mild ROP.

Conclusions: The normal development of photoreceptor and post-receptor function is altered by ROP. ROP affects the function of the rods more than the later maturing cones. Late maturing rod and cone functions in the posterior retina are also vulnerable to ROP. The results from the ROP subjects support the idea that the developing photoreceptors, which introduce enormous oxygen-based metabolic burdens on the immature preterm retina, are very much involved in the ROP disease process. The development of post-receptor function, both rod and cone driven, both posterior and peripheral, is altered by ROP and may well be influenced by neurovascular cross-talk that we have documented in rat models of ROP. Our research in progress evaluates the neurovascular cross-talk in our infant and child ROP subjects.

S15.7

RETINOBLASTOMA: EXPERIENCE IN INDONESIA

R.S. SITORUS

Ophthalmology, University of Indonesia, Faculty of Medicine/Cipto Mangunkusumo Hospital, Jakarta, INDONESIA

Retinoblastoma is the most common intraocular malignant tumour of the eye in infant and children.

As one of about three referral centers for retinoblastoma patients in Indonesia, we were alarmed by the high frequency of advanced diseases and the high mortality rate of our patients. Our study showed 48% survival rate in retinoblastoma patients, which is very low compared to 80-90% both in life and ocular survival rate achieved in developed countries.

Long delays to diagnosis, which lead to advanced disease stage, are frequently observed in the management of retinoblastoma in Indonesia. Our study showed that refusal of therapy does have an adverse effect on the survival outcome of retinoblastoma patients.

Since year 2005 we have develop an educational or campaign program in retinoblastoma for communities and health care practitioners to avoid the adverse effect of diagnosis and therapy delays on both ocular and life survival. Jakarta and its surrounding areas are our primary targets. The ultimate goal of the programs should be to minimise the delays, which mainly (90%) originate from parents, but also from health care professionals (10%). The efficacy of such program will depend on accompanying on the health care system, especially with respect to the provision of medical facilities in remote areas for early diagnosis, reduction in the cost of medical services, or financial support to help to cover the cost of travel to the diagnostic and therapeutic facilities. Problems in management of retinoblastoma patients in Indonesia, strategies to overcome the problems, and the current outcomes will be discussed.

S15.8

GENETICS OF RETINOBLASTOMA IN INDIA: OUR EXPERIENCE

G. KUMARAMANICKAVEL

Genetics Molecular Biology, Vision Research Foundation Sankara Nethralaya, Chennai, Tamil Nadu, INDIA

Background: Retinoblastoma is an intraocular malignancy that affects children less than 5 years of age. It is a rare tumour which has a worldwide incidence of 1 in 20,000. Knudson's two hit 'knocking-off' of the gene results in uncontrolled cell division. Diagnostic testing of the proband and closely related young siblings could avoid frequent eye examination under anesthesia and the ensuing clinical risks. Detecting these events is a complex process that needs dedicated personnel and laboratory.

Methods: On patients who came for genetic counseling to our clinic we performed tests like chromosomal analysis, mutational screening, methylation analysis of RB1 promoter and linkage analysis. Genetic testing was evaluated for cost when compared to clinical management.

Results: Deletion of 13q14 region was detected in 11.8% of patients. Thirteen mutations were identified including five novel mutations and arginine was involved in eight. Hypermethylation of RB1 promoter was 6.6%. Loss of heterozygosity (LOH) was found in 72.9% of patients with no specific association between presence or absence of LOH and tumour. Seven germline deletions (13%) were identified and maternal allele was more frequently lost than paternal ($p = 0.01$). A disease co-segregating haplotype was detected in 2 hereditary autosomal dominant families. Genetic testing was 22-fold cheaper compared to the clinical management

cost. Genotype-phenotype correlation showed severe clinical form of retinoblastoma when mutation happens in the N terminus of the protein.

Conclusions: Collectively this study has established an effective molecular genetic diagnostic laboratory for retinoblastoma patients at our Institution.

S15.9

COMITANT HORIZONTAL STRABISMUS PATTERNS IN SINGAPORE

A. CHIA

Paediatric Ophthalmology, Singapore National Eye Center, Singapore, SINGAPORE

Aim: Asian strabismus patterns have been noted to be different from that of the West. In this retrospective review, the profile of comitant horizontal strabismus was analysed.

Methods: All children aged <16 years presenting with strabismus for the first time between 2000 and 2004 to clinics at two major hospitals were included in this study. The type and size of the squint, vision, refractive error and stereopsis were noted.

Results: 696 children presented with comitant horizontal strabismus. Of these, 505 (72.5%) were exotropic (91.5% intermittent and 8.5% constant). 50% of children with constant exotropia had poor vision in one eye. In those with intermittent exotropia, the mean exodeviation was $27 \pm 12D$ and the median age of presentation was 5.2 ± 3.0 years. Most had divergence excess (59.5%) followed by basic (29.0%) and convergence weakness (11.5%). Children with convergence weakness had significantly more myopia and astigmatism. Stereopsis was present for near in 92% and distant in 50% in children with intermittent exotropia. Esotropia was noted in 191 (27.5%) children; 23.5% were congenital whilst 53.4% were accommodative. As expected, children with congenital esotropia presented significantly younger (2.8 years vs. 4.5 years), had larger squint size (35D vs. 26D) and were less hyperopic (+0.78D vs. +2.79D). Amblyopia was noted in 50% of children with congenital esotropia, and 43% with accommodative esotropia. Stereopsis was achieved in 57% of those with fully accommodative esotropia, and in none of the children with partially accommodative esotropia.

Conclusion: Exotropia is far more common than esotropia in Singapore. This differs from Western studies where the reverse is true. Questions remain whether these differences are limited to ratio, or whether other differences in clinical and surgical outcomes also exist.

S15.10

INVESTIGATING THE GENETIC BASIS OF NON-SYNDROMIC COMITANT STRABISMUS

C.F. INGLEHEARN

School of Medicine, Leeds University, Leeds, UNITED KINGDOM