535 Novel findings and approaches in myopia research

Thursday, May 11, 2017 11:30 AM–1:15 PM
Room 321  Paper Session
Program #/Board #/ Range: 5634–5640
Organizing Section: Anatomy and Pathology/Oncology

Program Number: 5634
Presentation Time: 11:30 AM–11:45 AM

A genome-wide association study (GWAS) for myopia susceptibility in chicks
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Purpose: Genome-wide association studies (GWAS) in human participants have successfully identified genetic variants associated with myopia susceptibility. However, GWAS approaches have had less success in identifying gene-environment (GxE) interactions. Aiming to reduce variability in environment exposures compared to children, we performed a GWAS for myopia susceptibility in the chick model.

Methods: White leghorn chicks 7 days old were monocularly form deprived for 4 days, in batches of approximately 20 individuals. The degree of induced myopia was quantified by A-scan ultrasonography and retinoscopy. From each batch, 20% of chicks with the highest or lowest treatment-induced axial length change (ΔAXL) were genotyped on a commercial Affymetrix SNP array. Using PLINK, ΔAXL elongation was analyzed as a continuous trait (dependent variable) with independent variables, SNP, sex, batch, and body weight.

Results: In 986 chicks from 48 batches, AAXL averaged 0.55 ± 0.17 mm (mean ± s.D.). Chicks with a relatively low degree of induced myopia (n=190; AAXL=0.31 ± 0.08 mm) and a relatively high degree of induced myopia (n=190; ΔAXL=0.78 ± 0.08 mm) were genotyped (average call rate 99.5%). SNPs with call rate <95% or minor allele frequency <0.1, and samples with heterozygosity >0.4 were excluded, leaving 379 samples and 354,147 SNPs. After genomic control correction for relatedness (lambda = 1.22), regions on chromosomes 1 and 7 exceeded the suggestive association threshold (P <1e-05). The nearest genes were a CAMP-dependent protein kinase subunit and a member of the UDP glucuronosyltransferase family, respectively. The former gene has previously been implicated in myopia development in the chick model. These association peaks are being followed-up using RNA-seq analysis.

Conclusions: This study is the first report of a GWAS for myopia susceptibility in an animal model of myopia. The human homologues of the genes located on chick chromosomes 1 and 7 are being excluded, leaving 379 samples and 354,147 SNPs. After genomic control correction for relatedness (lambda = 1.22), regions on chromosomes 1 and 7 exceeded the suggestive association threshold (P <1e-05). The nearest genes were a CAMP-dependent protein kinase subunit and a member of the UDP glucuronosyltransferase family, respectively. The former gene has previously been implicated in myopia development in the chick model. These association peaks are being followed-up using RNA-seq analysis.

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Presentation Time: 11:45 AM–12:00 PM

Persistent activity of Muller glia-derived PRSS56 is required for refractive development
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Purpose: Ocular axial growth is central to refractive development. Alteration in ocular axial length leads to refractive errors. Molecular factors governing ocular growth are poorly defined. We have previously implicated a serine protease, PRSS56, in ocular axial growth and their genetic variants are implicated in refractive errors. Here, combinations of genetic mouse models were utilized to study the role of PRSS56 in the ocular axial growth.

Methods: Ocular expression of Prss56 was determined by lineage-tracing and in situ hybridization. Conditional Prss56 mutant (Prss56flfl) mice were generated to delineate the spatiotemporal requirements of Prss56 on ocular growth. An inducible Rax-Cre ER was used to ablate Prss56 from retinal Muller glia following tamoxifen injection at P8. Ubiquitous inducible Ubc-Cre line was used to ablate Prss56 (Prss56flfl;Ubc-Cre ER) during distinct stages, both before [Postnatal (P) days 6, and 8] and after opening of the eye (at P13). Optical Coherence Tomography was employed to perform ocular biometry, including axial length (AL) measurements starting from P5 to P60. Mouse auto-photorefractor was employed to measure the ocular refraction.

Results: Prss56 is first detected in the late retinal progenitor cells, and in a subset of Muller glial cells following retinal cell differentiation. Prss56 eyes display a reduced ocular axial length starting at P5 compared to the controls (Prss56flfl or Prss56flox) and subsequently develop hyperopia. Conditional ablation of Prss56 specifically from differentiated Muller glia by inducing activation of Rax-Cre ER caused a significant reduction in ocular axial length as compared to control groups (2.724±0.036 mm for Prss56flfl vs 2.869±0.164 mm for Prss56Rax; p<0.001). Stage-specific ablation of Prss56 both before (P6 and P8) and following the opening of the eyes (P13) caused reduction in AL compared to the controls. Prss56 ablation at an earlier time point (P6) caused a greater reduction in AL compared to later ablation at P8 (P (0.115±0.027 mm at P6 vs 0.08±0.02 at P8).

Conclusions: PRSS56 derived from Muller glia contributes to ocular growth in a stage-specific manner. Persistent activity of PRSS56 both before (previson) and following the opening of the eyes is required to sustain ocular axial growth. Thus, we identify a factor that operates in a continuum through distinct stages to support ocular growth and normal refractive development.

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Presentation Time: 12:00 PM–12:15 PM

Dynamic noise promotes myopic eye growth
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Purpose: Spatial, temporal, and spectral characteristics of visual stimulation are known to influence enmetropization in the chick. Previous research (Hess et al., 2008) has suggested that the high spatial frequency information in static noise stimuli can act to slow eye growth and reduce the development of myopia. In this experiment, we tested whether high spatial frequency information has
the same effect when the noise stimulus is dynamic and whether the spectral content of the stimulus is relevant in controlling eye growth.  

Methods: 66 one-week old chicks were exposed to visual stimulation presented during a 12h light/dark cycle for three days. An Eizo computer monitor placed outside a Plexiglas cage was used to present the stimuli. The three stimulus conditions were i) Control: a steady full-screen of average luminance (110 cd/m²), ii) Flicker: full-screen sinusoidal flicker (frequency 10Hz, Michelson Contrast 0.82, RMS Contrast 0.56) iii) Noise: a dynamic full-screen noise stimulus (peak temporal frequency 10Hz; RMS contrast 0.56). Two spectral conditions, White and Yellow, were used for six conditions total. The White spectral condition used the red, green, and blue monitor components while the Yellow condition used only the red and green components. The average luminance for all stimulus conditions was equated. Ocular biometry (Lenstar LS 900) and refraction (Hartinger Coincidence Refractometer) were measured before and after three days of stimulus exposure.

Results: Eye growth showed a main effect of stimulus (F=6.58, p < 0.01) and an interaction between stimulus and the spectral characteristics (F=11.58, p < 0.001). Birds exposed to 10Hz White Flicker showed less eye growth than birds under the White Control (-0.03 mm/day, p < 0.05) or White Noise condition (-0.06 mm/day, p < 0.001). Birds exposed to White Noise showed more eye growth than those in the White Control condition (0.036 mm/day, p < 0.05). There was no difference in eye growth among the birds exposed to the stimulus conditions with Yellow light.

Conclusions: Consistent with previous research, broad-spectrum high-frequency flicker produced shorter, less myopic eyes than a steady light. Inconsistent with previous research using static noise, high-frequency flicker produced longer eyes.  

Commercial Relationships: Christopher Taylor, None; Burke Lieppman, None; Frances J. Rucker, None

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Presentation Time: 12:15 PM–12:30 PM

Altered dopamine release in VMAT2 mutant mice has little effect on refractive development  

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Purpose: The purpose of this work is to determine the role of dopamine (DA) as a potential stop signal for refractive development and myopia susceptibility in mice. Vascular monoamine transporter 2 (VMAT2) is responsible for packaging DA into vesicles for release. Thus, we hypothesize that increasing or decreasing expression of VMAT2 will enhance or diminish DA release, thereby altering refractive error during development.

Methods: Three groups of mice were used for these experiments; C57BL/6 mice over-expressing VMAT2 (VMAT2 HI), wild-type littermates (WT), and VMAT2 under-expressing mice (VMAT2 LO). To confirm changes in DA signaling, we measured VMAT2 expression in the retina with western blots and the retinal levels of DA and DOPAC with HPLC. The refractive development of each genotype was studied with biweekly measurements of refractive error, keratometry, and axial length from post-natal day 28 (P28) until P112 (HI n=16, WT n=8, LO n=7). To determine functional differences caused by changes in VMAT2 expression, optokinetic tracking was used to measure spatial frequency and contrast sensitivity thresholds of adult mice (HI n=3, WT n=4, LO n=3).

Results: Western blotting confirmed increased retinal VMAT2 expression in the HI mice relative to the WTs and LOs. HPLC analysis showed that retinal dopamine levels in the LO mice were reduced by 68.3% compared to WT mice (p<0.001). In addition, retinal DOPAC was reduced in VMAT2 LO mice by 40.8% relative to WTs (p<0.01). HI and WT mice had similar levels of dopamine and DOPAC. Refractive errors of VMAT2 LO mice were significantly more hyperopic than HI and WT mice at P28 only (HI 2.78±0.43D; WT 1.93±0.77; LO 4.49±0.91). No significant differences were found in keratometry or axial length between the genotypes. Spatial frequency and contrast sensitivity thresholds were similar across HI, WT and LO mice (SF HI: 0.40±0.003, WT: 0.40±0.006, LO: 0.40±0.004, CS HI: 9.78±0.13, WT: 9.76±0.42, LO: 9.62±0.44).

Conclusions: These results indicate that altered DA release modulates only early refractive development under normal laboratory conditions. The absence of changes in visual function suggest that modulations of VMAT2 may not alter DA release to critical levels or that potential compensatory mechanisms exist, which may also explain the lack of effect on refractive development. Future studies will test susceptibility of VMAT2 mutants to form deprivation myopia.

Commercial Relationships: Erica Landis, None; Victoria Yang, None; Li He, None; Kelly Lohr, None; P. Michael Iuvone, None; Machelle T. Pardue, None

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Presentation Time: 12:30 PM–12:45 PM

Differences in INL/ONL Ratios for Myopes and Emmetropes/ Hyperopes  

Christopher A. Clark, Ann E. Elsner, Casey Carr, Theodore Chow. School of Optometry, University of Indiana, Bloomington, IN.

Purpose: Retinal thickness has been shown to differ between myopes and emmetropes, with myopes typically having relatively thinner retinas outside of 5 degrees from the fovea, and, in some studies, myopes having thicker retinas at the fovea. The Inner Nuclear Layer (INL), composed primarily of bipolar, horizontal, and amacrine cell nuclei, and the Outer Nuclear Layer (ONL), composed of photoreceptor nuclei and Mueller cell processes, may contain some of the first signalling neurons in the emmetropization cascade. The purpose of this study is examine change in proportion of INL to ONL thickness as it relates to refractive error/axial elongation.

Methods: 376 subjects signed informed consents approved by the Indiana University IRB. Retinal thickness data was collected by SD OCT using 30 degree radial scans along with corneal topography (Medmont), refraction (Grand Seiko) and axial length/ anterior chamber depth (IOLmaster, Zeiss). After automated segmentation, thickness ratios were calculated by dividing the INL thickness by the ONL thickness at every location along the horizontal and vertical plane. Data were then collected at 1 degree intervals +/-14 degrees from the fovea. Statistics were performed using repeat measures ANOVA in SPSS (IBM.) Subjects were categorized into emmetropes/ hyperopes and myopes based upon a -0.75 diopter cutoff.

Results: Myopes exhibited significantly lower INL/ONL ratios at locations peripheral to the fovea, beginning at 5 deg temporally and 7 deg nasally (P = 0.008), compared to the emmetropes/ hyperopes. Both the INL and ONL were significantly thinner for myopes compared to emmetropes. Linear regression showed a strong correlation between refractive error and INL/ONL ratio at 5 degrees (y=0.0097X+0.3314, R² = 0.162) and 10 degrees (y=0.0074X+0.2757, R² = 0.121) temporal.

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**Conclusions:** While both the INL and ONL were thinner in myopes, the INL/ONL ratio was reduced in the myopic group compared to the emmetropes/hyperopes. This suggests that outside the central fovea, the INL was comparatively thinner than the ONL in myopes vs myopes in hyperopes. In a radial stretch hypothesis, the ONL would be expected to be thinned to a similar extent compared with the INL due to axial elongation, which was not supported by the data. The data also did not support a neural summation hypothesis whereby a larger number of photoreceptors connect to smaller number of cells, again expecting the ONL to be thinner relative to the INL.

**Commercial Relationships:** Christopher A. Clark, None; Ann E. Elsner, None; Casey Carr, None; Theodore Chow, None

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**Presentation Time:** 1:00 PM–1:15 PM

**Egr-1 mRNA Expression in Response to Myopic Defocus**

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**Purpose:** The expression of the transcription factor Egr-1 appears to act as a biomarker of ocular growth, showing, for the most part, a bi-directional response to opposing growth stimuli. However, in response to lens-induced hyperopia (LIH), Egr-1 mRNA levels do not follow the expected up-regulation normally associated with growth suppression, but rather are down-regulated similar to that seen during periods of enhanced growth. This study investigated if this unexpected directional change in Egr-1 expression may be associated with the growth state of the eye prior to lens-wear. Specifically, Egr-1 expression is up-regulated in response to myopic defocus associated with the removal of translucent diffusers or negative lenses from already myopic eyes. In both of these paradigms, the eye is in a state of increased growth before the optical device is removed. In contrast, in LIH, myopic defocus is applied to an eye that was previously in a state of normal growth.

**Methods:** Egr-1 mRNA levels were measured in the chick retina using RT-PCR after 4 and 24 hrs in response to the following conditions: 1) Fitment of +5D or +10D lenses to otherwise normal eyes (n=7); 2) Fitment of +5D lenses following compensation to -10D lenses (n=7); 3) Fitment of +10D lenses after compensation to -5D lenses (n=7); 4) Fitment of +5D lenses after partial compensation to -10D lenses (i.e. the eye is still in a state of excessive growth; n=7); 5) Fitment of +5D lenses following four days of diffuser-wear (i.e. again, the eye is in a state of excessive growth; n=7).

**Results:** Egr-1 mRNA expression was significantly down-regulated in the retina following 4 and 24 hrs of plus lens-wear irrespective of the previous state of eye growth (one-way ANOVA, treated (five conditions x two time points) vs untreated control values; F(10, 84) = 1816.8, p<0.0001).

**Conclusions:** Egr-1 mRNA levels are normally up-regulated in response to myopic defocus associated with the removal of translucent diffusers or negative lenses. In contrast, plus lens-wear, which also induces myopic defocus, induces a down-regulation in Egr-1 levels. This down-regulation occurs irrespective of whether the eye was in a normal or excessive state of growth prior to plus lens-wear. Together, these results suggest that myopic defocus induced by plus lens-wear is somehow processed differently at the level of the retina to that seen following diffuser or negative-lens removal.

**Commercial Relationships:** Regan Ashby, None; Cindy Karouta, None

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