147 Genetics and modeling of lens and anterior segment anomalie - Minisymposium
Sunday, May 07, 2017 3:15 PM–5:00 PM
Room 307 Minisymposium
Program #/Board # Range: 828–833
Organizing Section: Lens

Program Number: 828
Presentation Time: 3:18 PM–3:35 PM
PITX3 pathway in lens and anterior segment development
Elena Semina. Pediatrics, Medical College of Wisconsin, Milwaukee, WI.
Presentation Description: The PITX3 transcription factor is important for ocular development in vertebrates. Mutations in *PITX3* result in anterior segment dysgenesis (ASD), cataracts, and/or microphthalmia in humans (Verdin et al. 2014; Reis and Semina 2011; Semina et al. 1998; Aldahmesh et al. 2011); mouse *Pitx3* promoter deletions or nonsense mutations lead to aphakia with microphthalmia (Rieger et al. 2001; Semina et al. 2000; Wada et al. 2014) and lenticulo-corneal adhesions (Medina-Martinez et al. 2009; Varnum et al. 1968); disruption of *pitx3* with morpholino in zebrafish and frog results in lens and retinal anomalies (Shi et al. 2005; Zilinski et al. 2005; Dutta et al. 2005; Khosrowshahian et al. 2005). In *Pitx3*-deficient *aphakia* mice, the cells of the lens vesicle do not undergo proper differentiation and exhibit reduced proliferation and increased apoptosis (Medina-Martinez et al. 2009). Despite its importance in ocular development, there is little known about the molecular pathway(s) controlled by PITX3. In order to unbiasedly identify pathways directed by PITX3/pitx3 during eye development, we performed transcriptome analysis in wild-type and *pitx3*-deficient zebrafish embryonic lenses. This analysis identified several highly downregulated genes within the morphant lenses with solute carrier *slc7a8a,* extracellular matrix protein *spon1b,* Wnt inhibitory factor 1 *wif1* and proto-oncogene receptor tyrosine kinase *met* being the top four affected transcripts; additionally, another solute carrier gene, *slc3a2a*, encoding a partner of slc7a8a, was among the top 15 downregulated targets. The microarray data were confirmed by qPCR/ in situ hybridization using *pitx3* morphant lenses as well as additional analyses in *pitx3* genetic mutant lines that we recently generated. Moreover, we created *slc7a8a- and slc3a2a*-mutant lines and observed ocular abnormalities in both mutants with *slc7a8a*-deficiency resulting in severe lens defects. Analysis of ocular patients identified two missense variants in *SLC7A8* that affect conserved amino acids and are novel and predicted to be damaging. The ocular functions of solute carrier genes *SLC7A8* and *SLC3A2* are not known while our data indicate potential involvement on these genes in ocular disease and/or PITX3 pathway. Further studies of these factors and other identified PITX3 targets are ongoing and the latest data will be presented.
Commercial Relationships: Elena Semina, None

Program Number: 829
Presentation Time: 3:35 PM–3:52 PM
EPH-receptor A2 (EPHA2) in lens development and cataract
Alan Shiels. Ophthal & Vis Sciences, Washington University in St. Louis, St Louis, MO.
Presentation Description: Genetic variation at the human EPHA2 locus has been associated with inherited and age-related forms of cataract. Gene-targeted mice provide a valuable model system to characterize the role of EPH-receptors and their ephrin ligands in lens development and cataract formation. Data comparing and contrasting lens phenotypes of knockout mice functionally lacking EPHA2, ephrin-A5, or both receptor and ligand will be presented. Collectively, these data suggest that EPHA2 is critical for establishing lens optical quality and acts as a pleomorphic risk locus for cataract.
Commercial Relationships: Alan Shiels, None
Support: NIH Grants EY023549, EY02687

Program Number: 830
Presentation Time: 3:52 PM–4:09 PM
Genomics, functional genomics and new insights to cellular polarity, adhesion and other factors in the lens and anterior segment
Robyn Jamieson[1, 2]. [1]Children's Medical Research Institute, University of Sydney, Westmead, NSW, Australia; [2]Eye Genetics Research Group, Sydney Children's Hospital Network, Sydney, NSW, Australia.
Presentation Description: Genetic disorders of the lens and ocular anterior segment, are markedly genetically heterogeneous conditions, reflecting the multitude of factors and interacting pathways critical for precise anterior segment development. Genomic sequencing technologies have created improved opportunities for new insights to these pathways through investigation of patients with disorders of the lens and anterior segment. In addition, we have developed a functional genomics pipeline incorporating other 'omic approaches, cellular assays and generation of mouse models for understanding the underlying disease mechanisms. Genomic approaches proved useful in our studies identifying *MAF* and *SIPA1L3* as human disease genes from specific cases, and we have identified novel variants in several different anterior segement genes using targeted next-generation sequencing, whole exome and whole genome sequencing in a cohort of over 50 probands with lens and anterior segment abnormalities. SIPA1L3 is implicated in Rap1 signalling, and has predicted RAPGAP, PDZ and actin-binding domains. Our functional genomic studies including cellular assays, zebrafish studies and a loss of function *Sipa1l3* mouse line, have revealed the impact of abnormality of this protein on the cytoskeleton, cellular polarity and adhesion in the lens and anterior segment. A similar approach is underway in other studies, revealing further insights to factors critical for development of the lens and anterior segment.
Commercial Relationships: Robyn Jamieson, None
Support: ORIA; NHMRC

Program Number: 831
Presentation Time: 4:09 PM–4:26 PM
Molecular mechanisms of disease development in the Tcf8 knock-in mouse model of Fuchs corneal dystrophy
John Gottsch. Anterior Segment/Ex Diseases, Johns Hopkins University School of Medicine, Baltimore, MD.
Presentation Description: Fuchs corneal dystrophy (FCD) is one of the leading indications for corneal transplantation in the United States. In spite of the substantial burden of vision loss from this disease, there is limited evidence regarding the molecular mechanisms which lead to the disease phenotype in this corneal dystrophy. FCD is inheritable, and we have identified causal mutations in *SLC4A11*, *LOXHD1*, and *AGBL1*. Missense mutations in TCF8, a transcription factor whose haploinsufficiency causes posterior polymorphous corneal dystrophy (PPCD), were found in a cohort of late-onset FCD patients. The segregation of a recurring p.Q840P mutation in TCF8 in a large, multigenerational FCD pedigree suggested that this allele was causal for the disease. To substantiate that this allele is indeed causal for the disease phenotype, we undertook the development of a *Tcf8* knock-in (KI) mouse model harboring p.Q818P. *Tcf8*$^{Q818P/Q818P}$ KI mice were found through slit lamp examination, confocal microscopy and histochemistry to recapitulate the cardinal signs of FCD. Corneal endothelium and

Descemets membrane of these mice are characterized by aberrant proteomes as compared to age-matched controls suggesting perturbed molecular pathways.
Commercial Relationships: John Gottsch, None
Support: NIH RO1 EY O16835

Program Number: 832
Presentation Time: 4:26 PM–4:43 PM
Application of *iSyTE* knowledge-maps to expedite eye disease gene discovery
Salil Lachke. Department of Biological Sciences, University of Delaware, Newark, DE.
Presentation Description: The application of systems-level approaches hold high potential to impact gene discovery in the eye. A new updated version of a web-based publically available bioinformatics tool for eye gene discovery will be discussed. This web resource - termed *iSyTE* (*i*ntegrated *Sy*stems *T*ool for *E*ye gene discovery, http://bioinformatics.udel.edu/Research/iSyTE) - is based on comprehensive eye tissue-specific gene expression datasets integrated with evidence-based molecular functional data. The application of *iSyTE* in predicting new gene candidates in the lens, cornea and retina and their associated defects will be discussed. In particular, the characterization of RNA-binding proteins linked to cataracts in humans and animal models will be highlighted. Finally, the use of *iSyTE* to prioritize new candidate genes from human cataract patient exome sequencing data will be disucussed.
Commercial Relationships: Salil Lachke, None
Support: This research was supported by the National Eye Institute of the National Institutes of Health under Award Number R01EY021505.

Program Number: 833
Presentation Time: 4:43 PM–5:00 PM
Functional assessment confirms the indispensable role of FYCO1 in lens morphogenesis
S Amer Riazuddin. Department of Ophthalmology, The Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD.
Presentation Description: FYVE and coiled-coil domain containing 1 (FYCO1) plays a critical role in microtubule plus-end-directed transport of autophagic vesicles. We have previously shown that loss function mutations in FYCO1 are responsible for congenital cataracts. To delineate the physiological role of FYCO1 in the development of the ocular lens, we established *Fyco1*$^{-/-}$ conditional knockout (KO) mice. These mice develop bilateral cataracts as early as two weeks of age that progress to mature cataracts by 12 weeks. Histological examination of *Fyco1*$^{-/-}$ mice revealed severely disorganized lens with large vacuoles and swollen lens fiber cells. In parallel, we developed a human lens epithelial (HLE) knock-in (KI) cell line through CRISPR/Cas9 strategy harboring the nonsense mutation (c.2206C>T, p.Q736*), previously shown to be responsible for congenital cataracts. The off-target effects were ruled out by next-generation whole exome sequencing while the loss of *FYCO1* expression in the KI cell line was confirmed by RT-PCR. We observed a two-hour increase in the population doubling time for KI cell line while the cell cycle analysis illustrated an increase in G1 population ($p<0.005$). Flow cytometer based quantification revealed lower levels of LC3 lipidation in the KI cell line compared to wild-type HLE cells. Likewise, measurement of the autophagic flux in live cells through Cyto-ID staining of autophagic compartments illustrate a reduced autophagic flux. In summary, our data confirms the association of FYCO1 with autophagy in human lens epithelial cells and its indispensable role in ocular lens development and maintenance of its transparency. We are currently investigating the functional significance of FYCO1 in lens fiber cell differentiation.
Commercial Relationships: S Amer Riazuddin, None
Support: This study was supported in part by the National Eye Institute, grant R01EY022714.