136 Structural/functional genomics and gene variants
Sunday, May 07, 2017 1:30 PM–3:15 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 565–585/B0133–B0153
Organizing Section: Biochemistry/Molecular Biology

Program Number: 565 **Poster Board Number:** B0133
Presentation Time: 1:30 PM–3:15 PM
RNA-seq analysis of the human retina
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Purpose: Rod and cone photoreceptors are highly specialized light sensitive neurons that initiate this process of phototransduction. Though they are very similar cell types, rods and cones have distinct functionalities, synaptic connection, and are affected by different blinding disease alleles. Here we use Illumina RNA-sequencing (RNA-seq) analysis to characterize the mRNA transcriptome of rod and cone-enriched portions of the adult human retina in an effort to better understand the distinct molecular function of human photoreceptors.
Methods: Cone rich central macula and rod rich peripheral retina samples were biopsied from adult post mortem human donor eyes in biological triplicate. Total RNAs were extracted from retinal samples using a Qiagen AllPrep Mini Kit. RNAs were validated for quality and Illumina TrueSeq mRNA-seq libraries were prepared from total RNA and sequencing reads were generated using the Illumina NextSeq 500 sequencing platform. Raw sequence quality assessment and high precision mapping to the Human 2013 hg38 genome assembly were achieved using FastQC and TopHat software respectively. Differentially expressed genes (DEGs) between macula and peripheral retina were determined using CuffDiff software analysis of TopHat mapping outputs between the two sample groups.
Results: Sequencing yielded between 19-21 million high quality 125 bp paired end reads/run. The majority of these reads were successfully paired and mapped to the Human 2013 hg38 genome assembly and heat map analysis demonstrated tight clustering of sample triplicates. Cuffdiff analysis yielded 4,627 statistically significant DEGs between macula and peripheral retina samples (p-value <0.05). 2,240 DEGs were significantly upregulated and 2,387 DEGs significantly downregulated in the peripheral retina compared to the macula. Ongoing pathway analysis is currently being conducted to identify genes with previously unrealized roles in rod and cone-specific visual perception.
Conclusions: RNA-seq analysis coupled with pathway analysis will help to identify novel candidate genes involved in rod and cone-specific visual perception pathways in the adult human retina. Current studies are underway investigating transcriptional and epigenetic regulation of these candidate genes. This study will also provide tools for integrating RNA-seq bioinformatics analysis into undergraduate research and course-embedded undergraduate active learning modules.
Commercial Relationships: Ray Enke, None; **Andrea Gargiulo**, None; **Melika Rahmani-Mofrad**, None; **Ashton Holub**, None
Support: Commonwealth Health Research Board Grant #540472

Program Number: 566 **Poster Board Number:** B0134
Presentation Time: 1:30 PM–3:15 PM
The response of uveal melanoma (UM) cells to Bromodomain and Extra Terminal (BET) inhibitors
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Purpose: UM is an aggressive ocular malignancy in which ~50% of patients develop fatal metastases. Our aim is to generate 'kinase expression fingerprints' of GNAQ/11 mutated (mt) and wild type (WT) UM cell lines, and probe kinome responses to the BET-inhibitor JQ1.
Methods: Using digital nanoString profiling and kinome network models, baseline mRNA expression profiles were obtained for the human kinome in GNAQ/GNA11 mt UM cell lines 92.1 (GNAQ G209L) Mel270, OMM2.5 (both GNAQ Q209P) and OMM1 (GNA11 Q209L); the GNAQ/11 WT conjunctival melanoma cell lines CRMM1 (BRAF V600E) and CRMM2 (BRAF WT), and 5 non-melanoma cell lines (GNAQ/11 and BRAF WT). Cell line responses to TKIs, MEK or BET-domain inhibitors were analysed by MTT assay. Cell-cycle perturbations were examined by flow cytometry.
Results: In ocular melanoma cells, expression of several kinase mRNAs (ROR2, EGFR, CAMK1D and FGFRL1) was markedly decreased compared to non-melanoma cells, irrespective of GNAQ/11 status. Conversely, CDK2 mRNA expression was higher. All UM/CRMM cell lines showed sub-μM EC_{50} responses to the BETi JQ1, in contrast to TKIs
The kinome response to JQ1 was dependent upon GNAQ/11 status. In GNAQ/11 mt UM cell lines, only 0.4 % of kinome mRNAs were upregulated more than 2-fold; in contrast, JQ1 induced upregulation of 3.5 % and 14.8 % of the kinome in CRMM1 and CRMM2 cells (GNAQ/11 WT), respectively.
JQ1 significantly reduced BRD4 mRNA expression in UM cells but not in CRMM cells, or the non-melanoma cell line HCT116. Conversely, BRD2 mRNA expression significantly increased in OMM1 (GNA11 Q209L) and CRMM1/2 cells.
CDK4 mRNA levels decreased >3-fold in the GNAQ/11 mt UM cells exposed to JQ1. We found that JQ1 induced a G1 cell-cycle arrest in 92.1 and OMM1 cells, and reduced FOXM1 protein levels and FOXM1-regulated kinase mRNAs. In UM patient samples, FOXM1 mRNA expression positively correlated with the expression of the FOXM1-regulated kinases that JQ1 reduced in UM cell lines.
Conclusions: Our data suggest that GNAQ/11 mutation status of ocular melanomas might dictate how cells respond to BET inhibitors, and provides mechanistic insight into kinome-wide responses to this class of compound. We demonstrate that JQ1 reduces the levels of both BRD4 and CDK4 mRNA in GNAQ/11 mt cells and causes a G1 cell-cycle arrest. Furthermore, JQ1 reduces FOXM1 protein levels and impairs expression of the FOXM1 transcriptional network of kinases.
Commercial Relationships: Fiona P. Bailey, None; **Helen Kalirai**, None; **Haleh Shahidipour**, None; **Kim Clarke**, None; **Sarah E. Coupland**, None; **Patrick A. Eyers**, None
Support: North West Cancer Research Post Doctoral Research Grant CR1037

Program Number: 567 **Poster Board Number:** B0135
Presentation Time: 1:30 PM–3:15 PM
Reference Transcriptome Landscape of Adult Human Retina
Margaret R. Starostik[1], Rinki Ratnapriya[1], Rebecca Kapphahn[2], Ashley Walton[1], Alexandra Pietraszkiewicz[1], Matthew Brooks[1], Sandra R. Montezuma[2], Lars Fritsche[3], Goncalo Abecasis[3], Deborah A. Ferrington[2], Anand Swaroop[1]. [1]Neurobiology Neurodegeneration & Repair Laboratory, National Eye Institute, National Institutes of Health, Washington, DC; [2]Department of Ophthalmology and Visual Neurosciences, University of Minnesota, Minneapolis, MN; [3]Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI.
Purpose: Given the magnitude of retinal degenerative diseases, much effort is invested to find genetic disease variants; yet, a major challenge remains to better understand the biological mechanisms involved. In this study, we performed RNA-seq on 94 human adult retina to characterize in detail the known and novel transcripts of the retinal transcriptome and to understand the gene regulatory network in healthy retina.
Methods: Retina from 45 females and 49 males without retinal disease were procured. RNA was isolated using TRIzol® and libraries were made with the TruSeq® Stranded mRNA Library Preparation Kit. The Illumina Hiseq 2500 platform was used for paired-end RNA-seq. Quality control was evaluated using FastQC and RSeQC. Reads were trimmed for adapters and low quality using Trimmomatic, and then aligned to the Ensembl 38.5 genome with STAR 2-pass. RSEM was used to estimate gene- and transcript expression levels such that low read counts (0 CPM across all samples) were removed and stricter filtering (at least 1 CPM in at least 25% of samples) was applied for further comparative analyses. Putative novel transcripts were identified using Cufflinks RABT assembly, TransDecoder, HMMER, and CPAT.
Results: RNA-seq generated 2.2 billion reads (mean 23.5 million reads per sample), of which 94% mapped to the reference genome. 29,422 (64%) of all annotated genes observed were expressed in the retina while more stringent analysis identified 14,025 protein-coding and 1,675 noncoding genes (34% of all annotated genes observed). DAVID analysis of the top 1,000 most highly expressed genes yielded 9 vision-related clusters (total 171); the second cluster related to visual perception while the fourth cluster related to phototransduction, with enrichment scores of 16.12 and 9.76, respectively. 4,015 putative novel isoforms and unknown transcripts were expressed in the retina, including 392 novel lnc RNA. Tissue-specific expression and alternative splicing analyses are underway.
Conclusions: Our study expands on past studies and demonstrates unique aspects of gene expression that can serve as a valuable resource to further understand the transcriptome changes and global molecular processes that define the retina in health and disease.
Commercial Relationships: Margaret R. Starostik, None; **Rinki Ratnapriya**, None; **Rebecca Kapphahn**, None; **Ashley Walton**, None; **Alexandra Pietraszkiewicz**, None; **Matthew Brooks**, None; **Sandra R. Montezuma**, None; **Lars Fritsche**, None; **Goncalo Abecasis**, None; **Deborah A. Ferrington**, None; **Anand Swaroop**, None
Support: This research was supported by the Intramural Research Program of the National Eye Institute.

Program Number: 568 **Poster Board Number:** B0136
Presentation Time: 1:30 PM–3:15 PM
Transcriptomic and epigenomic analysis of retinal ganglion cell development
Xiuqian Mu[1], Fuguo Wu[1], Darshan Sapkota[1], Tao Liu[2], zihua hu[3, 1]. [1]Ophthalmology, University at Buffalo, Buffalo, NY; [2]Biochemistry, University at Buffalo, Buffalo, NY; [3]CCR, University at Buffalo, Buffalo, NY.
Purpose: In the gene regulatory network controlling the formation of retinal ganglion cells (RGCs), how individual regulators (transcription factors) carry out their functions is largely unknown. Likely these factors fulfill their roles through influencing and interacting with the epigenetic landscape to achieve the transcriptional outcome specific to and essential for RGC development. Our purpose is to understand how key transcription factors contribute to the RGC-specific epigenome and transcriptome during RGC development.
Methods: We used RNA-seq to identify genes that are dependent on three key transcription factors, Atoh7, Pou4f2 and Isl1. We also used ChIP-seq to identify RGC-specific enhancers. In addition, we made new mouse lines in which retinal cells at the different stages of RGC development are labeled by fluorescent proteins, which allows us to use purify cells at different stages of RGC development.
Results: Previous work have identified some downstream genes of Atoh7, Pou4f2 and Isl1 using custom microarrays, but those results are far from complete. Taking advantage of the mouse lines we have, we performed RNA-seq for *Pou4f2*-null, *Isl1*-null, and *Atoh7*-null retinas at E14.5 and identified differentially expressed genes in these mutants, as compared with the wild-type retinas. These results revealed new pathways and players in RGC development. Meanwhile, we have also performed ChIP-seq for H3K4me1 and H3K27ac, two active enhancer makers, with E14.5 wild-type and *Atoh7*-null retinas, and are in the process of identifying enhancers that are specifically active in RGCs at a global level. In addtition, by gene targeting, we have created new mouse lines in which RGC-competent retinal progenitor cells and fate-determined RGCs are labeled by different fluorescent proteins. We will use these lines to purify cells at different stages of RGC development by FACS and further study their properties by transcriptomic and epigenomic analysis.
Conclusions: Using a combined approach of RNA-seq, ChIP-seq and mouse genetics, we will be able to understand how the epigenetic landscape shifts during RGC development and how the shift is regulated. The results will further reveal the genetic mechanisms underlying RGC formation during development. The new mouse lines we have created will significantly facilitate our effort by allowing us to study this process with purified cell populations.
Commercial Relationships: Xiuqian Mu, None; **Fuguo Wu**, None; **Darshan Sapkota**, None; **Tao Liu**, None; **zihua hu**, None
Support: NIH Grant EY020545; Research to Prevent Blindness unrestricted grant; BrightFocus G2016024

Program Number: 569 **Poster Board Number:** B0137
Presentation Time: 1:30 PM–3:15 PM
Characterizing lincRNA expression during RPE development
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Purpose: Long intervening non-coding RNAs (lincRNAs) are increasingly being implicated in human disease, yet nothing is known about their expression or function in the human retina. Using

RNA-Seq, we analyzed both *human induced pluripotent stem cell-derived RPE (iPS-RPE) and age matched human fetal RPE* to characterize lincRNA expression during development.
Methods: iPS were grown in mTeSR media on matrigel coated plates. Embryoid bodies (EBs) were formed by lifting iPS colonies using accutase and were transitioned to neural iduction medium. EBs were then transferred to laminin coated plates, and RPE differentiation begun by adding retinal differentiation medium. At 90 days, RPE were dissected from the plates, pelleted, and flash frozen. Fetal RPE was isolated from 16 week whole eyes. An eye cup was made, and vitreous and neural retina were removed using hyaluronidase. RPE was isolated using dispase, pelleted, and flash frozen. RNA was isolated and RNA-Seq libraries were made using Agilent's SureSelect Strand Specific RNA Library Preparation Kit. Samples were sequenced to a depth of 50 million reads/sample on an Illumina HiSeq 2500. Data were aligned to human genome build 38 with STAR, and gene counts generated using a comprehensive transcriptome annotation database composed of all empirically-determined transcripts. Differential expression was performed using DESeq.
Results: In total, 22,816 transcripts (coding and non-coding) were expressed in iPS, 21,708 in iPS-RPE, and 20,978 in fetal RPE. We identified 1958 lincRNAs expressed in iPS-RPE and 1,964 in fetal RPE with a concordance of 73%. As many as 675 putative novel genes were also discovered, a majority of which are likely to be lincRNAs. We compared the expression of lincRNAs in undifferentiated iPS cells to iPS-RPE. In the differentiated cells, we found that 321 lincRNAs were turned on and 228 were turned off. Additionally, 279 lincRNAs were significantly ($p<0.01$) upregulated (>2-fold), and 234 were significantly downregulated.
Conclusions: This is the first characterization of lincRNA expression in human retinal tissue. We have identified thousands of expressed lincRNAs in the RPE and are likely to be important for RPE development and function. Since so little is known about lincRNA expression in the human retina, these data serve as a guide to expand our studies of lincRNA structure and function; thus providing the baseline for studying their role in visual dystrophies.
Commercial Relationships: Michael H. Farkas, None; **Elizabeth D. Au**, None; **Rosario Fernandez-Godino**, None; **Tadeusz J. Kaczynski**, None; **Maria E. Sousa**, None

Program Number: 570 **Poster Board Number:** B0138
Presentation Time: 1:30 PM–3:15 PM
Nonsyndromic Retinitis Pigmentosa in the Ashkenazi Jewish Population: Genetic and Clinical Aspects
Adva Kimchi[1], Samer Khateb[1], Rong Wen[2], Ziqiang Guan[3], Eran Pras[4, 5], Shoshi Kurtzman[1], Samuel G. Jacobson[6], Hadas Newman[7, 5], Tamar Ben-Yosef[8], Eyal Banin[1], Dror Sharon[1].
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Purpose: Nonsyndromic retinitis pigmentosa (RP) is the most common inherited retinal disease, with a prevalence estimated at 1:5,000 in Europe and the USA and 1:2,230 in the Jewish population in Israel. The higher prevalence of RP in the Jewish population results mainly from intra-community marriages. The Ashkenazi Jewish (AJ) population was genetically isolated for many generations and therefore many founder mutations in this population are known to cause various autosomal recessive diseases. The purpose of the current study is to analyze the genetic and clinical findings in RP patients of AJ descent, aiming to identify genotype-phenotype correlations.
Methods: 226 RP patients who reported AJ origin of both parents were included. Direct mutation screening by Sanger sequencing was performed to detect specific founder mutations. Ophthalmologic analysis includes visual acuity (VA) testing, visual field, electroretinography (ERG), and imaging.
Results: The causative mutation was identified in 36% of families. The most prevalent RP-causing mutations in our cohort are the previously described Alu insertion (c.1297_8ins353, p.K433Rins31*) in *MAK* (35% of cases with a known genetic cause for RP) and c.124A>G, p.K42E in *DHDDS* (34%). In addition, disease-causing mutations were identified in the following genes: *BBS2, CNGB1, EYS, FAM161A, HGSNAT, NR2E3, PRPH2, RHO, RP2, RPE65 and RPGR*. Analysis of clinical parameters of patients with these common founder mutations revealed that on average patients with biallelic *MAK* mutations had a later age of onset and a milder retinal phenotype based on VA testing and cone-flicker ERG analysis compared to patients with biallelic *DHDDS* mutations.
Conclusions: Our AJ cohort of RP patients is the largest reported to date. Our results show a substantial difference in the genetic causes for RP in the AJ population compared to cohorts of other populations, mainly a large proportion of AR cases and a unique composition of causative genes. The most prevalent RP-causing genes in our cohort, *MAK* and *DHDDS*, were not described as major causative genes in other (non-Jewish) populations. The clinical data analysis shows that the phenotype of the *MAK* group of patients is less severe than that of *DHDDS* patients.
Commercial Relationships: Adva Kimchi, None; **Samer Khateb**, None; **Rong Wen**, None; **Ziqiang Guan**, None; **Eran Pras**, None; **Shoshi Kurtzman**, None; **Samuel G. Jacobson**, None; **Hadas Newman**, None; **Tamar Ben-Yosef**, None; **Eyal Banin**, None; **Dror Sharon**, None
Support: Foundation Fighting Blindness USA (BR-GE-0214-0639), Israeli Science Foundation #1037_2015.

Program Number: 571 **Poster Board Number:** B0139
Presentation Time: 1:30 PM–3:15 PM
Clinical features of family members with Stickler syndrome from a mutation of COL11A1 gene
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Purpose: Stickler syndrome (hereditary arthro-ophthalmopathy) is a dominantly inherited connective tissue disorder with ocular, orofacial, auditory, and skeletal manifestations. The majority of families with Stickler syndrome have mutations in the gene encoding type II collagen (*COL2A1*) that exhibits a characteristic 'membranous' or type 1 vitreous phenotype. A novel mutation in the gene encoding the α1 chain of type XI collagen (*COL11A1*) was reported in a Stickler syndrome pedigree with a different 'beaded' or type 2 vitreous phenotype. We report ophthalmic and systemic characteristics of 11 family members with a *COL11A1* mutation.
Methods: The family described is the four-generation family with 18 family members having the *COL11A1* mutation, that was originally reported in the Am J Hum Genet paper[1] with genetic mutation of deletion in the nucleotide A in -2 position of intron 14 (del A^{-2}IVS14). Retrospective chart review was performed on 11 affected family members.

Results: There were 18 affected family members of the pedigree who carried a *COL11A1* gene mutation. The 17 year-old proband had optically empty vitreous with radial perivascular lattice degeneration in both eyes. The father and mother of the proband are first cousins both carrying the gene mutation. Among the affected family members, 100% had optically empty vitreous with perivascular radial lattice degeneration, 63.6% (7/11) had retinal detachment, 81.8% (9/11) had cataract, and 27.2% (3/11) had glaucoma. Of note, there was one patient with retinal degeneration and extinguished ERG and one patient with night blindness. Systemic manifestation showed short stature, high arch palate, hearing loss, obesity, rheumatoid arthritis, and hypertrophic cardiomyopathy.
Conclusions: Family members with Stickler syndrome with *COL11A1* mutation showed optically empty vitreous, perivascular lattice degeneration with early-complicated retinal detachment, and cataract progression.
Commercial Relationships: Miin Roh, None; **Shizuo Mukai**, None

Program Number: 572 **Poster Board Number:** B0140
Presentation Time: 1:30 PM–3:15 PM
Mutation survey of candidate genes in 22 Chinese patients with Axenfeld-Rieger syndrome
Xun Wang, Xing Liu, Xiaoyun Jia, Xueshan Xiao, Shiqiang Li, Xiangming Guo. State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Guangzhou, China.
Purpose: Axenfeld-Rieger syndrome (ARS) is a multiple organ genetic disease and carries a high risk of glaucoma. Its mutation spectrum of candidate genes in Chinese patients remains unknown. We performed a basic study to identify the spectrum and frequency of *PITX2*, *FOXC1* and *PRDM5* genes in Chinese patients with ARS.
Methods: A total of 22 unrelated Chinese probands with ARS were recruited, including three with a family history of an autosomal dominant pattern and 19 sporadic cases, 12 males and 10 females. All patients underwent full ocular and systemic examinations. Sanger sequencing was used to analyze all coding and adjacent regions of the *PITX2*, *FOXC1* and *PRDM5* genes. Segregation and bioinformatics analysis were performed to evaluate the pathogenicity of the variants. All variations detected in the patients were further evaluated by sequencing 96 unrelated healthy individuals as a control.
Results: Eleven mutations in *PITX2* were identified in 11 of 22 probands, including nine novel (c.47-1G>A, c.64C>T, c.90delC, c.108delG, c.211G>T, c.251G>C, c.272G>A, c.475_476delCT, c.484C>T) mutations and two known mutations (c.253-11A>G, c.356delA). Of the 11 mutations, seven were nonsense mutations, two were splicing mutations and the other two were missense mutations. Putative pathogenic mutations of the *FOXC1* and *PRDM5* genes were absent in this cohort of patients.
Conclusions: In this study, nine novel and two known mutations in the *PITX2* gene were identified in 11 of 22 Chinese patients with ARS. The results expand the mutation spectrum of the *PITX2* gene. It also suggested that *PITX2* mutations may be the major cause of Chinese patients with ARS and analysis of the mutation type showed that the nonsense mutation may be a main mutation type of *PITX2* in Chinese patients.
Commercial Relationships: Xun Wang, None; **Xing Liu**, None; **Xiaoyun Jia**, None; **Xueshan Xiao**, None; **Shiqiang Li**, None; **Xiangming Guo**, None
Support: National Natural Science Foundation of China: 81170847

Program Number: 573 **Poster Board Number:** B0141
Presentation Time: 1:30 PM–3:15 PM
***CC2D2A* mutations lead to variable phenotypes in a family with retinal dystrophy**
Cécile Méjécase[1], Saddek Mohand-Said[1, 2], Camille Andrieux[2], Aurélie Hummel[3], Saïd El Shamieh[1], Aline Antonio[1, 2], Fiona Boyard[1], Christel Condroyer[1], Christelle Michiels[1], Steven Blanchard[4], Mélanie Letexier[4], Jean-Paul Saraiva[4], Jose A. Sahel[1, 2], Christina Zeitz[1], Isabelle S. Audo[1, 2]. [1]Sorbonne Universités, UPMC Univ Paris 06, INSERM, CNRS, Institut de la Vision, Paris, France; [2]CHNO des Quinze-Vingts, DHU Sight Restore, INSERM-DHOS CIC1423, Paris, France; [3]Department of Nephrology, Necker-Enfants Malades Hospital, Paris, France; [4]IntegraGen SA, Genopole, CAMPUS, Evry, France.
Purpose: Inherited retinal dystrophies (IRDs) are a heterogeneous group of disorders characterized by progressive degeneration of photoreceptors. Many gene defects have been identified but they only account for 60% of the cases. The purpose of this study was to identify the gene defect(s) underlying IRD in three affected brothers from a consanguineous family.
Methods: Patients were clinically examined by standard methods. Targeted next-generation sequencing (NGS) covering 120 genes known to be mutated in IRD and subsequent whole exome sequencing (WES) were performed. Putative disease-causing variants identified after stringent filtering were confirmed by Sanger sequencing. Additional 960 probands with IRDs were analyzed by targeted NGS.
Results: Ophthalmic examination of the eldest brothers was in keeping with rod-cone dystrophy whereas the younger brother had early-onset cone-rod dystrophy. Renal function and ultrasound was normal in the eldest brothers whereas the youngest had high blood pressure, hematuria and proteinuria but no sign of glomerulonephritis on renal biopsy. Brain magnetic resonance imaging was normal for the three siblings. In addition, none of the siblings had learning disabilities and all were otherwise fit and well. Initial targeted NGS and subsequent WES did not identify any homozygous disease-causing variant. However, two heterozygous variants were identified in *CC2D2A* (c.2774G>C p.(Arg925Pro) and c.4730_4731delinsTGTATA p.(Ala1577Valfs*5)), confirmed by direct sequencing. Of note, *CC2D2A* was covered by targeted NGS approach, but the two variants were missed due to the expectation of homozygous mutations related to the reported consanguinity. Additional 960 probands with IRD screened by targeted NGS did not have *CC2D2A* mutations.
Conclusions: Mutations in *CC2D2A* were previously reported in Meckel-Gruber and Joubert Syndromes. Here we report new phenotypes associated with *CC2D2A* mutations in the same sibship including non-syndromic rod-cone dystrophy and cone-rod dystrophy with atypical renal feature. The link between renal and retinal abnormalities is not established in the later case and warrant further investigations. Mutations in *CC2D2A* underlying non-syndromic IRD account for less than 0.1%. Further studies are needed to understand the phenotype-genotype correlations in Meckel-Gruber Syndrome, Joubert Syndrome and in non-syndromic retinal dystrophy.
Commercial Relationships: Cécile Méjécase, None; **Saddek Mohand-Said**, None; **Camille Andrieux**, None; **Aurélie Hummel**, None; **Saïd El Shamieh**, None; **Aline Antonio**, None; **Fiona Boyard**, None; **Christel Condroyer**, None; **Christelle Michiels**, None; **Steven Blanchard**, None; **Mélanie Letexier**, None; **Jean-Paul Saraiva**, None; **Jose A. Sahel**, None; **Christina Zeitz**, None; **Isabelle S. Audo**, None

Support: Foundation Fighting Blindness center grant [C-CMM-0907-0428-INSERM04], Fondation Voir et Entendre, Prix Dalloz for "La recherche en ophtalmologie", LABEX LIFESENSES [reference ANR-10-LABX-65] supported by French state funds managed by the Agence Nationale de la Recherche within the Investissements d'Avenir program [ANR-11-IDEX-0004-0], Prix de la Fondation de l'Œil and doctoral funding from the Ministère de l'Enseignement Supérieur et de la Recherche (MESR).

Program Number: 574 **Poster Board Number:** B0142
Presentation Time: 1:30 PM–3:15 PM
Changes in macular structure and retinal function in patients with Leber congenital amaurosis with *RPGRIP1* mutations
Daisuke Miyamichi[1], Sachiko Nishina[2], Katsuhiro Hosono[1], Tadashi Yokoi[2], Kentaro Kurata[1], Miho Sato[1], Shinsei Minoshima[3], Maki Fukami[2], Yoshihiro Hotta[1], Noriyuki Azuma[2]. [1]Ophthalmology, Hamamatsu university school of medicine, Hamamatsu, Japan; [2]National Center for Child Health and Development, Setagaya, Japan; [3]Hamamatsu University School of Medicine, Hamamatsu, Japan.
Purpose: Leber congenital amaurosis (LCA) with *RPGRIP1* mutations manifests in patients as a loss of photoreceptors within the first decade life regardless of whether they have a normal central fundus. We evaluated the macular structure and retinal function in patients with LCA with *RPGRIP1* mutations.
Methods: We examined 4 Japanese patients in 2 families (family ID: LCA20, LCA55) at the National Center for Child Health and Development. Case 1: 13-year-old female in LCA20; Cases 2 and 3: 7-year-old male monozygotic twins in LCA20; Case 4: 15-year-old male in LCA55. We collected complete medical histories and conducted pedigree analyses and ophthalmic examinations, including optical coherence tomography (OCT). Electroretinography (ERG) was recorded according to International Society for Clinical Electrophysiology of Vision protocol. To identify causative mutations, 74 genes known to cause retinitis pigmentosa or LCA were examined by targeted-next generation sequencing (NGS), using a custom-designed Agilent HaloPlex target enrichment kit with illumine Miseq sequencer.
Results: Cases 1, 2, and 3 carried the homozygous c.3565_3571del mutation. Case 4 was compound heterozygous for the novel splice mutation c.1467+1G>T and the exon 17 deletion mutation. ERG showed that rod and cone responses were severely reduced in Case 1 at 7 years of age. The rod response was subnormal while the cone response was severely reduced in Case 2 and 3 at 1 year 3 months. The cone response was undetectable and the rod response was slightly reduced in Case 4 at 7 years. OCT indicated that the outer nuclear layer (ONL) was slightly thinning with increasing age in Case 1 between 7 and 11 years. The OLN retained a relatively uniform thickness in Cases 2 and 3 at 1 year 6 months of age. ONL thickness in Case 4 at 7 years was lower than normal. Although OCT showed a thinning of the ONL in all 4 cases, lamellar structures were retained at the foveal area.
Conclusions: We described the ONL thinning at the perifoveal area as well as retinal dysfunction in 4 LCA cases with *RPGRIP1* mutations. To our knowledge, this is the youngest OCT data of the patients with LCA with *RPGRIP1* mutation. Although four cases in 2 families exhibited various clinical findings of LCA, it is suggested that ONL on OCT and rod function on ERG are relatively retained in patients with LCA with *RPGRIP1* mutation in early childhood.
Commercial Relationships: Daisuke Miyamichi, None; **Sachiko Nishina**, None; **Katsuhiro Hosono**, None; **Tadashi Yokoi**, None; **Kentaro Kurata**, None; **Miho Sato**, None; **Shinsei Minoshima**, None; **Maki Fukami**, None; **Yoshihiro Hotta**, None; **Noriyuki Azuma**, None

Program Number: 575 **Poster Board Number:** B0143
Presentation Time: 1:30 PM–3:15 PM
Identification of mutations in *CACNA1F* in patients with incomplete CSNB applying next generation sequencing approaches
Christina Zeitz[1], Christelle Michiels[1], Marion Neuille[1], Christoph Friedburg[2], Christel Condroyer[1], Fiona Boyard[1], Aline Antonio[1], Markus N. Preising[2], Vincent Meyer[3], Anne Boland[3], Jean-François Deleuze[3], Lilia Mesrob[3], Bernhard Jurklies[4], Birgit Lorenz[2], Jose A. Sahel[1], Isabelle S. Audo[1]. [1]Institut de la Vision, Univ Pierre et Marie Curie Paris 6, Paris, France; [2]Justus-Liebig University Gießen, Gießen, Germany; [3]Centre National de Génotypage, Institut de Génomique, CEA, Evry, France; [4]Department of Ophthalmology, University of Essen, Essen, Germany.
Purpose: Patients with incomplete congenital stationary night blindness (icCSNB) show a normal a-wave in the scotopic bright flash electroretinogram (ERG) with a reduced b-wave giving an electronegative waveform. The 30-Hz and single-flash cone ERG are severely diminished and delayed and the long duration stimulation shows reduced ON and OFF responses. The purpose of this work to identify the gene defect in patients with icCSNB, previously excluded in the coding regions of *CACNA1F, CABP4* and *CACNA2D4*.
Methods: Patients of different European clinical centers underwent full ophthalmic examination. Informed consent was obtained from each patient and available family members. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee. The DNA of 4 index patients with a clinical diagnosis of icCSNB and available family members was extracted and screened for *CACNA1F*, *CABP4* and *CACNA2D4* mutations by Sanger sequencing. Subsequently targeted, whole exome (WES) or whole genome sequencing (WGS) was applied. Minigene approaches were used to investigate the effect of intronic variants on splicing.
Results: These patients revealed absence of mutations in the coding regions of *CACNA1F*, *CABP4* and *CACNA2D4*. WES of family A indicated that the DNA of the father and index was confounded showing a novel mutation, c.868C>T, p.(Arg290Cys) in *CACNA1F*. Investigation of family B and C by WES covering flanking intronic regions revealed a c.1496+5G>C and a c.4294-9G>A variant in *CACNA1F.* WGS on family D identified a c.522-16G>A variant in *CACNA1F*. The validated variants co-segregated with the phenotype in the families. Although these variants do not directly affect splice acceptor or donor sites, they are predicted to influence splicing. Indeed, preliminary data obtained from minigene approaches revealed a different RT-PCR profile in patients compared to control.
Conclusions: More than 120 different mutations in the coding regions of *CACNA1F* have been described to be the major cause of icCSNB. A few icCSNB cases lack mutations in the coding regions of known genes underlying this disorder. Our study presented herein highlight the possibility that the missing mutations may be found in overlooked intronic regions of *CACNA1F*, influencing natural splicing. This assumption may hold true for many other gene defects underlying inherited retinal disorders.
Commercial Relationships: Christina Zeitz; **Christelle Michiels**, None; **Marion Neuille**, None; **Christoph Friedburg**, None; **Christel Condroyer**, None; **Fiona Boyard**, None; **Aline Antonio**, None; **Markus N. Preising**, None; **Vincent Meyer**, None; **Anne Boland**, None; **Jean-François Deleuze**, None; **Lilia Mesrob**, None; **Bernhard Jurklies**, None; **Birgit Lorenz**, None; **Jose A. Sahel**, None; **Isabelle S. Audo**, None

Support: Voir et Entendre (http://www.fondave.org/) (CZ), Prix Dalloz for "La recherche en ophtalmologie" (CZ), LABEX LIFESENSES [reference ANR-10-LABX-65] supported by French state funds managed by the Agence Nationale de la Recherche within the Investissements d'Avenir program [ANR- 11-IDEX-0004-0] (http://www.agence-nationale-recherche.fr/investissements-davenir/), Foundation Fighting Blindness center grant [C-CMM-0907-0428-INSERM04] (http://www.blindness.org/), Prix de la Fondation de l'OEil (IA)

Program Number: 576 **Poster Board Number:** B0144
Presentation Time: 1:30 PM–3:15 PM

An Alu-mediated copy number variation mediates progressive optic nerve cupping in autosomal dominant optic pit

Eileen Hwang[1], Denise Morgan[1], James L. Zimmerman[1], Claude F. Burgoyne[2], Paul S. Bernstein[1], Margaret M. DeAngelis[1]. [1]Moran Eye Center, University of Utah, Salt Lake City, UT; [2]Devers Eye Institute, Legacy Health, Portland, OR.

Purpose: Progressive optic nerve cupping was previously described in a Midwestern family with autosomal dominant optic pit, i.e. "cavitary optic disc anomaly." Affected members of the Midwestern family were found to have two extra copies (four copies total) of a region upstream from the MMP19 gene (Hazlewood, 2015). We describe a second family with a similar but independent copy number variation.

Methods: Members of a three-generation Utah pedigree were characterized by clinical exam, fundus photography, and optical coherence tomography to determine disease status. Leukocyte DNA from 16 individuals was analyzed using two independent TaqMan copy number variation assays located in the region previously described in the Midwestern family.

Results: All four affected individuals in the Utah family had one extra copy (three copies total) of the non-coding region upstream of MMP19. The 13 unaffected individuals had two copies of the region, which is the normal genotype. In three of the four affected individuals, long term follow up with optic nerve photos demonstrated progressive cupping of the optic nerve head.

Conclusions: We are the second group to describe a family with autosomal dominant optic pit associated with a copy number variation in the non-coding region upstream of MMP19. Affected members of the Midwestern family had two extra copies of the region, whereas affected members of the Utah family had one extra copy. The description of an independent family with a different variation in the same region confirms the role of this locus in disease pathogenesis. Studies are ongoing to investigate possible phenotypic differences from the previously described family.

Reference: Hazlewood et al. (2015) Heterozygous Triplication of Upstream Regulatory Sequences Leads to Dysregulation of Matrix Metalloproteinase 19 in Patients with Cavitary Optic Disc Anomaly. Human Mutation. 36:369-378.

Commercial Relationships: Eileen Hwang, None; **Denise Morgan**, None; **James L. Zimmerman**, None; **Claude F. Burgoyne**, None; **Paul S. Bernstein**, None; **Margaret M. DeAngelis**, None

Support: Achievement Rewards for College Scientists, Research to Prevent Blindness, NIH NEI Core Grant 14800, NIH NEI EY014800, The Skaggs Foundation for Research

Program Number: 577 **Poster Board Number:** B0145
Presentation Time: 1:30 PM–3:15 PM

Phenotype and Genotype Features of *PAX6*-related Ocular Dysgenesis

Lizhu Yang[1, 3], Qi Zhou[1], Zixi Sun[1], Kaoru Fujinami[2, 3], Huajin Li[1], Zhisheng Yuan[1], Kazuo Tsubota[3], Ruifang Sui[1]. [1]Department of Ophthalmology, Peking Union Medical College Hospital, Beijing, China; [2]Laboratory of Visual Physiology, Division of Vision Research, National Institute of Sensory Organs, National Hospital Organization, Tokyo Medical Center, Tokyo, Japan; [3]Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan.

Purpose: Paired box gene 6 (*PAX6*) is a master regulatory gene of eye development. *PAX6* mutations can cause a series of ocular dysgenesis, including aniridia, Peter's anomaly, corneal anomaly, foveal hypoplasia, congenital cataract and so on. This study aims to identify the genetic defect for a cohort of ocular dysgenesis and to analyze the clinical features of *PAX6*-related developmental eye diseases.

Methods: Twenty patients from 8 aniridia pedigrees and 4 pedigrees with other developmental eye diseases were recruited and detailed ophthalmologic examinations were performed. Peripheral blood samples were taken to extract genomic DNA. (1) Sanger direct sequencing of *PAX6* was performed in aniridia subjects. (2) Next-generation sequencing (NGS) was utilized in the samples with the other developmental eye diseases. (3) Multiplex ligation-dependent probe amplification (MLPA) of *PAX6* was applied to detect large deletions/duplications in the samples negative for (1) and (2). Then co-segregation analysis was performed to further confirm the causative mutation.

Results: There were 10 pedigrees with autosomal dominant inheritance and 2 sporadic cases. Eleven aniridia patients from 8 families were all bilaterally affected and had nystagmus, respectively combined with bilateral ptosis (3/11, 27.3%), cornea anomalies (3/11, 27.3%) and foveal hypoplasia (5/5, 100%). The phenotypes of the other 4 pedigrees include: 3 patients in 1 family had bilateral cornea opacity, developmental glaucoma and iris aplasia; 4 patients in 1 family had bilateral nystagmus, premature cataract and foveal hypoplasia; The other 2 patients had bilateral microcornea, iris hypoplasia and congenital cataract. *PAX6* defects were found in all the 12 pedigrees including 9 heterozygous point mutations and 3 large chromosomal changes. 9 mutations include 2 nonsense mutations, 2 frameshift mutations, 2 splicing mutations and 3 missense mutations. Interestingly, all the 3 missense mutations were found in non-aniridia subjects. Totally, 6 novel point mutations and 3 novel large fragment changes were identified, which accounts for 75% of the total variants.

Conclusions: *PAX6* plays an important role in developmental eye diseases. Twelve *PAX6* pathogenic variants including 9 novel variants were determined in our study. Non-aniridia phenotypes are frequently associated with missense mutations, which elucidates the genotype-phenotype correlation in *PAX6*-related ocular dysgenesis.

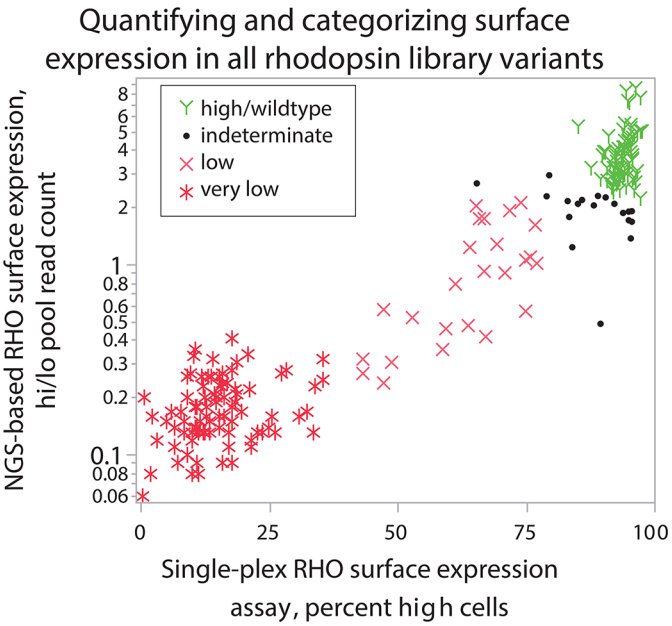
Commercial Relationships: Lizhu Yang, None; **Qi Zhou**, None; **Zixi Sun**, None; **Kaoru Fujinami**, None; **Huajin Li**, None; **Zhisheng Yuan**, None; **Kazuo Tsubota**, None; **Ruifang Sui**, None

Program Number: 578 **Poster Board Number:** B0146
Presentation Time: 1:30 PM–3:15 PM

A functional genomics approach for characterizing variants of unknown significance: assaying known and novel rhodopsin variants

Jason Comander, Aliete Wan. Ocular Genomics Institute, Massachusetts Eye & Ear Infirmary, Boston, MA.

Purpose: Characterizing the functional significance of DNA sequence variants of unknown significance (VUS) is currently a major bottleneck in interpreting human genetic variation. A library approach was developed to efficiently detect pathogenic rhodopsin (*RHO*) variants that fail to express on the cell surface. This approach, while initially focused on *RHO*, is meant to demonstrate a streamlined, general method for detecting pathogenic VUS.
Methods: A cDNA library was created with 210 known and novel *RHO* variants taken from the literature and from the results of genetic diagnostic testing for patients with inherited retinal degenerations performed in our department. To identify misfolding (class II) mutants, transfected cultured cells were sorted using flow cytometry based on RHO surface expression. Next generation sequencing and string-based bioinformatic methods were used to quantify the transfected DNA in high versus low RHO-expressing cell pools.
Results: The multiplexed assay successfully detected all pooled library variants and quantified their expression precisely (Z'=0.94, R^2=0.92-0.95). Updated *RHO* variant classifications were generated based on these functional data. For example, 82% of known folding mutants were confirmed to show pathogenic surface expression, while 18% unexpectedly showed normal or slightly reduced levels. Endocytosis and stability/posttranslational modification mutants showed an intermediate phenotype, although a more general pathogenicity assay will be needed to detect all pathogenic mutation classes. Among 10 novel VUS, 3 (G18D, G101V, P180T) showed low or very low levels and were present in probands manifesting retinitis pigmentosa, confirming their pathogenicity in these patients.
Conclusions: A multiplexed assay efficiently identified pathogenic VUS in *RHO* based on surface expression, solving 3 pedigrees. The updated pathogenicity findings may be useful to investigators and genetic counselors in interpreting pathogenicity of *RHO* variants, and the surface expression data may inform RHO structure-function relationships. This study demonstrates a parallelized, higher-throughput cell-based assay for the functional characterization of VUS in rhodopsin, and can be applied more broadly in other genes and disorders.



Surface expression levels of 210 *RHO* variants as measured by single-plex and multiplex (NGS) assays were highly correlated, R^2=0.91.

Commercial Relationships: Jason Comander, None; **Aliete Wan**, None
Support: NEI K12 EY016335, Foundation Fighting Blindness Enhanced Career Development Award, Research to Prevent Blindness Career Development Award

Program Number: 579 **Poster Board Number:** B0147
Presentation Time: 1:30 PM–3:15 PM

The Oculome: a genetic test to diagnose a diverse range of ocular birth anomalies

Vijay K. Tailor[2, 1], Jane Hayward[3], Aara Patel[3], Camila Gabriel[3], Annegret H. Dahlmann-Noor[2], Jane Sowden[3]. [1]Experimental Psychology, University College London, London, United Kingdom; [2]NIHR Biomedical Research Centre at Moorfields Eye Hospital NHS Trust, London, UK, Moorfields Eye Hospital, London, United Kingdom; [3]Birth Defects Research Centre, UCL Great Ormond Street Institute of Child Health, and NIHR Biomedical Research Centre at Great Ormond Street Hospital NHS Trust and University College London, London, UK, London, United Kingdom.

Purpose: Each year up to 4 in 10,000 children in the UK are diagnosed as severely sight impaired. More than 25% of cases may be inherited, with the majority remaining undiagnosed due to the large number of genes underlying these highly heterogeneous conditions. We developed a new next generation sequencing (NGS) panel test, the Oculome, which enables genetic diagnosis in a diverse range of ocular birth defects and inherited eye conditions, including syndromic, metabolic, developmental, or sensory abnormalities.
Methods: A custom-designed Agilent SureSelect QXT target capture method was used to capture 436 genes representing known eye disease genes. Panel development included sequencing n=288 clinical samples using Illumina HiSeq2500 with 125 bp paired end sequencing reads. We achieved >30 X read-depth for 99.5% of the targeted region. Bioinformatics analysis was performed using an in-house pipeline and annotated using Variant Effect Predictor v73.
Results: The Oculome panel covers 436 genes subdivided into 5 overlapping sub-panels for retinal dystrophies (212), anterior segment dysgenesis and glaucoma (47), microphthalmia-anophthalmia-coloboma (MAC) (86), congenital cataracts and lens-associated (84) and albinism (16). Analysis of n=160 paediatric samples resulted in a definitive diagnosis in n=41 (25.6%) with variability in diagnostic yield between phenotypic sub-panels e.g. retinal dystrophies (57.6% cases solved) and anterior segment/glaucoma (26.8% cases solved).
Conclusions: The Oculome enabled the molecular diagnosis of a significant number of cases in our sample cohort of varied congenital ocular conditions. The Oculome test is available at the Great Ormond Street North East Thames NHS Regional Genetics Service Laboratories, London UK. Genetics.labs@gosh.nhs.uk

Commercial Relationships: Vijay K. Tailor, None; **Jane Hayward**, None; **Aara Patel**, None; **Camila Gabriel**, None; **Annegret H. Dahlmann-Noor**, None; **Jane Sowden**, None

Program Number: 580 **Poster Board Number:** B0148
Presentation Time: 1:30 PM–3:15 PM
Hidden genetic variation in Stargardt disease: novel copy number variations, *cis*-regulatory and deep-intronic splice variants within the *ABCA4* locus
Miriam Bauwens[1], Riccardo Sangermano[2], Timothy Cherry[4, 3], Van Cauwenbergh Caroline[1], José Luis Gómez-Skarmeta[5], Nicole Weisschuh[7], Susanne Kohl[7], Bart P. Leroy[1, 6], Frans P. Cremers[2], Elfride De Baere[1]. [1]Center for Medical Genetics, University of Ghent, Ghent, Belgium; [2]Department of Human Genetics, RadboudUMC, Nijmegen, Netherlands; [3]Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute, Seattle, WA; [4]Dept. of Pediatrics, University of Washington School of Medicine, Seattle, WA; [5]Centro Andaluz de Biología del Desarrollo, CSIC/ Universidad Pablo de Olavide, Sevilla, Spain; [6]Dept of Ophthalmology, Ghent University Hospital & Ghent University, Gent, Belgium; [7]Institute for Ophthalmic Research, Centre for Ophthalmology, University of Tuebingen, Tuebingen, Germany.
Purpose: A large proportion of Stargardt disease (STGD1) patients have single coding variants in the disease gene *ABCA4*, suggestive of hidden genetic variations in non-coding regions. We aimed to assess the contribution of copy number variations (CNVs) and non-coding sequence variations in mono-allelic STGD1 patients. Moreover, we attempted to gain more insight into the *cis*-regulatory landscape of *ABCA4*.
Methods: A total of 116 monoallelic STGD1 patients underwent targeted resequencing of a conserved block of synteny encompassing the *ABCA4* gene (HaloPlex enrichment). Filtering of variants was based on criteria such as publicly available minor allele frequencies and frequency in the total cohort of sequenced patients. Altered splice predictions, presence in *cis*-regulatory regions of *ABCA4* and RegulomeDB data served as a secondary filter. Candidate splice variants were tested by mini-gene assays while putative *cis*-regulatory variants were investigated by *ex vivo* electroporation in mouse retinas. 4C-seq was performed on human retinal cells, using an anchor in the *ABCA4* promoter region. Customized arrayCGH (arrEYE) was used for CNV analysis of 5 patients.
Results: Mini-gene assays were performed for a subset of 12 intronic variants, confirming a splice effect for 3 variants. The *cis*-regulatory effect of 3 promoter and 2 deep intronic variants were tested in mouse retinal explants, revealing a significant effect on regulation for 2 of the promoter variants. A chromatin interaction map of the *ABCA4* region was generated by 4C-seq in human adult retinal cells. 11 putative *cis*-regulatory variants are being tested by luciferase assays in Y79 cells and nine putative splice variants are being assessed by mini-gene assays. CNV analysis revealed a novel *ABCA4* deletion (ex 40-50) and a duplication (ex 2-6) in 2/5 patients without candidate non-coding variants.
Conclusions: Resequencing of the whole *ABCA4* locus uncovered novel deep intronic splice variants and *cis*-acting regulatory variants in unsolved cases of STGD1, representing the first report of non-coding regulatory *ABCA4* variants. In addition, a chromatin interaction dataset for the *ABCA4* locus was generated in retinal cells. Finally, apart from a novel deletion, the first duplication was identified in *ABCA4*, expanding the CNV spectrum which represents a very small fraction of the known mutational load in *ABCA4*.
Commercial Relationships: Miriam Bauwens, None; **Riccardo Sangermano**, None; **Timothy Cherry**, None; **Van Cauwenbergh Caroline**, None; **José Luis Gómez-Skarmeta**, None; **Nicole Weisschuh**, None; **Susanne Kohl**, None; **Bart P. Leroy**, None; **Frans P. Cremers**, None; **Elfride De Baere**, None

Program Number: 581 **Poster Board Number:** B0149
Presentation Time: 1:30 PM–3:15 PM
Phenotype and Genotype Characterization of a small cohort of Chinese patients with Occult Macular Dystrophy
Ruifang Sui, Lizhu Yang, Xuan Zou, Huajin Li, Fangtian Dong. Ophthalmology, Peking Union Med College Hosp, Beijing, China.
Purpose: Occult Macular Dystrophy (OMD) is an inherited progressive ocular disease, featured by normal appeared fundus without clear functional impairment of the retina. Retinitis pigmentosa 1 like 1 gene (*RP1L1*) is the only confirmed gene related to OMD. This study aims to investigate the clinical characteristics of OMD and genetic changes in *RP1L1* for a small cohort of Chinese patients.
Methods: Thirteen patients from 10 unrelated families with clinically diagnosed OMD were recruited. Ophthalmologic examinations including optic coherence tomography (OCT), visual field, full-field electroretinogram (ERG), pattern ERG (pERG) and multifocal ERG (mfERG) were performed. Sanger sequencing of *RP1L1* were utilized to identify the pathogenic variants. Co-segregation analysis was performed to further confirm the causative variants.
Results: There were 3 pedigrees with autosomal dominant (AD) inheritance and 7 sporadic cases. Twelve patients were bilaterally affected and one case was unilateral. The best corrected visual acuity (BCVA) among ranged from 0.02 to 1.0 with the median 0.2. The median onset age was 17 years with a range from 5 to 45 years. All patients appeared with normal fundus and localized dysfunction of the macular detected by electrophysiological examinations. Two *RP1L1* mutations was identified in 3 families (3/10, 33.3%), including the hot-spot variant p. R45W identified in two AD pedigrees, and a novel variant p. R397X localized in an isolated patient. There are characteristic phenotype differences in OCT manifestation between *RP1L1* positive group and negative group: all positive cases showed classical OCT manifestations with both blurred ellipsoid zone (EZ) and absence of interdigitation zone (IZ). While the *RP1L1* negative group lacked at least one of these two features, which is defined as nonclassical OCT type.
Conclusions: This is the first study of genotype and phenotype features based on Chinese patient cohort. Our study identified one novel RP1L1 mutation (p. R397X) and confirmed that the hot spot pathogenic variants is associated with OMD in Chinese population. The microstructural difference between *RP1L1* positive and negative groups indicates the possible genotype-phenotype correlation of OMD.
Commercial Relationships: Ruifang Sui, None; **Lizhu Yang**, None; **Xuan Zou**, None; **Huajin Li**, None; **Fangtian Dong**, None
Support: Ministry of Science and Technology of the People's Republic of China (Grant No.:2010DFB33430), the Foundation Fighting Blindness USA (CD-CL-0214-0631-PUMCH), the National Natural Science Foundation of China (81470669) and Beijing Natural Science Foundation (7152116).

Program Number: 582 **Poster Board Number:** B0150
Presentation Time: 1:30 PM–3:15 PM
Identification of novel *BEST1* mutations in Bestrophinopathy families
Anshuman Verma[1], Thong Nguyen[2], Poornachandra B[3], Somasekar Seshagiri[2], Andrew Peterson[2], Sameer Phalke[4], Anuprita Ghosh[1], Arkasubhra Ghosh[1]. [1]GROW Research Laboratory, Narayana Nethralaya Foundation, Bangalore, India; [2]Dept of molecular biology, Genentech, San Francisco, CA; [3]Narayana Nethralaya 1, Bangalore, India; [4]MedGenome pvt, Bangalore, India.
Purpose: The *BEST1* gene encodes for the Bestrophin protein, a chloride channel required for normal visual functions of the retina.

Mutations in *BEST 1* have been reported in a few related genetic ocular diseases, autosomal recessive bestrophinopathy (ARB) or Best vitelliform macular dystrophy (BVMD). However, novel mutations associated with new phenotypic variations and the inheritance patterns are still being discovered. In this study, we analyzed four Indian families using whole-exome sequencing and identified unreported *BEST1* mutations with their phenotype.
Methods: This study was performed with the approval of the Narayana Nethralaya ethics committee and written informed consent of the patients. The subjects underwent ophthalmic examination including fundus imaging, slit lamp examination, fundus autoflourescence and electrophysiology. A total 8 patients with ARB (6 males, 2 females) (age range 11-26 years) and 12 related unaffected members from four unrelated southern Indian families were clinically investigated. Whole exome sequencing was performed for all 20 members using DNA isolated from peripheral blood and data was processed using a DNA sequencing workflow.
Results: The data from 20 samples from the four families in our study resulted in 2,630,722 variants. After filtration, we found *BEST1* mutations in all 4 families (hom. p.Y131C in Family A ; hom. p.R150P in Family B ; het.p.R47H and het.p.V216I in Family C; and het.p.T91I in Familly D). The features of families A, B and C suggest ARB while family D is suggestive of BVMD.
Conclusions: The genetic analysis characterized 4 novel *BEST1* mutations in four families, two identified with homozygous inheritance and rest two with heterozygous mutations. The presence of compound heterozygous mutations in ARB family C is a novel discovery. In BVMD family D, the heterozygous mutation in asymptomatic parent and the proband suggests the possibility of either incomplete dominance or additional undiscovered genetic features. This report adds to the spectrum of genotypes and phenotypes of *BEST1* mutations and will be useful for its accurate clinical and molecular diagnosis.
Commercial Relationships: Anshuman Verma; **Thong Nguyen**, None; **Poornachandra B**, None; **Somasekar Seshagiri**, None; **Andrew Peterson**, None; **Sameer Phalke**, None; **Anuprita Ghosh**, None; **Arkasubhra Ghosh**, None
Support: Narayana Nethralaya Foundation, India ; Genentech, US.

Program Number: 583 **Poster Board Number:** B0151
Presentation Time: 1:30 PM–3:15 PM
First missense mutation in *CEP78* in a family with cone-rod dystrophy, sensorineural hearing loss, obesity and subfertility
Frauke Coppieters[1], Giulia Ascari[1], Stijn Van De Sompele[1], Lara Derycke[2], Holtappels Gabriële[2], Olga Krysko[2], Jo Van Dorpe[3], David Creytens[3], Irina Balikova[4, 5], Jan Gerris[6], Claus Bachert[2], Bart P. Leroy[1, 4]. [1]Center for Medical Genetics Ghent, Ghent University, Ghent, Belgium; [2]Upper Airways Research Laboratory, Ghent University Hospital, Ghent, Belgium; [3]Department of Pathology, Ghent University Hospital, Ghent, Belgium; [4]Dept of Ophthalmology, Ghent University Hospital, Ghent, Belgium; [5]Free University of Brussels, Brussels, Belgium; [6]Gynaecology Dept, Ghent University Hospital, Ghent, Belgium.
Purpose: Bi-allelic truncating mutations in *CEP78* were recently found to cause a recognizable phenotype characterized by cone-rod dystrophy (CRD) with sensorineural hearing loss. Here, we aimed to identify the causal mutation in a family presenting with CRD and sensorineural hearing loss, and to explore ciliary phenotypic features in homozygous affected individuals and heterozygous carriers.
Methods: Whole exome sequencing was performed in 2 affected siblings using the SureSelectXT V6 kit (Agilent), followed by sequencing on a NextSeq 500 instrument (Illumina). Segregation analysis in 5 additional family members was done using Sanger sequencing. Skin biopsies and nasal brush samples were obtained from both affected individuals and their parents to study ciliary structure and length.
Results: We identified the following novel missense mutation in *CEP78* in a homozygous state in both affected individuals: c.449T>C, p.(Leu150Ser). This mutation was found to segregate with disease in 7 individuals. The affected nucleotide and amino acid are highly conserved, and the change is predicted to be damaging by several *in silico* prediction programs (SIFT, MutationTaster, PolyPhen2). Detailed phenotyping revealed the presence of additional features reminiscent to a ciliopathy such as recurrent airway infections in the affected individuals and heterozygous carriers, obesity in both parents and both affected individuals, and subfertility in one affected male. Functional studies are currently ongoing on fibroblasts, nasal epithelial cells and semen samples to investigate if this *CEP78* mutation affects ciliary structure, and if this is associated with the recurrent airway infections and subfertility observed.
Conclusions: In conclusion, we identified the first missense mutation in *CEP78* causing CRD and sensorineural hearing loss, a recently identified syndrome distinct from Usher syndrome. The affected individuals as well as heterozygous carriers studied here displayed additional features, suggesting a potential involvement of *CEP78* in more complex ciliopathies than previously anticipated.
Commercial Relationships: Frauke Coppieters, None; **Giulia Ascari**, None; **Stijn Van De Sompele**, None; **Lara Derycke**, None; **Holtappels Gabriële**, None; **Olga Krysko**, None; **Jo Van Dorpe**, None; **David Creytens**, None; **Irina Balikova**, None; **Jan Gerris**, None; **Claus Bachert**, None; **Bart P. Leroy**, None

Program Number: 584 **Poster Board Number:** B0152
Presentation Time: 1:30 PM–3:15 PM
Identification of rhodopsin exon mutations underlying retinitis pigmentosa that alter splicing
Maureen Neitz, Jay Neitz. Ophthalmology, University of Washington, Seattle, WA.
Purpose: Mutations in the rhodopsin gene (RHO) are associated with autosomal dominant retinitis pigmentosa (ADRP), with >120 disease-causing mutations identified. The disease mechanism for individual mutations is unclear but important for understanding and developing therapeutic strategies. Individual nucleotide changes in exons have been presumed to exert pathology at the level of protein structure/function, overlooking the potential for effects on splicing. We tested the hypothesis that some exon mutations in rhodopsin alter splicing.
Methods: We tested 3 rhodopsin mutations based on bioinformatics predictions that they may alter splicing, and 2 randomly chosen mutations not predicted to alter splicing (Table 1). We used wild-type RHO as the control. Splicing assays were performed using full length rhodopsin genes driven by the ubiquitous CMV promoter. Plasmids carrying the rhodopsin genes were sequence-verified, then transfected into duplicate cultures of HEK293T cells. mRNA was isolated 48 hours later and subjected to reverse transcription (RT) and the polymerase chain reaction (PCR). Assays were performed in triplicate for each mutant. Gel purified RT-PCR products were directly sequenced by fluorescent sequencing on an ABI 3500 capillary sequencer.
Results: The wild-type RHO gene, the p.A164Q and p.W184S mutants only gave rise to correctly spliced, full-length mRNA (confirmed by sequencing); however the other three mutations altered splicing. Both the p.Y136X and the p.C167W produced only mRNA in which exon 2 was skipped. The p.Q312X mutant used a cryptic splice site in exon 4, deleting the last 4 bases of exon 4.
Conclusions: If spliced correctly, the p.Y136X and p.C167W mutants are predicted to have quite different disease mechanisms.

The p.Y136X mRNA is likely targeted to nonsense mediated decay (NMD) due to the premature translation termination codon, whereas the p.C167W mRNA is predicted to be translated into a mutant opsin protein. However, both mutations cause exon 2-skipping, which introduces premature stop codons for both that likely target the mRNAs to NMD, and thus they share the same disease mechanism. Rather than producing a truncated protein, the altered splicing observed for the p.Q312X mutation shifts the reading frame and encodes a protein that is 10 amino acids longer than RHO.

Table 1. RHO mutations investigated

codon change	protein change
cDNA.408 C>A	p.Y136X
cDNA.501 C>G	p.C167W
cDNA.934 C>T	p.Q312X
cDNA.491 C>A	p.A164D
cDNA.557 C>G	p.S186W

Rhodopsin mutations investigated.

Commercial Relationships: Maureen Neitz, None; **Jay Neitz**, None

Support: Unrestricted funds from Research to Prevent Blindness

Program Number: 585 **Poster Board Number:** B0153
Presentation Time: 1:30 PM–3:15 PM

NEI eyeGENE® clinical research data accessibility through a Biomedical Research Informatics Computing System

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Purpose: As advances in data technology and data sharing requirements continue to feature in scientific efforts, the combined analysis of disparate data is a challenge to be addressed. eyeGENE®, a National Eye Institute(NEI) genomic research initiative created in response to growing therapeutic opportunities, has taken advantage of the Biomedical Research Informatics and Computing System (BRICS), a bioinformatics platform that allows users to store and query large amounts of data in a relational manner.

Methods: BRICS forms the foundation for a number of biomedical informatics platforms and data repositories within the NIH, extramural research communities, and the Department of Defense. It provides web-based functionality and downloadable tools to support data definition, data access and contribution for continuous research use. eyeGENE® developed forms for use in the system: Demographics, Genetics, Imaging, Legacy Clinical, and LOINC® Clinical. Data collected in the eyeGENE® legacy data capture system was exported into csv files corresponding to the appropriate form. Data was validated using the BRICS submission tool before being available for query.

Results: 475 Unique Data Elements were created to standardize eyeGENE® data collected from 2006-2015. The first data sets available were the demographics and genetics data containing 43,283 rows of data. Over 11,000 genes were tested in the program. Genetics data includes pathogenic mutations and variants of unknown significance. Imaging data, including 18,825 supplemental files, and clinical data will soon be available. The legacy data is also mapped to LOINC® standards to allow for cross-analysis. Research results are being collected in the BRICS Meta Study feature, which serves as a Data Commons.

Conclusions: Biomedical “Big Data” is diverse and complex, and drawing conclusions from large amounts of clinical research data relies heavily on being able to access, compare and query across these data sets. Using BRICS, we provide vision community access to data and tools for analysis of eyeGENE® clinical and genetic data. Summary level genetics results are also freely available to the public (https://eyegene.nih.gov/). LOINC® nomenclature (http://loinc.org) allows for data and information comparison across studies. Over time, it is expected that these efforts will maximize the value of the eyeGENE® data, leading to continued research discoveries.

Commercial Relationships: Santa J. Tumminia, None; **Yvonne O. Akporji**, None; **Chelsea Bender**, None; **Jemma Iano-Fletcher**, None; **Andrew Hughes**, None; **Matthew McAuliffe**, None; **Leonie Misquitta**, None; **Rebecca S. Parrish**, None; **Melissa J. Reeves**, None; **Kerry E. Goetz**, None