Whole Exome Sequencing of a large family with Primary Open Angle Glaucoma reveals its vast complexity.

Mohd Hussain Shah1*, Manojkumar Kumaran2,5*, Mohideen Abdul Kader3, R. Ramakrishnan3, Subbiah. R. Krishnadas4, Bharanidharan Devarajan2@, and Periasamy Sundaresan1@

1Department of Molecular Genetics, Aravind Medical Research Foundation, Dr.G.Venkataswamy Eye Research Institute, Madurai, Tamil Nadu, India
2Department of Bioinformatics, Aravind Medical Research Foundation, Dr.G.Venkataswamy Eye Research Institute, Madurai, Tamil Nadu, India
3Glaucoma Clinic, Aravind Eye Hospital, Tirunelveli, Tamil Nadu, India
4Glaucoma Clinic, Aravind Eye Hospital, Madurai, Tamil Nadu, India
5School of Chemical and Biotechnology, SASTRA (Deemed to be University), Thanjavur, Tamil Nadu, India

*First two authors contributed equally to this work

@ Corresponding Authors.

@ Corresponding Authors: Bharanidharan, Devarajan E-Mail: bharani@aravind.org Telephone: 91-452-253 0984 Extn.467

Periasamy, Sundaresan E-Mail: sundar@aravind.org Telephone: 91-452-253 0984 Extn.423
Abstract

Multiple studies have identified several pathogenic variants, majorly contribute to the pathogenesis of primary open-angle glaucoma (POAG). However, these genetic factors can only explain 5-6% of POAG. To identify pathogenic variants associated with POAG by using Whole Exome Sequencing (WES) data of an Egyptian origin of a large family with POAG settled in South India. We recruited a large five-generation family with a positive family history of POAG from Kayalpatnam, Tamil Nadu, India who basically from Egyptian origin. All participants had a comprehensive ocular evaluation (367 study subjects, including 22 POAG and 20 Suspects). We performed WES for 16 samples (9 POAG and 7 controls). We identified one novel potential pathogenic variants, with low-frequency and several pathogenic variants. The heterozygous pathogenic variant c.G3719A in the RPGR-interacting domain of RPGRIP1 gene is segregated in six POAG cases, which may affect the function of RPGR protein complex. In contrast, the RPGRIP1 variant (G1240E) is relatively common in most populations especially in Africans. Furthermore, we identified a novel c.A1295G variant in Rho guanine nucleotide exchange factors Gene 40 (ARHGEF40) and in Retinitis Pigmentosa GTPase regulator (RPGR) gene, may affect the intraocular pressure regulation by altering the RhoA signaling pathway through RPGR protein complex. Moreover, it is difficult to determine the population frequency for this variant. Even though our study reports rare pathogenic variants in multiple genes and pathways associated in the large family with POAG, epigenetic changes and copy number variations may explored to understand the incredibly complexity of the POAG pathogenesis.

Key words: Whole Exome Sequencing, Large Family with Primary Open Angle Glaucoma, Pathogenic Variants, RPGRIP1 and ARHGEF40 gene.
1. Introduction

Primary open angle glaucoma (POAG) is a leading cause of irreversible blindness that affects approximately 60.5 million people worldwide with a worrisome exponential increase. Glaucoma is a well-known second-leading cause of global irreversible blindness after cataract (Resnikoff, et al. 2004). Due to the rapid increase in aging population worldwide, this number is expected to rise to 80 million by 2020 (Quigley and Broman 2006) and 111.8 million people by 2040 compared with 2013 inexplicably affecting people belonging to Asia and Africa (Tham, et al. 2014). Asia alone accounted for approximately 60% of the world’s glaucoma cases, and Africa had the second highest number of glaucoma cases with 8.3 million (13%). In India, glaucoma affects 12 million people; this figure is predicted to increase up to 16 million by 2020 (Thylefors, et al. 1995, Vijaya, et al. 2008).

Many risk factors for POAG include advanced age, central corneal thickness, myopia, steroid responsiveness and elevated intraocular pressure (IOP) is associated with this disease (Fingert 2011). However, these risk factors do not capture the full spectrum of the disease. Though, the positive family history is also one of the risk factors for POAG; family-based studies have been useful in discovering candidate genes (MYOC, OPTN, and TBK1) (Fingert 2011, Rezaie, et al. 2002, Stone, et al. 1997) that are capable of causing POAG. These candidate genes were discovered through large pedigrees with a positive family history of glaucoma. In addition, the previous studies have shown that POAG development is associated with various genetic risk factors, including variants of genes with different functions, such as CDKN2B-AS (Nakano, et al. 2012, Osman, et al. 2018, Ramdas, et al. 2011, Wiggs 2012) CAV1/CAV2 (Thorleifsson, et al. 2010), TMCO1 (Koolwijk, et al. 2016) AFAP1 (Gharahkhani, et al. 2014), TXNRD2, FOXC1/GMDS, ATXN2 (Cooke Bailey, et al. 2014).
2016), FNDC3B (Hysi, et al. 2014, Liu, et al. 2013), GAS (Koolwijk, et al. 2016), PMM2 (Chen, et al. 2014), TGFBR3 (Li, et al. 2015), and SIX1/SIX6 (Osman, et al. 2018, Ramdas, et al. 2011). Therefore, understanding its genetic causes is thus of prime socio-economic importance.

Earlier, we have reported that known candidate gene variants screening in a single large south Indian family with POAG history failed to detect genetic risk factors (abdulkader, et al. 2016). Therefore, we have performed whole exome sequencing (WES), coding all the exonic regions of the human genome, for sixteen samples including nine POAG and seven unaffected family members a large five generation of South Indian family.

2. Materials and Methods

2.1 POAG subjects and controls

The study was approved by the Institutional Review Board at the Aravind Eye Care System, Madurai, Tamil Nadu, India (IRB2011008BAS). The research followed the tenets of the Declaration of Helsinki. Study subjects were screened as previously described (Abdulkader, et al. 2016). Briefly, the study subjects were recruited from a large five generation of South Indian family at Kayalpatanam region, Tamil Nadu, India. After informed consent, a brief medical history was obtained from each study subject that included information regarding demographics, history of systemic disease, and ocular history after informed consent. The study subjects were clinically examined using slit-lamp, pachymetry, optic disc examination with 90 D lens, and applanation tonometry. Additional examinations including standard automated perimetry with a Humphrey Visual Field Analyzer (Zeiss-Humphrey Systems, Dublin, CA) using SITA 24-2 and 10-2 algorithms were performed for suspected glaucoma subjects at the Aravind Eye Care System and Institute of Ophthalmology at Tirunelveli, Tamil Nadu.
Subject with open angles on gonioscopy, glaucomatous optic disc cupping, and consistent visual field defects but not necessarily elevated IOP were characterized as POAG, as previously described (abdul kader, et al. 2016). The study subjects who did not fulfill the above conditions for POAG but still displayed suspicious characteristics like ocular hypertension, suspicious visual fields, or dubious optic discs were characterized as glaucoma suspects. Study subjects were excluded if they were diagnosed with a secondary cause of glaucoma such as ocular surgery, developmental abnormalities, exfoliation syndrome, inflammation, ocular trauma, and pigment dispersion syndrome.

2.2 Sample preparation and Selection

We collected 5 ml of peripheral blood in anticoagulant (EDTA) coated tube from each study subjects. The collected samples were stored at 4°C. Within 12 hours of collection, the genomic DNA was extracted by using a salting-out precipitation method (Miller, et al. 1988). The isolated DNA samples were quantified. Sixteen samples were selected based on their relationship with proband to perform whole exome sequencing.

2.3 Whole exome sequencing

Before proceeding for WES, the DNA concentration and the purity of the samples were analyzed by Nanodrop spectrophotometer. Good quality DNA had an absorbance A260/A280 ratio of 1.7–2.0; also, the samples were run on an agarose gel as a quality control before performing the WES. The ratio of absorption at 260 nm vs. 280 nm was used to determine the purity of DNA. RNA capture baits against approximately 60 Mb of the Human Exome (targeting >99% of regions in CCDS, RefSeq and Gencode databases) were used to enrich regions of interest from fragmented genomic DNA with Agilent’s SureSelect Human All Exon V6 kit. Briefly, three μg of each genomic DNA was sheared into 300-350 bp fragments by Covaris. The quality of the fragmentation
was assessed on the Agilent high sensitivity chip by using Bioanalyzer 2100. The fragmented DNA was repaired by using SureSelect XT library kit followed by the adenylation step at the 3’ end by an appropriate volume of adenylation mixture, and the paired-end adapters were ligated, and then the adaptor-ligated library was amplified. Each step was followed by purification by AMPure XP beads. Again, the quality of the library was assessed by using bioanalyzer. The prepared libraries were pooled in equal amount to give ~500 ng. The prepared genomic DNA library was then hybridized to a target-specific capture library and then captured using streptavidin-coated beads. The captured library was amplified with the indexing primers contacting 8-bp indexes and cleaned up using AMPure XP beads.

The library preparation and the WES was performed at Centogene, Germany. The generated libraries were sequenced on an Illumina HiSeq 4000 platform to obtain an average coverage depth of ~150x. Typically, ~97% of the targeted bases are covered >10x.

2.4 Data Analysis

We developed an automated pipeline (Supplementary Figure.1) for variant identification from WES data using UNIX script (https://github.com/bharani-lab/WES-pipelines/tree/master/Script). First, raw reads (FASTQ file) were processed to remove the adapter and low-quality sequences using Cutadapt. The processed reads were further aligned against the human genome build GRCh37 using BWA-mem version 0.7.12. GATK version 4.1.0. was used to identify single-nucleotide variants (SNVs) and small Insertion and Deletions (InDels) followed by annotation using ANNOVAR (Wang, et al. 2013). We first filtered rare and low-frequency variants keeping minor allele frequency (MAF) less than or equal to 0.5% in 1000genome, ESP, ExAC and genomeAD. Next, all the protein-coding variants that were either introducing or
removing of stop codon, altering transcripts (frameshift InDels), altering a canonical splice acceptor or splice donor site, and introducing an amino acid change (non-synonymous/missense variant) selected. The non-synonymous variants were further filtered as deleterious variants with two-step process; firstly, variants were selected with the conservation score >2.5 (GERP score) and CADD score greater than 10; secondly, the variants should be predicted to be deleterious with at least three prediction tools among the five (Polyphen2, SIFT, Mutation Taster, FATHMM and LRT). Also, we checked all the variants manually with the help of IGV viewer to avoid mapping errors. All predicted deleterious variants were further filtered based on their presence in at least more than three affected individuals. Finally, the variants were sorted out by their presence in number of affected individuals in the family members and their associated with glaucoma phenotype. We used VarElect software (Stelzer, et al. 2016) to sort the genes based on their direct or indirect association with a glaucoma disease.

We performed pathway and gene ontology analysis using DAVID for all the genes identified final set of variants. A gene network was created using Cytoscape with the enriched pathways and biological processes.

2.5 Confirmation of variant by Sanger sequencing

A novel variant of ARHGEF40 was confirmed by Sanger sequencing and targeted with the following primer set FW-5′-CTGAGCTGACGCCTGAACTT-3′; RV-5′-GGCCGTGGGTACTGAGAAAG-3′. Polymerase Chain Reaction (PCR) was carried out in a 50 µl reaction mixture containing 100 ng of genomic DNA, 1X buffer (PCR buffer (10 mM TRIS hydrochloride, pH 8.3; 50 mM potassium chloride; 1.5 mM magnesium chloride and 0.001% gelatin)), 0.5 pmol of each primer 200 µM of deoxynucleotide triphosphate and 1 U of Taq DNA polymerase (Sigma Aldrich). We performed the amplification in a DNA Thermal cycler (Applied Biosystems-Invitrogen)
with initial denaturation of 10 minutes at 96°C, followed by 37 cycles at 96°C for 30 seconds, annealing at 58°C for 30 seconds, and final extension at 72°C for 5 mins. The amplified DNA products were purified by QIA quick PCR purification kit method (Bio Basic Inc.,) followed by cyclic PCR. Bi-directional sequencing was performed (3130 Genetic Analyser; Applied Biosystems) and the results were compared with the reference sequence of ARHGEF40 gene using BLAST and Chromas lite (2.1) software.

3. Results

3.1 Clinical Evaluation of patients

We enrolled 84 members of the family after screening 240 family members based on their relation to proband (as seen in the Supplementary Figure 2). Among 84, 14 were diagnosed with POAG. POAG in the family has a relatively early age of onset with a mean of 50 ± 14 years and a range of 23-68 years. Maximum recorded IOP in family members ranged from 14-36 mmHg with a mean of 22.5 ± 6.5mmHg. CCT had a mean value of 529 ± 37.8 microns and cup-disc ratio ranges from 0.6 to 0.9 with a mean of 0.74 ± 0.14. Moderate to severe visual field losses were detected.

3.2 Exome Sequencing and Variant Filtering

We selected nine POAG cases and seven unaffected family members for Whole Exome Sequencing study. The human exonic regions of about 60 Mb (targeting >99% of regions in CCDS, RefSeq and Gencode databases) was enriched from fragmented genomic DNA with Agilent’s SureSelect Human All Exon V6 kit. The enriched libraries were sequenced on an Illumina HiSeq 4000 platform to obtain an average coverage depth of ~150x. The raw data were initially pre-processed and analyzed to produce a VCF file containing all the variants with annotations. Approximately 60,000 single-nucleotide variants (SNVs) and small insertion and deletion (InDels) changes were identified in each patient’s exome by comparison with the human reference build
Further, we followed stringent variant filtering and prioritization strategy (as mentioned in the methods section, Figure 1) to provide pathogenic variants in the POAG samples.

### 3.3 Pathogenic variants

We identified six pathogenic variants (5 non-synonymous, one frameshift variants) based on their co-segregated in the family (Table 1). We found a heterozygous variant c.G3719A altering amino acid (p.G1240E) with a deleterious effect might affect the Retinitis Pigmentosa GTPase regulator-interacting protein1 (RPGRIP1) gene, which showed direct association with glaucoma disease (Varlect score of 8.35). Among the WES screening, the variant was segregated in the family with the phenotype (Supplementary Figure 3). Also, Fernández-Martínez et al showed that mutations in the RPGRIP1 gene might cause or increase the susceptibility to various forms of glaucoma including POAG (Fernández-Martínez, et al. 2011). Followed by RPGRIP1 gene, we found a novel variant c.A1295G (p.Q432R) in the ARHGEF40 gene, which also segregated with phenotype in the family. Further, we validated the c.A1295G (p.Q432R) variant in other family members by Sanger sequencing, and we detected in eight POAG and two unaffected family members (Supplementary Figure 2). The reaming variants were detected in OR11G2 (c.847delC p.H282fs), OR4K14 (c.A355G p.M119V), RNASE13 (c.C338T p.S113F) and OR11H12 (c.T719G p.V240G) genes.

Interestingly, all the pathogenic variants were found in the genetic loci of chromosomal location between 14q19 and 14q21.

We also identified 54 pathogenic variants in 51 genes. Of that were not co-segregated based on exome data, 52 were missense and 2 were InDel variants with frameshifting the coding region as shown in table 2. From the top list based on the glaucoma phenotype, RPGR gene variants may affect its protein partner RPGRIP1 in the RPGR
proteasome complex (Roepman, et al. 2000). The next on the list, the PLK4 gene has been reported to be involved eye abnormalities (Martin, et al. 2014) Mutations in PLK4, encoding a master regulator of centriole biogenesis, cause microcephaly, growth failure and retinopathy. Interestingly, six variants were identified as novel variants. The top variants (c.A1841T p.D614V) in neural cell adhesion molecule 1 (NCAM1) gene, was further confirmed in six POAG and two unaffected family members by Sanger sequencing. The NCAM1 has been reported to be altered in the optic nerve, which is associated with elevated intraocular pressure (Ricard, et al. 2000).

**3.4 Functional network analysis**

To investigate the pathways and the biological processes involved in the glaucoma pathogenesis, we constructed a functional network of all genes identified with pathogenic variants. Initially, DAVID database was used to integrate all genes with KEGG pathways and Gene Ontology (GO) process. In total, 60 genes submitted were significantly enriched into three pathways, and 17 GO biological processes (P < 0.01). These pathways are Focal adhesion, ECM-receptor interaction and PI3K-Akt signaling pathway. Further in the Gene-functional network (Figure. 2), NCAM1, LAMB4 and PDGFRA genes connected all the three pathways to other GO process. Of these genes, NCAM1 was shown to be connected to the top gene list RPGRIP1 and ARHGEF40 with pathogenic variants through RPGR protein interaction, and GO processes of positive regulation of GTPase activity and visual perception.

**4. Discussion**

Due to the late onset of disease, recruiting families with POAG was a challenging assignment. With the support of study subjects, we have recruited a large South Indian family with 240 participants. We clinically characterized 22 members as POAG and 20 members as POAG suspect; the rest of the members are unaffected and considered as
controls. Earlier, we have reported the clinical characterization and mutational screening of reported POAG gene of these five generational south Indian family (abdul kader, et al. 2016). We have shown that the reported POAG gene screening failed to detect pathogenic variants in the family through conventional Sanger Sequencing (abdul kader, et al. 2016) method. Therefore, in this study, we have performed whole exome sequencing (WES) of family members with POAG and unaffected. To perform WES, we have selected nine POAG cases and seven unaffected family members based on their relation to the proband (III-2). More than sixty thousands of SNVs and InDels were detected in each family members. Following low population frequency analysis and pathogenic predictions using several bioinformatics tools (Figure 1), several potential variants were detected. Further, we have prioritized the variants based on their segregation and their associated with phenotype. This filtering led us to identify six pathogenic variants including five non-synonymous in ARHGEF40, RPGRIP1, OR4K14, RNASE13 and OR11H12, and one frameshift InDel in OR11G2 gene. All these pathogenic variants were present in the chromosome 14q, which has previously been reported to have potential POAG loci (Fan, et al. 2011, Osman, et al. 2018, Wiggs, et al. 2000). In addition, we have shown that fifty-four variants including fifty-one non-synonymous and two frameshift InDel.

In this study, we used phenotype sorting tool to sort the variants that directly or indirectly associated with the glaucoma phenotype. The pathogenic variant in retinitis pigmentosa GTPase regulator-interacting protein 1 (RPGRIP1) gene is observed with highest phenotype score and existed in six POAG cases, suggesting that it may play an important role in POAG. Fernandez-Martinez et al., has shown that the heterozygous non-synonymous variants in C2 domain of RPGRIP1gene might cause the various forms of glaucoma including POAG (Fernandez-Martinez, et al. 2011). Further, they
have shown that RPGRIP1 interaction with NPHP4 protein plays an important role in the pathogenesis of glaucoma (Fernández-Martínez, et al. 2011). In this study, the heterozygous non-synonymous variant is detected in the RPGR-interacting domain of RPGRIP1. This is in contrast to the previous study (Fernández-Martínez, et al. 2011) and also all homozygous or compound heterozygous variants detected in RPGRIP1 that are associated to photoreceptor dystrophies (Booij, et al. 2005, Dryja, et al. 2001). Interestingly, we observed a pathogenic variant in RPGR gene, which is existed in four POAG cases. RPGRIP1 and its interacting partner RPGR, have been shown to express in human retina and also outside of the retina (Castagnet, et al. 2003, Ferreira 2005, Mavlyutov, et al. 2002, Roepman, et al. 2000) may regulate cilia genesis, maintenance, and function mainly through signalling pathways (Patnaik, et al. 2015). Luo et al., 2014 has reported that the primary cilia of trabecular meshwork (TM) mediates intraocular pressure sensation through signaling pathway in the eye, and further highlighted that defect in the signaling pathway leads to Lowe syndrome that developed congenital glaucoma at birth (Luo, et al. 2014). RPGR and its protein partners play an important role in actin cytoskeleton remodeling of cilia through these signaling pathways by activating the small GTPase, RhoA (Gakovic, et al. 2011).

In the current study, we have identified one novel pathogenic variant in Rho guanine nucleotide exchange factor 40 (ARHGEF40) gene using WES data. Further, the variant was confirmed in the family members (Supplementary Figure 3). Study show that Rho guanine nucleotide exchange factors Gene Family protein (ARHGEF12) has been implicated as a risk factor of glaucoma by increasing intraocular pressure through RhoA/RhoA kinase pathway (Abiko, et al. 2015). Furthermore, the activation of the Rho/ROCK pathway results in trabecular meshwork (TM) contraction, and the inhibition of this pathway would aggravate relaxation of TM with a consequent increase
in outflow facility and, thereby, decrease intraocular pressure (Wang and Li 2010). In the present study, we speculate that ARGHEF40 variant may affect the RhoA signaling through RPGRIP1 and its interacting partner RPGR in actin cytoskeleton remodeling of trabecular meshwork (TM) cilia, which may subsequently increase the intraocular pressure.

The pathogenic variants detected in other genes have not been reported to be directly associated with POAG. Therefore, we constructed a network of genes using GO and pathway enrichment. We have shown three pathways Focal adhesion, ECM-receptor interaction and PI3K-Akt signaling pathway to be associated with the pathogenesis of POAG. Furthermore, the highlighted genes ARHGEF40, RPGRIP1 and RPGR were enriched through visual perception and positive regulation of GTPase activity. Intriguingly, the genes NCAM1, HSP1 and PDGFRA including ARHGEF40 and RPGR in the biological process of positive regulation of GTPase activity is prioritized as top pathogenic variants based on the phenotype score. A study has shown that neural cell adhesion molecule (NCAM) participate in the optic nerve changes associated with elevated intraocular pressure (Ricard, et al. 2000).

5. Conclusion

Altogether, this study provides an panel of pathogenic variants in multiple genes, and the interaction of these genes may directly or indirectly be associated with pathogenesis of POAG in the five generational South Indian family. Although this study needs a larger sample size to confirm the results, this study supports the idea of genetic heterogeneity in POAG.

Funding details:

This research was supported by Indian Council of Medical Research, Government of
India (2012-0383/F1)

Acknowledgments

We thank all the patients and their families for their participation in this study. We are grateful to the Indian Council of Medical Research (ICMR) for their financial support. We are also thankful to Mr. Saravanan, Mrs. Kalarani and Mrs. Muthu Selvi for helping us in recruiting the samples and maintaining the clinical data in our lab.

Ethics approval and consent to participate

The study adhered to the tenets of the Declaration of Helsinki, and ethics committee approval was obtained from the Institutional Review Board of the Aravind Eye Care System (IRB2011008BAS). All study participants read and signed informed consent after explaining the nature and possible significances of the study.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Author’s contributions

Mohd Hussain Shah done the sample preparation, sanger sequencing and wrote the manuscript. Manojkumar Kumaran analysed the whole exome sequencing data and wrote the manuscript. Periasamy Sundaresan and Bharanidharan Devarajan designed the work and co-wrote the manuscript. Mohideen Abdul Kader, R Ramakrishnan and Subbiah R Krishnadas assisted in clinical diagnosis and sample collection. All authors read and approved the final manuscript.

Availability of data and material

The pipeline lines used for analysis in this study and a detailed tutorial is openly available for the public at

(https://github.com/bharani-lab/Wole-Exome-Analysis-Pipeline).
We have submitted the data that support the finding of this study to SRA project ID PRJNA555016. Data can be accessed upon request.

Supportive/Supplementary Materials

Figure Legends

Figure 1. Work Flow for variant prioritization

Figure 2. Functional network enriched with pathways and Gene Ontology (GO) on genes identified with pathogenic variants. Hexagon represents the gene; rectangle represent pathways and Diamond represents GO.

Table Legends

Table 1. List of the Pathogenic variants co-segregated with phenotype. Varlect score with symbol † represents the direct association with glaucoma phenotypes and ‡ represent the indirect association. * represent the Novel variant.

Table 2. List of pathogenic variants. Varlect score with symbol † represents the direct association with glaucoma phenotypes, and ‡ represent the indirect association. * represent the Novel variant.

Supplementary Figure Legends

Supplementary Figure 1. Modular Pipeline

Supplementary Figure 2. Pedigree from south India Family. Family members diagnosed with POAG are shaded with black.

Supplementary Figure 3. Pedigree of selected family members from large south India family as shown in supplementary figure 1 (A). Sanger sequencing results of novel variant c.A1295G in ARGEF40 gene (marked with down arrow). The variant is detected in the family members II-2, III-1, III-2 and III-3 (B).
Periasamy Sundaresan - https://orcid.org/0000-0002-0599-8653
Bharanidharan Devarajan - https://orcid.org/0000-0002-2193-4609
Manojkumar Kumaran - https://orcid.org/0000-0003-0255-6144

Reference

abdul kader M, Namburi P, Ramugade S, Ramakrishnan R, Krishnadas S, Roos B, Periasamy S, Robin A, Fingert J (2016) Clinical and genetic characterization of a large primary open angle glaucoma pedigree. Ophthalmic Genetics 38: 1-4 doi: 10.1080/13816810.2016.1193883

Abiko H, Fujiwara S, Ohashi K, Hiatari R, Mashiko T, Sakamoto N, Sato M, Mizuno K (2015) Rho-guanine nucleotide exchange factors involved in cyclic stretch-induced reorientation of vascular endothelial cells. Journal of cell science 12810.1242/jcs.157503

Booij J, Florijn R, ten Brink J, Loves W, Meire F, van Schooneveld M, Jong P, Bergen A (2005) Identification of mutations in the AIPL1, CRB1, GUCY2D, RPE65, and RPGRIP1 genes in patients with juvenile retinitis pigmentosa. Journal of medical genetics 42: e67 doi: 10.1136/jmg.2005.035121

Castagnet P, Mavlyutov T, Cai Y, Zhong F, Ferreira P (2003) RPGRIP1s with distinct neuronal localization and biochemical properties associate selectively with RanBP2 in amacrine neurons. Human molecular genetics 12: 1847-1863 doi: 10.1093/hmg/ddg202

Chen Y, Lin Y, Vithana E, Jia L, Zuo X, Wong TY, Chen LJ, Zhu X, Tam P, Gong B, Qian S, Li Z, Liu X, Baskaran M, Luo Q, Guzman C, Leung C, Li X, Cao W, Yang Z (2014) Common variants near ABCA1 and in PMM2 are associated with primary open-angle glaucoma. Nature genetics 4610.1038/ng.3078

Cooke Bailey J, Loomis S, Kang J, Allingham RR, Gharakhhani P, Khor CC, Burdon K, Aschard H, Chasman D, Igo R, Hysi P, Glastonbury C, Ashley-Koch A, Brilliant M, Brown A, Budenz D, Buil A, Chen P, Choi H, Wiggs J (2016) Genome-wide association analysis identifies TXNRD2, ATXN2 and FOXC1 as
susceptibility loci for primary open-angle glaucoma. Nature genetics 4810.1038/ng.3482

Dryja T, Adams S, Grimsby J, McGee T, Hong DH, Li T, Andréasson S, Berson E (2001) Null RPGRIP1 alleles in patients with Leber congenital amaurosis. American journal of human genetics 68: 1295-1298 doi: 10.1086/320113

Fan BJ, Wang D, Pasquale L, Haines J, Wiggs J (2011) Genetic Variants Associated with Optic Nerve Vertical Cup-to-Disc Ratio Are Risk Factors for Primary Open Angle Glaucoma in a US Caucasian Population. Investigative ophthalmology & visual science 52: 1788-1792 doi: 10.1167/iovs.10-6339

Fernández-Martínez L, Letteboer S, Mardin C, Weisschuh N, Gramer E, Weber B, Rautenstrauss B, Ferreira P, Kruse F, Reis A, Roepman R, Pasutto F (2011) Evidence for RPGRIP1 gene as risk factor for primary open angle glaucoma. European journal of human genetics : EJHG 19: 445-451 doi: 10.1038/ejhg.2010.217

Ferreira P (2005) Identification of Novel Murine- and Human-Specific RPGRIP1 Splice Variants with Distinct Expression Profiles and Subcellular Localization. Investigative ophthalmology & visual science 46: 1882-1890 doi: 10.1167/iovs.04-1286

Fingert J (2011) Primary open-angle glaucoma genes. Eye (London, England) 25: 587-595 doi: 10.1038/eye.2011.97

Gakovic M, Shu X, Kasioulis I, Carpanini S, Moraga I, Wright A (2011) The role of RPGR in cilia formation and actin stability. Human molecular genetics 20: 4840-4850 doi: 10.1093/hmg/ddr423

Gharahkhani P, Burdon K, Fogarty R, Sharma S, Hewitt A, Martin S, Law M, Cremin K, Cooke Bailey J, Loomis S, Pasquale L, Haines J, Hauser M, Viswanathan A, McGuffin P, Topouzis F, Foster P, Graham S, Casson R, Craig J (2014) Common variants near ABCA1, AFAP1 and GMDS confer risk of primary open-angle glaucoma. Nature genetics 4610.1038/ng.3079
Hysi P, Cheng C-y, Springelkamp H, Macgregor S, Cooke Bailey J, Wojciechowski R, Vitart V, Nag A, Hewitt A, Hoehn R, Venturini C, Mirshahi A, Ramdas W, Thorleifsson G, Vithana E, Khor CC, Stefansson A, Liao J, Haines J, Aung T (2014) Genome-wide analysis of multiethnic cohorts identifies new loci influencing intraocular pressure and susceptibility to glaucoma. Nature genetics 46:10.1038/ng.3087

Koolwijk L, Ramdas W, Ikram K, Jansonius N, Pasutto F, Hys P, Macgregor S, Janssen S, Hewitt A, Viswanathan A, ten Brink J, Hosseini S, Amin N, Despriet D, Willemse-Assink J, Kramer R, Rivadeneira F, Struchalin M, Aulchenko Y, Duijn C (2016) Common Genetic Determinants of Intraocular Pressure and Primary Open-Angle Glaucoma.

Li Z, Allingham RR, Nakano M, Jia L, Chen Y, Ikeda Y, Baskaran M, Chen L-J, Kee C, Garway-Heath D, Sripriya S, Fuse N, Abu-Amero K, Huang C, Namburi P, Burdon K, Perera S, Gharakhhani P, Lin Y, Vithana E (2015) A common variant near TGFBR3 is associated with primary open angle glaucoma. Human Molecular Genetics 24: 3880-3892 doi: 10.1093/hmg/ddv128

Liu Y, Vitart V, Burdon K, Khor CC, Bykhovskaya Y, Mirshahi A, Aw H, Koehn D, Pg H, Ramdas W, Zeller T, En V, Bk C, Tay WT, Tai ES, Cheng C-y, Liu J, Foo JN, Saw S-M, Wong TY (2013) Genome-wide association analyses identify multiple loci associated with central corneal thickness and keratoconus. Nature Genetics

Luo N, Conwell M, Chen X, Insinna C, Westlake C, Cantor L, Wells C, Weinreb R, Corson T, Spandau D, Joos K, Iomini C, Obukhov A, Sun Y (2014) Primary cilia signaling mediates intraocular pressure sensation. Proceedings of the National Academy of Sciences of the United States of America 111:10.1073/pnas.1323292111

Martin C-A, Ahmad I, Klingseisen A, Hussain M, Bicknell L, Leitch A, Nürnberg G, Toliat M, Murray J, Hunt D, Ali Z, Tinschert S, Ding J, Keith C, Harley M, Heyn P, Müller R, Hoffmann I, Jackson A (2014) Mutations in PLK4, encoding a master regulator of centriole biogenesis, cause microcephaly, growth failure...
Mavlyutov T, Zhao H, Ferreira P (2002) Species-specific subcellular localization of RPGR and RPGRIP isoforms: Implications for the phenotypic variability of congenital retinopathies among species. Human molecular genetics 11: 1899-1907 doi:

Miller SA, Dykes D, Polesky H (1988) Miller SA, Dykes DD, Polesky HF. A simple salting-out procedure for extracting DNA from nucleated cells. Nucleic Acids Res 16: 1215. Nucleic acids research 16: 1215 doi: 10.1093/nar/16.3.1215

Nakano M, Ikeda Y, Tokuda Y, Fuwa M, Omi N, Ueno M, Imai K, Adachi H, Kageyama M, Mori K, Kinoshita S, Tashiro K (2012) Common Variants in CDKN2B-AS1 Associated with Optic-Nerve Vulnerability of Glaucoma Identified by Genome-Wide Association Studies in Japanese. PloS one 7: e33389 doi: 10.1371/journal.pone.0033389

Osman W, Low S-K, Takahashi A, Kubo M, Nakamura Y (2018) A genome-wide association study in the Japanese population confirms 9p21 and 14q23 as susceptibility loci for primary open angle glaucoma.

Patnaik S, Kotapati Raghupathy R, Zhang X, Mansfield D, Shu X (2015) The Role of RPGR and Its Interacting Proteins in Ciliopathies. Journal of Ophthalmology 2015: 1-10 doi: 10.1155/2015/414781

Quigley H, Broman AT (2006) The number of people with glaucoma worldwide in 2010 and 2020. The British journal of ophthalmology 90: 262-267 doi: 10.1136/bjo.2005.081224

Ramdas W, Rizopoulos D, Wolfs R, Hofman A, Jong P, Vingerling J, Jansonius N (2011) Defining Glaucomatous Optic Neuropathy from a Continuous Measure of Optic Nerve Damage – The Optimal Cut-off Point for Risk-factor Analysis in Population-based Epidemiology. Ophthalmic epidemiology 18: 211-216 doi: 10.3109/09286586.2011.595038

Resnikoff S, Pascolini D, Etya'ale D, Kocur I, Pararajasegaram R, Pokharel G, Mariotti
S (2004) Global data on visual impairment in the year 2002. Bulletin of the World Health Organization 82: 844-851 doi: 10.1590/s0042-96862004001100009

Rezaie T, Child A, Hitchings R, Brice G, Miller L, Coca-Prados M, Héon E, Krupin T, Ritch R, Kreutzer D, Crick R, Sarfarazi M (2002) Adult-Onset Primary Open-Angle Glaucoma Caused by Mutations in Optineurin. Science (New York, NY) 295: 1077-1079 doi: 10.1126/science.1066901

Ricard C, Pena J, Hernandez MR (2000) Differential expression of neural cell adhesion molecule isoforms in normal and glaucomatous human optic nerve heads. Brain research Molecular brain research 74: 69-82 doi: 10.1016/S0169-328X(99)00264-8

Roepman R, Bernoud-Hubac N, Schick D, Mauger A, Berger W, Ropers H-H, Cremers F, Ferreira P (2000) The retinitis pigmentosa GTPase regulator (RPGR) interacts with novel transport-like proteins in the outer segments of rod photoreceptors. Human Molecular Genetics 910.1093/hmg/9.14.2095

Stelzer G, Plaschkes I, Oz-Levi D, Alkelai A, Olender T, Zimmerman S, Twik M, Belinky F, Fishilevich S, Nudel R, Guan-Golan Y, Warshawsky D, Dahary D, Kohn A, Mazor Y, Kaplan S, Stein T, Baris H, Rappaport N, Lancet D (2016) VarElect: The phenotype-based variation prioritizer of the GeneCards Suite. BMC Genomics 1710.1186/s12864-016-2722-2

Stone E, Fingert J, Alward W, Nguyen T, Polansky J, Sunden S, Nishimura D, Clark A, Nystuen A, Nichols B, Mackey D, Ritch R, Kalenak J, Craven E, Sheffield V (1997) Identification of a Gene That Causes Primary Open Angle Glaucoma. Science (New York, NY) 275: 668-670 doi: 10.1126/science.275.5300.668

Tham Y-C, Li X, Wong TY, Quigley H, Aung T, Cheng C-y (2014) Global Prevalence of Glaucoma and Projections of Glaucoma Burden through 2040 A Systematic Review and Meta-Analysis. Ophthalmology 12110.1016/j.ophtha.2014.05.013

Thorleifsson G, Walters G, Hewitt A, Masson G, Helgason A, Dewan A, Sigurdsson A, Jonasdottir A, Gudjonsson S, Magnusson K, Stefansson H, Lam D, Tam P,
Gudmundsdottir G, Southgate L, Burdon K, Gottfredsdottir M, Aldred M, Mitchell P, Stefansson K (2010) Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma. Nature genetics 42: 906-909 doi: 10.1038/ng.661

Thylefors B, Négrel AD, Pararajasegaram R, Dadzie Y (1995) Global Data on Blindness. Bulletin of the World Health Organization 73: 115-121 doi:

Vijaya L, George R, Baskaran M, Arvind H, Sriram P, S Ve R, Kumaramanicakvel G, McCarty C (2008) Prevalence of Primary Open-angle Glaucoma in an Urban South Indian Population and Comparison with a Rural Population The Chennai Glaucoma Study. Ophthalmology 115: 648-654.e641 doi: 10.1016/j.ophtha.2007.04.062

Wang J, Liu X, Zhong Y (2013) Rho/Rho-associated kinase pathway in glaucoma (Review). International journal of oncology 4310.3892/ijo.2013.2100

Wang K, Li M (2010) Wang K, Li M, Hakonarson HANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nuc Acids Res 38(16): e164. Nucleic acids research 38: e164 doi: 10.1093/nar/gkq603

Wiggs J (2012) The Cell and Molecular Biology of Complex Forms of Glaucoma: Updates on Genetic, Environmental, and Epigenetic Risk Factors. Investigative ophthalmology & visual science 53: 2467-2469 doi: 10.1167/iovs.12-9483e

Wiggs J, Allingham RR, Hossain A, Kern J, Auguste J, Delbono E, Broomer B, Graham F, Hauser M, Pericak-Vance M, Haines J (2000) Genome-wide scan for adult onset primary open angle glaucoma. Human molecular genetics 9: 1109-1117 doi: 10.1093/hmg/9.7.1109
| Chromosome Position | Accession number | Nucleotide changes | Gene Name   | Amino acid change | dbSNP       | Varlect | Number of cases (sample ID) |
|---------------------|------------------|--------------------|-------------|-------------------|-------------|---------|-----------------------------|
| 14:21816432         | NM_020366.3      | c.G3719A           | RPGRIP1     | p.G1240E          | rs34725281  | 8.35†   | 6 (III-3;III-2;II-2;III-32;IV-26;IV-27) |
| 14:21550588         | NM_001278529.2   | c.A1295G           | ARHGEF40*   | p.Q432R           | .           | 1.59‡   | 6 (III-3;III-2;II-2;III-32;IV-26;IV-27) |
| 14:20666340         | NM_001005503.1   | c.847delC          | OR11G2      | p.H282fs          | rs528205284 | 0.99‡   | 6 (III-3;III-2;II-2;III-32;IV-26;IV-27) |
| 14:20482998         | NM_001004712.1   | c.A355G            | OR4K14      | p.M119V           | rs7157076   | 0.95‡   | 6 (III-3;III-2;II-2;III-32;IV-26;IV-27) |
| 14:21502110         | NM_00101264.4    | c.C338T            | RNASE13     | p.S113F           | rs114504351 | 0.71‡   | 6 (III-3;III-2;II-2;III-32;IV-26;IV-27) |
| 14:19378312         | NM_001013354.1   | c.T719G            | OR11H12     | p.V240G           | rs61969158  | 0.22‡   | 6 (III-3;III-2;II-2;III-32;IV-26;IV-27) |

**Table 1.** List of the Pathogenic variants co-segregated with phenotype. Varlect score with symbol † represents the direct association with glaucoma phenotypes and ‡ represent the indirect association. * represent the Novel variant.
| Chromosome Position | Accession number | Nucleotide changes | Gene Name | Amino acid change | dbSNP     | Varlect | Number of cases (sample ID) | Number of controls (sample ID) |
|---------------------|------------------|-------------------|-----------|-------------------|-----------|---------|-----------------------------|--------------------------------|
| X:38144822          | NM_001034853.2   | c.G3430A          | RPGR      | p.V1144I          | rs12688514| 9.02†   | 4 (III-3;II-2;II-15)         | 1 (III-41)                     |
| 4:128816154         | NM_001190799.2   | c.C2513T          | PLK4      | p.T838I           | rs557954721| 6.01†   | 6 (III-3;II-2;III-32;IV-26;IV-27;II-15) | 1 (III-4)                     |
| 14:45605338         | NM_001308133.2   | c.C104T           | FANCM*    | p.P35L            |           | 4.84‡   | 6 (III-3;II-2;III-32;IV-26;IV-27;II-15) | 1 (III-4)                     |
| 6:56394545          | NM_015548.5      | c.A9427G          | DST       | p.N3143D          | rs530170321| 4.2‡    | 6 (III-3;II-2;II-5;II-15;III-32;IV-27) | 2 (III-4;III-16)               |
| 13:10175992         | NM_001350750.2   | c.A2408G          | NALCN     | p.Y803C           | rs549182297| 4.11†   | 8 (III-3;II-2;II-5;III-19;III-32;IV-27;II-15) | 3 (III-41;III-4IV-10)          |
| 1:145534254         | NM_001303041.1   | c.A1330G          | ITGA10    | p.T444A           | rs782732004| 3.98‡   | 3 (III-3;II-2;II-2)          | 1 (III-4)                     |
| 1:145541806         | NM_001303041.1   | c.T2900C          | ITGA10*   | p.L967P           |           | 3.98‡   | 3 (III-3;II-2;II-2)          | 1 (III-4)                     |
| 2:179528378         | NM_001267550.2   | c.G36508A         | TTN       | p.E12170K         | rs2163008  | 3.78‡   | 4 (III-3;II-2;II-2;III-32)    | 1 (IV-28)                     |
| 19:55350963         | NM_001281971.2   | c.509_510insCCC   | KIR2DS4   | p.S151fs          | rs551456772| 3.56†   | 5 (III-3;II-5;III-32;IV-26;IV-27) | 2 (IV-11;III-4)               |
| 4:55147769          | NM_001347827.2   | c.C2345T          | PDGFRA    | p.T782M           | rs2291591  | 3.56†   | 4 (III-32;IV-26;IV-27;II-15)  | 1 (IV-28)                     |
| Genomic Position | Gene Symbol | Transcript | Mutation | Protein Change | rs | Distance from Reference Position | Haplotype | Distance from Reference Haplotype |
|------------------|-------------|------------|----------|---------------|----|-------------------------------|-----------|---------------------------------|
| 4:141483476      | NM_021833.5 | c.C680T    | UCP1     | p.T227I       | rs148598275 | 3.51‡                         | (III-3;III-2;III-32;IV-26;IV-27;II-15) | 1 (III-4) |
| 1:175046835      | NM_022093.2 | c.C281T    | TNN      | p.T94M        | rs41266080  | 3.23‡                         | (III-3;III-2;II-5;II-15) | 2 (III-41;III-34) |
| 3:141526640      | NM_139209.2 | c.G1204A   | GRK7     | p.D402N       | rs150840377 | 3.21‡                         | (III-3;II-15) | 4 (III-34;IV-11;IV-28;III-41) |
| 5:33951693       | NM_001012509.4 | c.G1204A   | GRK7     | p.D402N       | rs150840377 | 3.21‡                         | (III-3;II-15) | 4 (III-34;IV-11;IV-28;III-41) |
| 11:113126641     | NM_000615.7  | c.A1841T   | NCAM1*   | p.D614V       | .            | 3.16†                         | (III-3;III-2;III-32;IV-26;IV-27;II-15) | 2 (IV-28;III-4) |
| 19:45853924      | NM_177417.3  | c.C1298T   | KLC3*    | p.S433F       | .            | 3.05‡                         | (III-3;III-2;II-5;IV-27;III-32;IV-26) | 3 (IV-28;III-4;III-16) |
| 19:50752298      | NM_024729.3  | c.T1360G   | MYH14    | p.W454G       | rs572234218  | 2.94‡                         | (III-3;III-2;II-5;IV-27;III-32;IV-26) | 3 (IV-28;III-16;III-4) |
| 1:209791929      | NM_001318046.2 | c.C2777A  | LAMB3    | p.A926D       | rs2076222   | 2.6‡                          | (III-3;III-2;II-5;III-32;IV-26;IV-27;III-19) | 7 (III-11;III-4;IV-10) |
| 10:100189242     | NM_001322492.1 | c.C808T    | HPS1     | p.P270S       | rs34533614  | 2.55‡                         | (III-3;III-2;II-2) | 1 (III-4) |
| 10:100202987     | NM_000195.5  | c.T11C     | HPS1     | p.V4A         | rs58548334  | 2.55†                         | (III-3;III-2;II-5;IV-26;V-27;II-15) | 3 (III-41;III-34;IV-28) |
| 10:97192237      | NM_001034957.1 | c.C173T    | SORBS1   | p.P58L        | rs200179325 | 2.49‡                         | (II-5;III-32;IV-26;IV-27;II-15;III-19) | 1 (IV-10) |

*preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.*

https://doi.org/10.1101/2020.09.21.306191
| Chromosome | Gene Symbol | HGVS Change | Protein Change | Reference SNP | odds Ratio | Cosegregation | Genotype |
|------------|-------------|--------------|---------------|--------------|------------|--------------|----------|
| 7:107746432 | NM_001350531.2 | c.C700T | LAMB4 p.H234Y | rs2074749 | 2.47‡ | 8 (III-3;III-2;II-5;III-32;IV-26;IV-27;II-15;III-19) | 2 (III-41;III-16) |
| 10:90530612 | NM_001289967.1 | c.G683C | LIPN p.G228A | rs201135817 | 2.33‡ | 9 (III-3;III-2;II-2;II-5;II-15;III-32;IV-26) | 4 (III-16;IV-28;IV-27;III-41;III-19;III-4) |
| 3:130361856 | NM_001102608.2 | c.G5216A | COL6A6 p.R1739Q | rs16830494 | 2.17‡ | 6 (III-3;III-2;II-5;III-32;IV-26;IV-27) | 3 (III-4;III-16;IV-28) |
| 19:44571252 | NM_013361.6 | c.1506dup | ZNF223 p.R424fs | rs56259351 | 2.05‡ | 7 (III-3;III-2;II-5;III-32;IV-26;II-15;III-19) | 3 (IV-11;III-4;III-16) |
| 5:90016871 | NM_032119.4 | c.G9743A | ADGRV1 p.G3248D | rs16869032 | 2.05‡ | 8 (III-3;III-2;II-5;III-32;IV-26;II-15;III-19) | 4 (III-16;IV-28;IV-27;III-41;III-34) |
| 10:73434888 | NM_001171930.2 | c.G1469C | CDH23 p.G490A | rs1227049 | 2.05‡ | 6 (III-3;III-2;II-5;III-32;IV-27;III-19) | 2 (III-34;III-16;) |
| 10:71160787 | NM_001322367.1 | c.C2554G | HK1* p.P852A | . | 2.05‡ | 6 (III-3;III-32;IV-26;II-15;III-2;II-5) | 2 (III-16;III-4) |
| 5:96117554 | NM_001040458.3 | c.C2290T | ERAP1* p.P764S | . | 1.95‡ | 7 (III-3;III-2;II-2;II-5;IV-26;IV-27;III-19) | 0 () |
| 3:182788862 | NM_001293273.1 | c.A335G | MCCC1 p.E112G | rs142629318 | 1.83‡ | 6 (III-3;III-32;IV-26;II-15;II-5) | 2 (III-16;III-4) |
| 3:179408072 | NM_003940.3 | c.A338G | USP13 p.N113S | rs771971543 | 1.78‡ | 7 (III-3;III-2;II-5;III-32;IV-26;IV-27;II-15) | 3 (III-4;III-16;IV-28) |
| Chromosome | Gene Accession | SNP | Gene Symbol | Protein Alteration | rs ID | p-Value | Risk Allele | Peptide Positions | Associated Genotypes |
|------------|----------------|-----|-------------|--------------------|-------|---------|-------------|------------------|---------------------|
| 4:141483476 | NM_021833.5    | c.C680T | TMEM63B    | p.R82P             | rs371238478 | 1.69‡    | 6 (III-3;II-5;III-32;IV-26;IV-27;II-15) | 3 (III-4;IV-10;III-16) |
| 19:40363916 | NM_003890.2    | c.G14726A | FCGBP     | p.T1975M           | rs372872173 | 1.66‡    | 6 (III-3;II-5;IV-27;III-32;IV-26) | 3 (IV-28;III-4;III-16) |
| 19:40366240 | NM_003890.2    | c.C13994T | FCGBP     | p.R4909H           | rs77005739  | 1.66‡    | 3 (III-3;II-2;IV-27) | 1 (IV-28)          |
| 3:141526640 | NM_139209.2    | c.G1204A | FCGBP     | p.R4909H           | rs62106922  | 1.66‡    | 3 (III-3;II-2;IV-27) | 1 (IV-28)          |
| 5:96117554  | NM_001040458.3 | c.C2290T | EIF2AK3    | p.S136C            | rs867529    | 1.62†    | 7 (III-3;II-5;III-32;IV-27;II-15;III-19) | 2 (III-4;III-16)    |
| 2:179528378 | NM_001267550.2 | c.G36508A | TIGD4     | p.C204F            | rs576908904 | 1.5‡     | 6 (III-3;II-5;III-32;IV-26;IV-27;IV-10) | 3 (III-16;IV-28)    |
| 15:82934639 | NM_001322400   | c.G941A  | GOLGA6L1   | p.R314H            | rs200928526 | 1.564‡   | 7 (III-3;II-5;III-32;IV-26;IV-27;II-15) | 3 (III-4;III-16;IV-28) |
| X:38144822  | NM_001034853.2 | c.G3430A | PEX5L      | p.F173C            | rs141827659 | 1.54‡    | 7 (III-3;II-5;III-32;IV-26;IV-27;II-15) | 3 (III-4;III-16;IV-28) |
| 17:39622068 | NM_001017402.1 | c.C665A  | KRT32      | p.S222Y            | rs2071561   | 1.1†     | 6 (III-3;II-3;II-32;IV-26;IV-27;II-15) | 1 (III-4)          |
| 11:10300663 | X:0 NM_001080463.2 | c.G2527A | DYNC2H1    | p.A843T            | rs548461924 | 0.97‡    | 6 (III-3;II-3;II-32;IV-26;IV-27;II-15) | 2 (III-4;IV-28)    |
| 4:128816154 | NM_001190799.2 | c.C2513T | PDZD3      | p.Q226K            | rs147147532 | 0.85‡    | 6 (III-3;II-3;II-32;IV-26;IV-27;II-15) | 0                 |
| 3:179529649 | NM_001349404.2 | c.T518G  | OR51E1     | p.A156T            | rs202113356 | 0.84‡    | 6 (III-3;II-3;II-32;II-15;IV-26;IV-27) | 3 (III-4;III-16;IV-28) |
| Chromosome | Gene Symbol | Gene Accession Number | cDNA Change | Protein Change | Variant ID | Frequency | Pedigree | Chromosome | Gene Symbol | Gene Accession Number | cDNA Change | Protein Change | Variant ID | Frequency | Pedigree |
|------------|-------------|----------------------|-------------|---------------|------------|-----------|----------|------------|-------------|-------------|------------|-------------|------------|-----------|----------|
| 11:11905866 | NM_001168468.2 | c.C676A | OR11H2 | p.P269S | rs2815979 | 0.82‡ | 6 (III-3;III-2;III-32;IV-26;IV-27;II-15) | 0 (III-4;IV-28) |
| 21:33735605 | NM_014825.3 | c.T1369A | URB1 | p.S457T | rs148292685 | 0.81‡ | 7 (III-3;II-5;III-32;IV-26;IV-27;II-15) | 3 (III-4;III-16;IV-28) |
| 11:30974115 | NM_001350255.1 | c.G1449T | DCDC1 | p.R483S | rs158633 | 0.78‡ | 6 (III-3;III-2;IV-26;IV-27;II-15;III-19) | 2 (III-41;III-34) |
| 11:31123752 | NM_001350255.1 | c.A812G | DCDC1 | p.D271G | rs183555899 | 0.78‡ | 6 (III-3;III-2;IV-26;IV-27;II-15;III-19) | 2 (III-41;III-34) |
| 19:49969382 | NM_001145396.2 | c.G1627C | ALDH16A1 | p.A543P | rs555667637 | 0.42‡ | 6 (III-3;II-5;III-32;IV-27;III-32;IV-26) | 3 (III-4;III-16;C|IV-28;C) |
| 2:88913273 | NM_004836.7 | c.C407G | EFHC1 | p.R353W | rs527295360 | 0.42‡ | 6 (III-3;III-32;IV-26;IV-27;II-15;II-5) | 2 (III-4;III-16) |
| 1:209791929 | NM_001318046.2 | c.C2777A | KLHL24 | p.D197A | rs116961268 | 0.42‡ | 7 (III-3;II-5;III-32;IV-26;IV-27;II-15) | 3 (III-4;IV-28;III-16) |
| 19:50752298 | NM_024729.3 | c.T1360G | MAGIX | p.P221L | rs781930221 | 0.28‡ | 7 (III-2;II-5;III-32;IV-26;IV-27;III-19) | 3 (III-4;III-34;III-16) |
| 5:33951693 | NM_001012509.4 | c.G1122C | PIEZO1 | p.I2265V | rs1803382 | 0.24‡ | 6 (III-3;II-5;III-32;IV-26;IV-27;II-15) | 1 (III-4) |
| 10:90530612 | NM_001289967.1 | c.G683C | LARP1B* | p.I178F | rs1803382 | 0.21‡ | 7 (III-3;II-5;III-32;IV-26;IV-27;II-15) | 3 (III-4;III-16;IV-28) |
| 6:44103070 | NM_001318792.1 | c.G245C | SSTR1 | p.T390delinSTLX | rs775405351 | 0.18† | 6 (III-3;II-5;III-32;IV-26;IV-27;II-15) | 2 (IV-28;III-16) |
Table 2. List of pathogenic variants. Varlect score with symbol † represents the direct association with glaucoma phenotypes, and ‡ represent the indirect association. * represent the Novel variant.
