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Genetic variants through exome sequencing in Spanish patients affected by primary congenital glaucoma and juvenile open-angle glaucoma

Javier Laguna,[1] Sara Labay-Tejado,[2] Míriam Potrony,[1,3] Laura Pinyol,[1] Cristina Vendrell,[4] Aurora Sánchez,[1,3] Celia Badenas,[1,3] Valeria Opazo-Toro,[5] Meritxell Jodar,[1,6] Elena Milla[2]

[1]Department of Biochemistry and Molecular Genetics, CDB, Hospital Clínic de Barcelona, Barcelona, Spain; [2]Department of Ophthalmology (ICOF), Hospital Clínic de Barcelona, Faculty of Medicine and Health Sciences, Universitat de Barcelona (UB), Barcelona, Spain; [3]CIBER of Rare Diseases, Barcelona, Spain; [4]Department of Ophthalmology, Hospital de Viladecans, Barcelona, Spain; [5]Skövde's Ophthalmology Department, Skaraborg's Hospital, Lövänsvägen, Sweden; [6]Department of Biomedicine, Faculty of Medicine and Biomedical Sciences, University of Barcelona, Barcelona, Spain

Purpose: To evaluate the genetic characteristics using whole-exome sequencing (WES), aiming to assess the potential of this approach for accurate diagnoses and to explore the genetic factors underlying these conditions.
Methods: A total of 28 patients, including 6 with primary congenital glaucoma and 22 with juvenile open-angle glaucoma, were studied. Genetic analysis involved initial Sanger sequencing for the *CYP1B1* and *MYOC* genes. WES was subsequently performed in 11 patients with negative initial results, using a panel of genes most associated with glaucoma and related ophthalmic syndromes. Variant interpretation was performed based on American College of Medical Genetics and Genomics guidelines. Segregation analysis was performed when possible.
Results: Pathogenic variants in *CYP1B1* and *MYOC* genes were identified in three patients (10%). WES identified disease-causing variants in three additional patients (27% of those with negative results from the initial testing conducted so far), all of them with syndromic features. In families where segregation analysis was possible, variants were confirmed to segregate with the clinical presentation. The diagnostic yield was 21%.
Conclusions: WES is an effective diagnostic tool for early-onset glaucoma, enhancing clinical management and genetic counseling. It supports its use in routine diagnostics to enable early detection and intervention for at-risk relatives. Further research is needed to uncover additional genetic factors and refine testing guidelines.

Glaucoma is a group of progressive optic neuropathies leading to blindness through optic disc neurodegeneration from retinal ganglion cell apoptosis [1,2]. Glaucoma is divided into early-onset glaucoma (before 40 years) and late-onset glaucoma (after 40 years). Early-onset includes primary congenital glaucoma (PCG), with neonatal (0-1 month) and infantile (1-24 months) subtypes, and juvenile open-angle glaucoma (JOAG) starting from age 3 years [3-5].

PCG and JOAG have strong genetic bases, with pathogenic variants in genes such as *CYP1B1* and *MYOC* being among the most frequently identified, making them key targets for genetic testing [5-7]. Next-generation sequencing (NGS) and whole-exome sequencing (WES) have advanced the understanding of monogenic forms of PCG and JOAG by identifying genes linked to ocular balance and intraocular pressure (IOP) regulation [8,9]. These new techniques support accurate diagnosis and genetic counseling, sometimes enabling preventive measures to avoid visual loss. However, the cause remains unknown for many families. Early detection of glaucoma is crucial, as decreasing IOP is the only current tool to halt its progression [1,10]. Suspected cases require initial treatment with IOP-lowering eye drops; if ineffective, surgery may be needed. Early management improves prognosis and may prevent surgery. The objectives of this study are to genetically evaluate Spanish patients with PCG and JOAG. In addition, it aims to assess the potential of WES to provide accurate diagnoses and to explore the genetic factors contributing to these conditions.

Correspondence to: Meritxell Jodar, Department of Biochemistry and Molecular Genetics, CDB, Hospital Clínic de Barcelona, C/ Villarroel, 170, Esc. 7 Pl. 5. 08036 Barcelona, Spain. mjodar@clinic.cat

METHODS

Patients: We studied 28 patients with PCG (6 patients) or JOAG (22 patients). Patients with angle-closure glaucoma and secondary glaucoma were excluded. The diagnostic criteria for PCG and JOAG were based on the European Glaucoma Society Guidelines [11] and the Childhood Glaucoma Research Network guidelines [12]. PCG was diagnosed in patients who had an onset before 2 years of age, presented with elevated IOP, and had a medical history of corneal

enlargement, Haab striae, and optic nerve cupping. JOAG was defined by onset after early childhood, with open angles on gonioscopy, elevated untreated IOP, glaucomatous optic nerve damage, and often a family history, frequently involving *MYOC* variants.

All patients underwent a complete ophthalmic examination, including slit-lamp evaluation of the anterior segment and IOP measurement with a Goldmann tonometer. In addition, ancillary tests were performed, such as gonioscopy, pachymetry, Humphrey visual field, and Cirrus optical coherence tomography of the optic nerve and macula. Glaucoma stage was categorized by the mean deviation (MD) from the visual field test using the Hoddap-Parrish-Anderson criteria: early (MD ≥ −6 dB), moderate (−6 dB > MD ≥ −12 dB), and severe (MD < −12 dB) stages [13]. Both eyes were taken into consideration unless there was phthisis bulbi, ocular hypertension or lack of pathology, or a missing fellow eye due to evisceration or enucleation. The investigation followed the principles established in the Declaration of Helsinki. The research protocol received approval from the ethics committee (HCB/2023/0220).

Genetic studies: Written informed consents for genetic testing were obtained from all patients and relatives when necessary. *CYP1B1* and *MYOC* genes were amplified by specific oligonucleotides (Appendix 1) and directly sequenced. WES was performed in a subset of patients who tested negative for pathogenic or likely pathogenic variants in *CYP1B1* and *MYOC*. These families were selected based on clinical and familial criteria suggestive of a stronger genetic component. Specifically, selection was guided by features such as early age of onset, high familial burden, high penetrance, a severe disease course with poor visual prognosis, the need for multiple surgical interventions, and poor response to conventional treatments. Moreover, three patients (11%) exhibited syndromic features (additional physical or developmental anomalies suggestive of an underlying syndrome).

Additionally, the Nextera Flex for Enrichment massive sequencing protocol was followed with complete exome probes (Illumina, San Diego, CA, USA) and subsequent sequencing on the NextSeq550 platform. Primary and secondary bioinformatic analysis of the data obtained was performed using the coreBM-EX-1.1.0 pipeline, developed at the Hospital Clínic de Barcelona. WES analysis relied on a virtual custom panel, including genes commonly linked to glaucoma and glaucoma-related syndromes (Appendix 2). The virtual panel of genes was assembled based on a comprehensive review of the literature and curated databases, including OMIM, Orphanet, HGMD, and PanelApp. Genes were selected for their known association with early-onset glaucoma or glaucoma-related syndromes, as well as for their biologic relevance to ocular development and IOP regulation.

The Jnomics platform was used for annotation and variant prioritization according to frequency, quality, coverage, and possible pathogenicity. A rare variant filter (allele frequency <0.5%) was applied to coding exons and flanking intronic regions (15 pb) of the analyzed exons. Untranslated regions were not analyzed. The coverage of the analyzed regions was a minimum of 20×. Furthermore, copy number variants were studied using the ExomeDepth tool [14] in Jnomics. Population allele frequencies were obtained from the gnomAD database (version 4.1.0), using general population data as the reference. Final interpretation of variants was performed based on the American College of Medical Genetics and Genomics guidelines [15], classifying variants as benign, likely benign, uncertain significance (VUS), likely pathogenic, or pathogenic.

Segregation analysis was performed in available relatives (families 1 to 5). Relatives were unavailable for families 6 and 7. Primers used are listed in Appendix 1.

RESULTS

Of the 28 patients included in the study, 22 patients had a family history of glaucoma (79%), and 25 patients had bilateral glaucoma (89%). Information about the stage of glaucoma and surgery per eye is summarized in Table 1. Pathogenic or likely pathogenic variants in *CYP1B1* and *MYOC* were identified in three patients (10%). The clinical and epidemiological characteristics of the probands from each family with genetic variants are summarized in Table 2. Since WES is newly implemented in our laboratory, it was performed on only 11 (44%) patients with negative *CYP1B1* and *MYOC* results, identifying disease-causing variants in 3 patients (27%; Table 3), all of whom had syndromic features. In addition, a VUS was found in one patient without other pathogenic or likely pathogenic variants. No copy number variants were detected.

Genetic variants were detected in four patients with PCG (one neonatal and three infantile cases) and in three patients with JOAG (Table 3). Figure 1 and Figure 2 show the detected variants and the pedigrees of each family, respectively.

Family 1: A 22-year-old woman with neonatal glaucoma (PCG) underwent goniotomy and trabeculectomy in both eyes as an infant, followed by cataract–Ahmed valve surgery in her right eye (RE). She had severe refractory glaucoma in her left eye (LE), despite medication leading to a successful Paul device implantation with satisfactory IOP control since then. Genetic testing revealed two pathogenic *CYP1B1* variants in

Table 1. Clinical features of the patients included in the study.

Various	Onset	PCG n=6 patients (10 eyes)	JOAG n=22 patients (43 eyes)
PCG subcategory	Neonatal onset	1 (17%)	-
	Infantile onset	5 (83%)	-
Gender	Female	3 (50%)	9 (41%)
	Male	3 (50%)	13 (59%)
Family history of glaucoma	Yes	2 (33%)	20 (91%)
	No	4 (67%)	2 (9%)
Eye laterality	Unilateral *	2 (33%)	1 (5%)
	Bilateral	4 (67%)	21 (95%)
Stage of glaucoma per eye	Initial	3 (30%)	19 (44%)
	Moderate	1 (10%)	10 (23%)
	Severe	6 (60%)	14 (33%)
Surgery/laser per eye	Glaucoma surgery	4 (40%)	10 (23%)
	Glaucoma surgery + Other surgery	4 (40%)	12 (28%)
	Glaucoma laser #	-	7 (16%)
	Other surgery	-	5 (12%)
	No surgery	2 (20%)	9 (21%)

JOAG: juvenile open-angle glaucoma; PCG: primary congenital glaucoma. * Unilateral because of *phthisis bulbi*, ocular hypertension, or absence of the other eye. # Iridotomy or laser trabeculoplasty

compound heterozygosity: c.840C>A (p.Cys280Ter; Figure 1A) and c.1064_1076del (p.Arg355HisfsTer69; Figure 1B), both classified as disease-causing with a low population frequency (0.001% and 0.02%, respectively). Segregation studies confirmed that the patient inherited the *CYP1B1* c.840C>A variant from her mother and the c.1064_1076del variant from her father (Figure 2A). Both parents were asymptomatic, and the brother did not carry any pathogenic variant.

Family 2: A 66-year-old woman with severe JOAG was diagnosed at age 20 years. She had a family history of glaucoma (father and siblings) and required trabeculectomy in her RE and sclerectomy in her LE (Figure 3). We identified the variant c.1139A>C (p.Asp380Ala) in heterozygosity in the *MYOC* gene (Figure 1C). A previous study showed that this variant has reduced secretion levels compared to wild-type myocilin protein [16], which has a deleterious effect on the protein function. This variant, reported in several patients with glaucoma, was detected in all affected relatives and absent in symptom-free relatives (Figure 2B), supporting its classification as pathogenic.

Family 3: A 23-year-old man was diagnosed with JOAG at age 7 years and initially managed his IOP with timolol-dorzolamide in both eyes. However, his IOP spiked to 38 mm

Table 2. Clinical and epidemiological characteristics of the probands from each family with genetic variants reported in this study.

Family	Gender	Age at diagnosis	Group	Stage of glaucoma	Eye laterality	Family history of glaucoma
1	Female	Birth	Neonatal	Severe	Bilateral	No
2	Female	20 years	Juvenile	Severe	Bilateral	Yes (father, two siblings)
3	Male	7 years	Juvenile	Moderate	Bilateral	Yes (mother, sister)
4	Male	1 year	Infantile	Severe	Bilateral	Yes (brother, father)
5	Female	4 years	Juvenile	Moderate	Bilateral	Yes (mother, brother)
7	Female	4 months	Infantile	Moderate	Bilateral	No

Table 3. Genetic variants identified in the seven families.

Family	Gene transcript	Variant/s		dbSNP ID	Frequency (gnomAD v.4.1.0)	Interpretation	ACMG criteria	Inheritance pattern
		cDNA	Protein					
1	*CYP1B1* (NM_000104.4)	c.840C>A (mat); c.1064_1076del (pat)	p.Cys280Ter (mat); p.Arg355HisfsTer69 (pat)	rs778202993; rs72549380	0.001%; 0.02%	Pathogenic; Pathogenic	PVS1, PM2, PM3; PVS1, PM2, PM3	AR
2	*MYOC* (NM_000261.2)	c.1139A>C	p.Asp380Ala	-	Not found	Pathogenic	PM2, PM5, PS3, PS4, PP1, PP3	AD
3	*MYOC* (NM_000261.2)	c.1150G>A	p.Asp384Asn	-	0.0007%	Likely pathogenic	PM2, PS4, PP1, PP3	AD
4	*FOXC1* (NM_001453.3)	c.257T>C	p.Leu86Pro	-	Not found	Pathogenic	PM2, PM5, PP1, PP3	AD
5	*COL2A1* (NM_001844.5)	c.3137dup	p.Gly1047TrpfsTer11	-	Not found	Pathogenic	PVS1, PM2, PP1	AD
	CYP1B1 (NM_000104.4)	c.1103G>A	p.Arg368His	rs79204362	0.2%	VUS	PM5, PP3	AR
6	*TRPC6* (NM_004621.5)	c.2683C>T	p.Arg895Cys	rs121434394	Not found	Likely pathogenic	PM2, PM5, PP3	AD
7	*COL18A1* (NM_001379500.1)	c.1311+5G>C	-	rs200610309	0.02%	VUS	PM2	AD

ACMG: American College of Medical Genetics and Genomics; AD: autosomal dominant; AR: autosomal recessive; dbSNP ID: Identifier for a variant in the Database of Single Nucleotide Polymorphisms; VUS: Variant of uncertain significance.

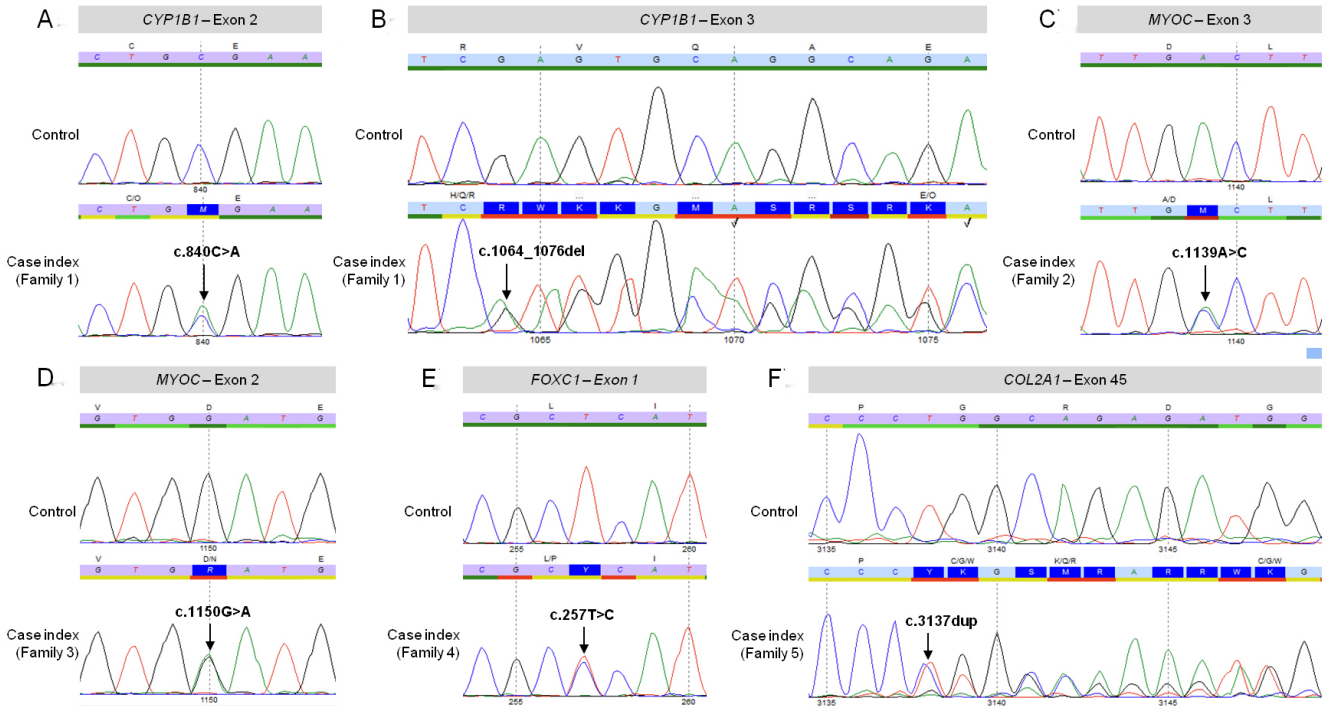

Figure 1. Chromatograms show the presence of some genetic variants detected in this study. *CYP1B1* variants were detected in the index case in family 1 (**A**, **B**). *MYOC* variants were found in the index cases in families 2 (**C**) and 3 (**D**). The *FOXC1* variant was detected in the siblings of family 4 (**E**), and the *COL2A1* variant was found in family 5 (**F**).

Hg in the RE and 34 mm Hg in the LE. Despite a preserved optic nerve head appearance (Figure 4A), an initial optic nerve fiber loss was detected (Figure 4B). Finally, the patient was scheduled for trabeculectomy in both eyes, resulting in controlled IOP without further medication. Genetic testing revealed the variant c.1150G>A (p.Asp384Asn) in heterozygosity in the *MYOC* gene (Figure 1D). This variant, with a low population frequency (0.0007%), had only been reported in Chinese affected families [17-19]. Segregation studies confirmed its presence in affected relatives and absence in healthy individuals (Figure 2C). Hence, the variant was classified as likely pathogenic. The patient's nieces (aged 5 and 2 years), while carrying the familial variant, have not shown any glaucoma manifestation to date.

Family 4: A 33-year-old man was diagnosed with infantile PCG glaucoma at age 1 year, requiring multiple interventions. He also exhibited micrognathia, dental crowding, hypertelorism, a broad forehead, impaired balance (possibly related to cerebellar vermis hypoplasia), and severely impaired visual acuity. His brother had a similar clinical presentation. NGS analysis revealed the heterozygous variant c.257T>C (p.Leu86Pro) in the *FOXC1* gene (Figure 1E), associated with Axenfeld-Rieger syndrome. This variant has not been previously described in the general population but was recently reported in a patient with glaucoma, congenital nasolacrimal duct obstruction, corectopia, posterior embryotoxon, and iridocorneal adhesions [20]. Furthermore, multiple variants at the same position (p.Leu86) had been reported, all of them causing disease [21,22]. Although the p.Leu86Pro variant results in a stable protein product, it still has the potential to disrupt FOXC1 function and severely affect the nuclear localization of the protein, affecting DNA binding [21]. Segregation analysis confirmed the variant in the brother but not in the unaffected mother, while the father was not available for the study (Figure 2D). Consequently, the variant was classified as pathogenic.

Family 5: A 36-year-old woman was diagnosed with JOAG at age 4 years. She underwent combined cataract-trabeculectomy surgery in late childhood and recently had a Paul device implanted in her LE due to glaucoma progression. The patient showed significant lordosis, leading to joint pain and bowlegs. Her mother and brother also exhibited similar clinical manifestations. The c.3137dup (p.Gly1047TrpfsTer11) variant was detected in the *COL2A1* gene (Figure 1F), associated with Stickler syndrome type I. Loss-of-function variants in the *COL2A1* gene have been considered pathogenic [23].

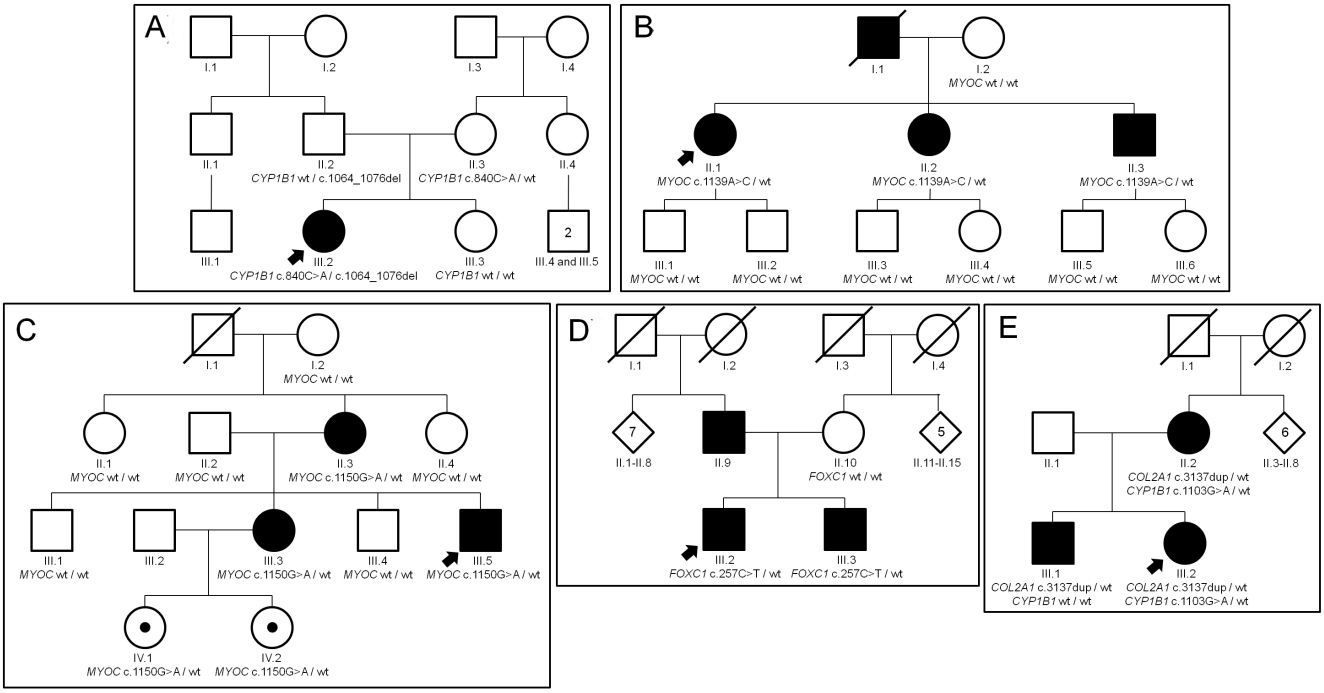

Figure 2. Representations of family trees. Family 1 (**A**), Family 2 (**B**), Family 3 (**C**), Family 4 (**D**), and Family 5 (**E**) are represented. Round symbols denote females, square symbols denote males; fully filled symbols indicate patients with congenital or juvenile glaucoma; unfilled symbols represent unaffected individuals; and symbols with a black dot inside correspond to unaffected individuals carrying genetic variants (observed only in Family 3).

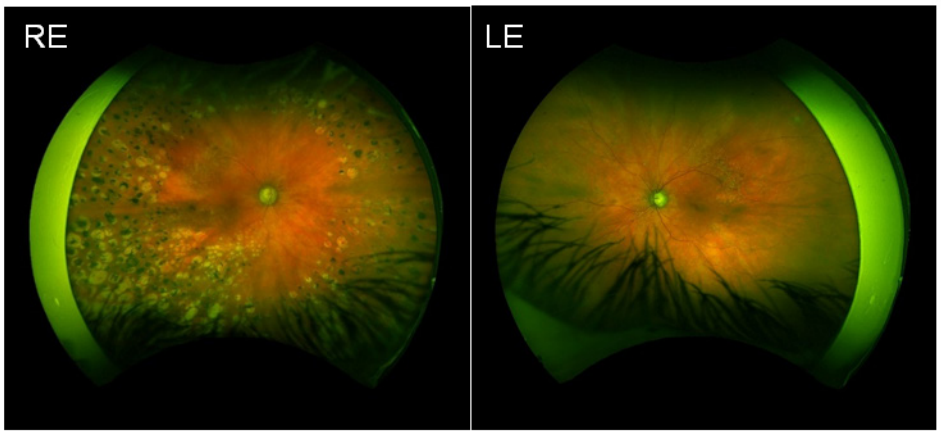

Figure 3. Fundoscopic examination of the index case in family 2 showed optic discs with well-defined margins that were somewhat pale, with optic disc cupping of 0.5 in the right eye (RE) and 0.7 in the left eye (LE). The superior neuroretinal rim was decreased in the RE and diffusely thinned in the LE. No hemorrhages were observed. These findings are consistent with moderate-to-severe glaucoma. Drusen were dispersed in the posterior pole. The RE displayed filiform vessels and had undergone panretinal photocoagulation due to a previous central retinal vein occlusion. The RE presents filiform vessels and underwent panretinal photocoagulation due to a previous central retinal vein occlusion (RE). LE, left eye; RE, right eye.

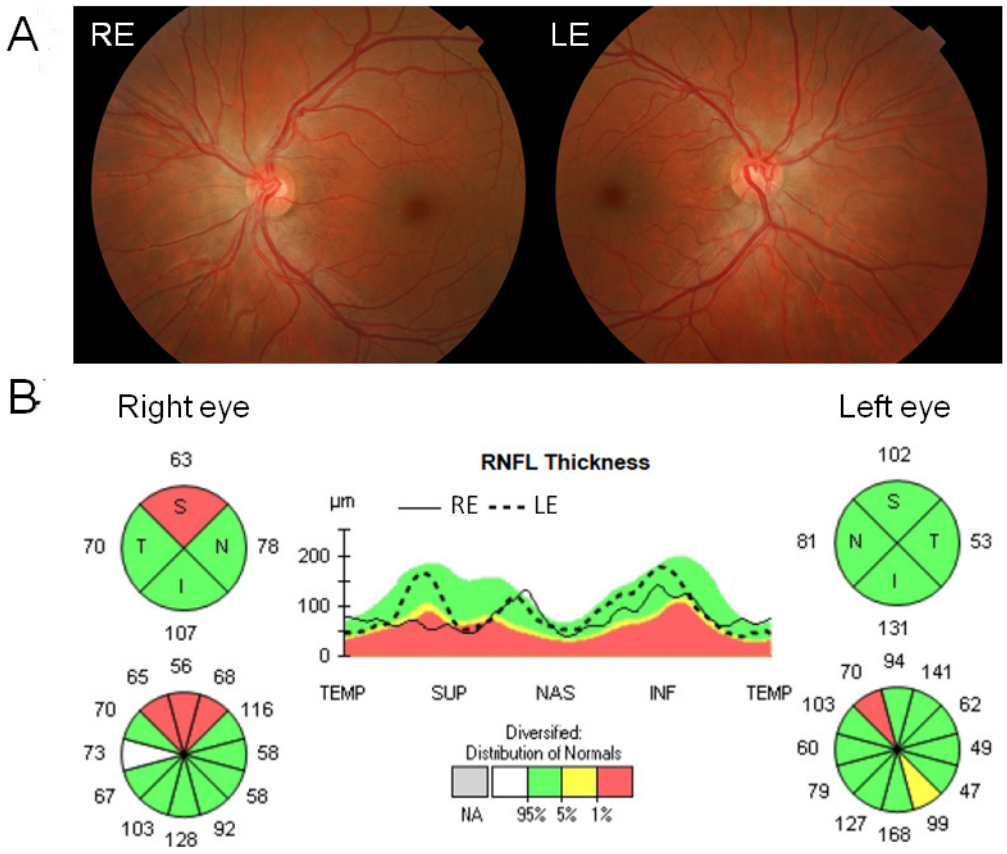

Figure 4. The figure shows the ophthalmologic findings of the index case in family 3. Optic disc photographs of the right eye (RE) and left eye (LE) show a relatively preserved neuroretinal rim in both eyes (**A**). Retinal nerve fiber layer (RNFL) analysis performed with Cirrus optical coherence tomography (OCT) reveals a superior defect in the RE, indicating early RNFL loss consistent with mild to moderate glaucoma in this eye (**B**). LE, left eye; RE, right eye; RNFL, retinal nerve fiber layer.

This variant has not been found in the general population but was identified in a patient with Stickler syndrome [23]. Additionally, it was confirmed to be present in both the affected mother and brother (Figure 2E), leading to its classification as pathogenic.

Moreover, the variant c.1103G>A in the *CYP1B1* gene was detected in heterozygosity in the index case and her mother (but not her brother). Prediction programs suggest possible pathogenicity, but this variant is present, even in homozygosity, in the general population (0.2%), mainly in South Asian populations [24]. For this reason, it was classified as a VUS.

Family 6: A 39-year-old woman with severe PCG in her LE and pupillary seclusion due to her condition (Figure 5) was diagnosed at age 1 year. She also had ocular hypertension in her RE and a history of collapsing focal and segmental glomerulosclerosis (cFSGS). The variant c.2683C>T (p.Arg895Cys) was detected in heterozygosity in the *TRPC6* gene. This variant has not been described in the general population and has been reported as a de novo occurrence in other patients with cFSGS [25]. Although an association between the *TRPC6* gene and glaucoma has been suggested in the literature [26], evidence is insufficient to support this link. Therefore, the variant was classified as likely pathogenic, pending further validation of its association with glaucoma.

Family 7: The heterozygous variant c.1311+5G>C in the *COL18A1* gene was found in a 33-year-old woman with infantile-onset PCG detected at 4 months. She underwent trabeculectomy in both eyes in early infancy, followed by cataract extraction and retinal detachment surgery in the LE. This variant has a low frequency of 0.02%. Prediction programs for splice-affecting variants have been inconsistent, and their impact on splicing needs confirmation via RNA analysis, so this variant was classified as a VUS.

DISCUSSION

Currently, there are no established guidelines for genetic testing in glaucoma. The American Academy of Ophthalmology suggests testing only if it impacts treatment or surveillance, prioritizing patients with clinical signs of Mendelian disorders. Thus, genetic testing is ideal for those with PGC or JOAG during childhood or young adulthood [27].

Our center initially offered genetic testing of *CYP1B1* and *MYOC*, key genes in early-onset glaucoma [5,6]. However, WES has emerged as a comprehensive and cost-effective approach for identifying pathogenic variants, making direct WES testing feasible for patients with suspected genetic early-onset glaucoma [28,29]. The diagnostic yield in our sequenced cohort was 21%, aligning with the 19% reported by others [7]. However, we acknowledge that this is a preliminary diagnostic rate, as WES was performed in only 11 patients who were negative for *CYP1B1* and *MYOC*. Therefore, the overall yield is expected to increase as WES is extended to the remaining unsolved cases.

Based on genetic findings, patients in our cohort can be classified into two groups: (1) those with isolated PCG or JOAG linked to pathogenic variants in *CYP1B1* (family 1) and *MYOC* (families 2 and 3) and (2) those with syndromic forms of early-onset glaucoma (families 4, 5, and 6). Notably, three of the six genetically diagnosed cases with pathogenic or likely pathogenic variants were associated with syndromic glaucoma, highlighting the added diagnostic value of WES beyond isolated ocular findings. These results emphasize the importance of adopting a broader genetic approach in patients with atypical phenotypes or systemic involvement. Early identification of syndromic conditions can guide multidisciplinary management and genetic counseling. Prior to genetic testing, a comprehensive evaluation of family history and associated clinical features is essential to identify potential syndromic presentations.

After genetic testing, a visit to a genetic counselor is essential to identify at-risk relatives for early diagnosis and treatment, understand inheritance patterns, and explore family planning options [6]. For example, the index case in family 1 will transmit a mutated *CYP1B1* allele to all offspring, but disease manifestation depends on the other parent's carrier status. In contrast, other index cases had early-onset glaucoma with autosomal dominant inheritance, giving offspring a 50% risk of inheriting the altered allele and developing the disease.

Cascade genetic testing enables targeted testing and surveillance for relatives of patients with early-onset glaucoma with the same pathogenic variant as the index case, while reassuring those without the variant that their glaucoma risk is no higher than the general population. For instance, in family 1, the index case had two pathogenic *CYP1B1* variants (one from each parent), but her brother did not inherit either, placing him at no increased risk. Similarly, in family 2, descendants of individuals with *MYOC* mutations did not carry the familial variant, meaning they are not at higher risk for JOAG.

It is noteworthy that, in family 3, genetic testing allowed presymptomatic diagnosis in two nieces of the index case, aged 5 and 2 years, who carried the variant but showed no

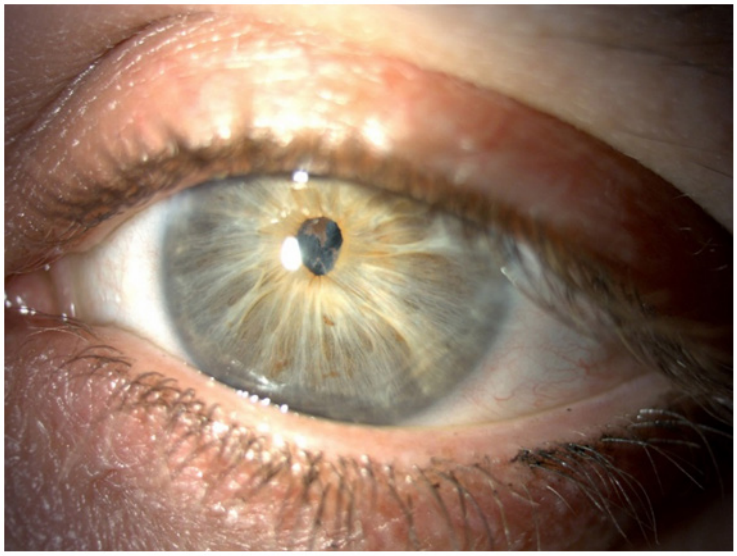

Figure 5. This figure shows an anterior segment photograph of the index case in family 6. The image shows pupillary seclusion in the left eye secondary to her clinical condition of severe primary congenital glaucoma.

glaucoma signs. Early diagnosis will allow prompt treatment before irreversible damage occurs. This case underscores the benefits of genetic testing for relatives of patients with PCG or JOAG, especially minors, with parental consent [30]. For positive cases without optic nerve or visual field damage, regular IOP monitoring is essential. In the case of a positive genetic test result, Leysen et al. [6] recommend four exams in the first year, followed by every 6 months for 2 years, and annually thereafter if no signs of glaucoma appear. While these follow-up recommendations are derived from PCG surveillance protocols, their application in MYOC-related JOAG may require adaptation, given the broader range of age of onset and variable expressivity associated with this form of glaucoma.

In family 5, a pathogenic variant in *COL2A1* (associated with Stickler syndrome) was identified, along with a heterozygous *CYP1B1* variant (c.1103G>A). Although CYP1B1 typically causes primary congenital glaucoma through autosomal recessive inheritance, several studies have proposed that heterozygous loss-of-function or hypomorphic variants may increase susceptibility to adult-onset primary open-angle glaucoma, rather than causing early-onset forms of the disease [31-33]. These variants have been shown to impair enzymatic activity and alter protein stability, suggesting a possible modifying effect on glaucoma risk. Nonetheless, as noted by Nolan et al. [34], the clinical interpretation of such variants remains difficult and must be approached with caution. In a recent study by our group, heterozygous *CYP1B1* variants were found in 5 of 61 patients with primary late-onset open-angle glaucoma, making it the most frequently affected gene in that cohort [35]. In the case of family 5, the coexistence of the *CYP1B1* variant with a pathogenic *COL2A1* mutation complicates interpretation, but the *COL2A1* variant is the most plausible explanation for the phenotype. Interestingly, the *CYP1B1* variant was absent in the patient's affected brother, prompting us to explore phenotypic differences within the family; however, follow-up was not possible due to relocation.

Regarding family 6, a likely pathogenic variant in the *TRPC6* gene was identified. This gene, along with other TRPC channels and transmembrane proteins such as podocin, forms mechanosensitive complexes that are involved in sensing glomerular pressure. This pressure-sensing function is critical in the pathogenesis of glomerular diseases such as cFSGS, where *TRPC6* variants lead to abnormal calcium influx and podocyte damage. Given the shared pressure-related mechanisms in both the kidney and the eye, it is plausible that similar TRPC6-mediated pathways may contribute to retinal ganglion cell damage in glaucoma. In both contexts, elevated mechanical stress—glomerular pressure in the kidney and IOP in the eye—can lead to cellular dysfunction and degeneration via calcium overload [36,37]. Therefore, *TRPC6* may represent a common molecular link between glomerular disease and glaucoma [26,38], supporting its role as a potential therapeutic target in pressure-related neurodegeneration, but further investigation is needed.

This study highlights the value of genetic analysis in diagnosing early-onset glaucoma. In our cohort, 21% of patients were diagnosed (6 of 28, corresponding to families 1 to 6), but many cases remain undiagnosed (22 of 28), indicating unidentified disease-causing variants.

Currently, therapies are focused on lowering IOP with medication, laser, or surgery. However, technological advances have brought gene therapy to the forefront. Recently, CRISPR/Cas9 system has emerged as a promising genome-editing tool in many medical fields, including glaucoma [39]. Jain et al. [40] used CRISPR/Cas9 to disrupt mutant *MYOC* genes in human and mouse trabecular meshwork cells and in a mouse model (Tg-MYOC^{Y437H}). This disruption reduced IOP by decreasing the accumulation of misfolded protein inside trabecular meshwork cells. Additionally, since the accumulation of misfolded protein causes *MYOC*-associated JOAG, chaperones that assist in proper protein folding may offer an alternative treatment strategy [41]. In this context, genetic studies will become increasingly relevant in identifying candidates for future gene-based therapies and enabling more personalized approaches to glaucoma management. Given the limited sample size and partial application of WES, this work is presented as a pilot descriptive case series. It is intended to generate preliminary insights into the genetic landscape of early-onset glaucoma.

In conclusion, the advent of NGS has improved the diagnosis of early-onset glaucoma by allowing the study of a larger number of genes. In some cases, it enables presymptomatic diagnosis in at-risk relatives, before glaucoma symptoms appear. This represents a clinical benefit, as in a state where optic nerve alterations are not yet present, it is crucial to conduct regular assessments to measure IOP, facilitating early diagnosis of glaucoma and preventing vision loss.

APPENDIX 1. SUPPLEMENTARY TABLE 1.

To access the data, click or select the words "Appendix 1." Amplification and direct sequencing of *CYP1B1* and *MYOC* genes, and the exons where variants were detected in the *FOXC1* and *COL2A1* genes, using specific oligonucleotides.

APPENDIX 2. SUPPLEMENTARY TABLE 2.

To access the data, click or select the words "Appendix 2." List of genes included in the gene panel for the study of glaucoma.

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REFERENCES

1. Kang JM, Tanna AP. Glaucoma. Med Clin North Am 2021; 105:493-510. [PMID: 33926643].
2. Zukerman R, Harris A, Vercellin AV, Siesky B, Pasquale LR, Ciulla TA. Molecular genetics of glaucoma: Subtype and ethnicity considerations. Genes (Basel) 2020; 12:1-36. [PMID: 33396423].
3. Karaconji T, Zagora S, Grigg JR. Approach to childhood glaucoma: A review. Clin Exp Ophthalmol 2022; 50:232-46. [PMID: 35023613].
4. Badawi AH, Al-Muhaylib AA, Al Owaifeer AM, Al-Essa RS, Al-Shahwan SA. Primary congenital glaucoma: An updated review. Saudi J Ophthalmol 2019; 33:382-8. [PMID: 31920449].
5. Selvan H, Gupta S, Wiggs JL, Gupta V. Juvenile-onset open-angle glaucoma - A clinical and genetic update. Surv Ophthalmol 2022; 67:1099-117. [PMID: 34536459].
6. Leysen L, Cassiman C, Vermeer S, Casteels I, Balikova I. Genetics in primary congenital glaucoma: Implications in disease management and counseling. Eur J Med Genet 2022; 65:104378[PMID: 34748994].
7. Kumar A, Han Y, Oatts JT. Genetic changes and testing associated with childhood glaucoma: A systematic review. PLoS One 2024; 19:e0298883[PMID: 38386645].
8. Carstens N, Goolam S, Hulley M, Brandenburg JT, Ramsay M, Williams SEI. Exome-based mutation screening in South African children with primary congenital glaucoma. Eye (Lond) 2023; 37:362-8. [PMID: 35094026].
9. Xiao X, Huang C, Cao Y, Chen S, Xu Y, Chen H, Pang C, Zhang M. Exome sequencing reveals a heterozygous OAS3 mutation in a Chinese family with juvenile-onset open-angle glaucoma. Invest Ophthalmol Vis Sci 2019; 60:4277-84. [PMID: 31618764].
10. Jiang L, Eaves S, Dhillon N, Ranjit P. Postoperative outcomes following trabeculectomy and nonpenetrating surgical procedures: a 5-year longitudinal study. Clin Ophthalmol 2018; 12:995-1002. [PMID: 29872259].
11. European Glaucoma Society Terminology and Guidelines for Glaucoma. 5th Edition. Br J Ophthalmol. 2021;105(Suppl 1):1–169.
12. Knight LSW, Ruddle JB, Taranath DA, Goldberg I, Smith JEH, Gole G, Chiang MY, Willett F, D'Mellow G, Breen J, Qassim A, Mullany S, Elder JE, Vincent AL, Staffieri SE, Kearns LS, Mackey DA, Luu S, Siggs OM, Souzeau E, Craig JE. Childhood and Early Onset Glaucoma Classification and Genetic Profile in a Large Australasian Disease Registry. Ophthalmology 2021; 128:1549-60. [PMID: 33892047].
13. Susanna R Jr, Vessani RM. Staging glaucoma patient: why and how? Open Ophthalmol J 2009; 3:59-64. [PMID: 19834563].
14. Plagnol V, Curtis J, Epstein M, Mok KY, Stebbings E, Grigoriadou S, Wood NW, Hambleton S, Burns SO, Thrasher AJ, Kumararatne D, Doffinger R, Nejentsev S. A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. Bioinformatics 2012; 28:2747-54. [PMID: 22942019].
15. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL. ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17:405-24. [PMID: 25741868].
16. Gobeil S, Letartre L, Raymond V. Functional analysis of the glaucoma-causing TIGR/myocilin protein: integrity of amino-terminal coiled-coil regions and olfactomedin homology domain is essential for extracellular adhesion and secretion. Exp Eye Res 2006; 82:1017-29. [PMID: 16466712].
17. Jia LY, Gong B, Pang CP, Huang Y, Lam DSC, Wang N, Yam GH. Correction of the disease phenotype of myocilin-causing glaucoma by a natural osmolyte. Invest Ophthalmol Vis Sci 2009; 50:3743-9. [PMID: 19234343].

18. Zhou XM, Yin Y, Fan N, Cheng HB, Li XH, Wang Y, Yu WH, Cai SP, Liu XY. Single nucleotide polymorphism of MYOC affected the severity of primary open angle glaucoma. Int J Ophthalmol 2013; 6:264-8. [PMID: 23826516].

19. Liu T, Tang C, Shi X. Analysis of variants in Chinese individuals with primary open-angle glaucoma using molecular inversion probe (MIP)-based panel sequencing. Mol Vis 2020; 26:378-91. [PMID: 32476818].

20. Zhang Y, Chen X, Wang L, Sun X, Chen Y. Heterogeneity of Axenfeld-Rieger Syndrome: Molecular and Clinical Findings in Chinese Patients. Front Genet 2021; 12:732170[PMID: 34745210].

21. Saleem RA, Murphy TC, Liebmann JM, Walter MA. Identification and analysis of a novel mutation in the FOXC1 forkhead domain. Invest Ophthalmol Vis Sci 2003; 44:4608-12. [PMID: 14578375].

22. Reis LM, Maheshwari M, Capasso J, Atilla H, Dudakova L, Thompson S, Zitano L, Lay-Son G, Lowry RB, Black J, Lee J, Shue A, Kremlikova Pourova R, Vaneckova M, Skalicka P, Jedlickova J, Trkova M, Williams B, Richard G, Bachman K, Seeley AH, Costakos D, Glaser TM, Levin AV, Liskova P, Murray JC, Semina EV. Axenfeld-Rieger syndrome: more than meets the eye. J Med Genet 2023; 60:368-79. [PMID: 35882526].

23. Hoornaert KP, Vereecke I, Dewinter C, Rosenberg T, Beemer FA, Leroy JG, Bendix L, Björck E, Bonduelle M, Boute O, Cormier-Daire V, De Die-Smulders C, Dieux-Coeslier A, Dollfus H, Elting M, Green A, Guerci VI, Hennekam RC, Hilhorts-Hofstee Y, Holder M, Hoyng C, Jones KJ, Josifova D, Kaitila I, Kjaergaard S, Kroes YH, Lagerstedt K, Lees M, Lemerrer M, Magnani C, Marcelis C, Martorell L, Mathieu M, McEntagart M, Mendicino A, Morton J, Orazio G, Paquis V, Reish O, Simola KO, Smithson SF, Temple KI, Van Aken E, Van Bever Y, van den Ende J, Van Hagen JM, Zelante L, Zordania R, De Paepe A, Leroy BP, De Buyzere M, Coucke PJ, Mortier GR. Stickler syndrome caused by COL2A1 mutations: genotype-phenotype correlation in a series of 100 patients. Eur J Hum Genet 2010; 18:872-80. [PMID: 20179744].

24. Alsaif HS, Khan AO, Patel N, Alkuraya H, Hashem M, Abdulwahab F, Ibrahim N, Aldahmesh MA, Alkuraya FS. Congenital glaucoma and CYP1B1: an old story revisited. Hum Genet 2019; 138:1043-9. Internet[PMID: 29556725].

25. Nagano C, Yamamura T, Horinouchi T, Aoto Y, Ishiko S, Sakakibara N, Shima Y, Nakanishi K, Nagase H, Iijima K, Nozu K. Comprehensive genetic diagnosis of Japanese patients with severe proteinuria. Sci Rep 2020; 10:270-[PMID: 31937884].

26. Fan Q, Huang WB, Zhang XL. TRPC6: an underlying target for human glaucoma. Int J Ophthalmol 2012; 5:523-6. [PMID: 22937518].

27. Miller MA, Fingert JH, Bettis DI. Genetics and genetic testing for glaucoma. Curr Opin Ophthalmol 2017; 28:133-8. .

28. Shashi V, McConkie-Rosell A, Rosell B, Schoch K, Vellore K, McDonald M, Jiang YH, Xie P, Need A, Goldstein DB. The utility of the traditional medical genetics diagnostic evaluation in the context of next-generation sequencing for undiagnosed genetic disorders. Genet Med 2014; 16:176-82. [PMID: 23928913].

29. Kolovos A, Maxwell G, Souzeau E, Craig JE. Progress in Translating Glaucoma Genetics Into the Clinic: A Review. Clin Exp Ophthalmol 2025; 53:246-59. [PMID: 39929609].

30. Souzeau E, Glading J, Ridge B, Wechsler D, Chehade M, Dubowsky A, Burdon KP, Craig JE. Predictive genetic testing in minors for Myocilin juvenile onset open angle glaucoma. Clin Genet 2015; 88:584-8. [PMID: 25582056].

31. López-Garrido MP, Blanco-Marchite C, Sánchez-Sánchez F, López-Sánchez E, Chaqués-Alepuz V, Campos-Mollo E, Salinas-Sánchez AS, Escribano J. Functional analysis of CYP1B1 mutations and association of heterozygous hypomorphic alleles with primary open-angle glaucoma. Clin Genet 2010; 77:70-8. [PMID: 19793111].

32. Pasutto F, Chavarria-Soley G, Mardin CY, Michels-Rautenstrauss K, Ingelman-Sundberg M, Fernández-Martínez L, Weber BH, Rautenstrauss B, Reis A. Heterozygous loss-of-function variants in CYP1B1 predispose to primary open-angle glaucoma. Invest Ophthalmol Vis Sci 2010; 51:249-54. [PMID: 19643970].

33. Banerjee A, Chakraborty S, Chakraborty A, Chakrabarti S, Ray K. Functional and structural analyses of CYP1B1 variants linked to congenital and adult-onset glaucoma to investigate the molecular basis of these diseases. PLoS One 2016; 11:e0156252[PMID: 27243976].

34. Nolan DK, Chaudhari B, Franklin SJ, Wijeratne S, Pfau R, Mihalic Mosher T, Crist E, McBride KL, White P, Wilson RK, Hickey SE, Koboldt DC. Hypomorphic alleles pose challenges in rare disease genomic variant interpretation. Clin Genet 2021; 100:775-6. [PMID: 34476810].

35. Milla E, Laguna J, Alforja MS, Pascual B, Gamundi MJ, Borràs E, Hernán I, Muniesa MJ, Pazos M, Duch S, Carballo M, Jodar M. EMEIGG group. Next-generation sequencing-based gene panel tests for the detection of rare variants and hypomorphic alleles associated with primary open-angle glaucoma. PLoS One 2024; 19:e0282133[PMID: 38241218].

36. Irnaten M, O'Malley G, Clark AF, O'Brien CJ. Transient receptor potential channels TRPC1/TRPC6 regulate lamina cribrosa cell extracellular matrix gene transcription and proliferation. Exp Eye Res 2020; 193:107980[PMID: 32088241].

37. Li CSZ, Yu B, Gao Q, Dong HL, Li ZL. The critical role of ion channels in kidney disease: perspective from AKI and CKD. Ren Fail 2025; 47:2488139[PMID: 40289808].

38. Chen S, Fan Q, Gao X, Wang X, Huang R, Laties AM, Zhang X. Increased expression of the transient receptor potential cation channel 6 gene in patients with primary open-angle glaucoma. Clin Exp Ophthalmol 2013; 41:753-60. [PMID: 23566105].

39. Hu X, Zhang B, Li X, Li M, Wang Y, Dan H, Zhou J, Wei Y, Ge K, Li P, Song Z. The application and progression of CRISPR/

Cas9 technology in ophthalmological diseases. Eye (Lond) 2023; 37:607-17. [PMID: 35915232].

40. Jain A, Zode G, Kasetti RB, Ran FA, Yan W, Sharma TP, Bugge K, Searby CC, Fingert JH, Zhang F, Clark AF, Sheffield VC. CRISPR-Cas9-based treatment of myocilin-associated glaucoma. Proc Natl Acad Sci U S A 2017; 114:11199-204. [PMID: 28973933].

41. Zode GS, Kuehn MH, Nishimura DY, Searby CC, Mohan K, Grozdanic SD, Bugge K, Anderson MG, Clark AF, Stone EM, Sheffield VC. Reduction of ER stress via a chemical chaperone prevents disease phenotypes in a mouse model of primary open angle glaucoma. J Clin Invest 2011; 121:3542-53. [PMID: 21821918].