AMERICAN ACADEMY OF OPHTHALMOLOGY®

Childhood and Early Onset Glaucoma Classification and Genetic Profile in a Large Australasian Disease Registry

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Purpose: To report the relative frequencies of childhood and early onset glaucoma subtypes and their genetic findings in a large single cohort.

Design: Retrospective clinical and molecular study.

Participants: All individuals with childhood glaucoma (diagnosed 0 to <18 years) and early onset glaucoma (diagnosed 18 to <40 years) referred to a national disease registry.

Methods: We retrospectively reviewed the referrals of all individuals with glaucoma diagnosed at <40 years of age recruited to the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG). Subtypes of glaucoma were determined using the Childhood Glaucoma Research Network (CGRN) classification system. DNA extracted from blood or saliva samples underwent sequencing of genes associated with glaucoma.

Main Outcome Measures: The phenotype and genotype distribution of glaucoma diagnosed at <40 years of age.

Results: A total of 290 individuals (533 eyes) with childhood glaucoma and 370 individuals (686 eyes) with early onset glaucoma were referred to the ANZRAG. Primary glaucoma was the most prevalent condition in both cohorts. In the childhood cohort, 57.6% of individuals (167/290, 303 eyes) had primary congenital glaucoma (PCG), and 19.3% (56/290, 109 eyes) had juvenile open-angle glaucoma. Juvenile open-angle glaucoma constituted 73.2% of the early onset glaucoma cohort (271/370, 513 eyes). Genetic testing in probands resulted in a diagnostic yield of 24.7% (125/506) and a reclassification of glaucoma subtype in 10.4% of probands (13/125). The highest molecular diagnostic rate was achieved in probands with glaucoma associated with non-acquired ocular anomalies (56.5%). Biallelic variants in *CYP1B1* (n = 29, 23.2%) and heterozygous variants in *MYOC* (n = 24, 19.2%) and *FOXC1* (n = 21, 16.8%) were most commonly reported among probands with a molecular diagnosis. Biallelic *CYP1B1* variants were reported in twice as many female individuals as male individuals with PCG (66.7% vs. 33.3%, $P = 0.02$).

Conclusions: We report on the largest cohort of individuals with childhood and early onset glaucoma from Australasia using the CGRN classification. Primary glaucoma was most prevalent. Genetic diagnoses ascertained in 24.7% of probands supported clinical diagnoses and genetic counseling. International collaborative efforts are required to identify further genes because the majority of individuals still lack a clear molecular diagnosis. *Ophthalmology 2021;■:1–12 © 2021 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Supplemental material available at www.aaojournal.org.

The term "early onset glaucoma" encompasses a heterogeneous group of vision-threatening optic neuropathies with onset before age 40 years.[1] Childhood glaucoma represents a subcategory of early onset glaucoma defined as disease onset at <18 years of age.[2] The different subtypes of childhood glaucoma have been described using various definitions and classification systems that lacked consensus. To address this issue, the Childhood Glaucoma Research Network (CGRN) recently developed a classification system describing the subtypes of childhood glaucoma that has been adopted by the World Glaucoma Association and the American Board of Ophthalmology.[2]

In accordance with the CGRN classification, primary glaucoma includes primary congenital glaucoma (PCG) and

https://doi.org/10.1016/j.ophtha.2021.04.016
ISSN 0161-6420/21

juvenile open-angle glaucoma (JOAG), whereas secondary glaucomas are subcategorized depending on their underlying pathology. These secondary glaucomas include glaucoma associated with nonacquired ocular anomalies (e.g., Axenfeld-Rieger spectrum [ARS], iris hypoplasia), glaucoma associated with nonacquired systemic disease (e.g., connective tissue disorders), and glaucoma associated with acquired conditions (e.g., uveitis, trauma, or intraocular surgery). Glaucoma after cataract surgery falls under a separate classification.[2]

Likewise, classification systems exist for later adult-onset glaucoma (disease onset >40 years) with subtypes defined as primary open-angle glaucoma, primary angle-closure glaucoma, and, more broadly, glaucoma secondary to other pathology. However, there is no formalized system for glaucoma diagnosis in individuals diagnosed between the ages of 18 and <40 years, henceforth referred to as "early onset glaucoma." This inhibits the understanding of disease patterns in this age group, which is of particular relevance given this cohort is of working age, and may experience more significant visual disability and impact on quality of life compared with those with later adult-onset disease.[3]

Childhood and early onset glaucoma are typically caused by variants in genes with a Mendelian pattern of inheritance.[1] The most common genes implicated are *CYP1B1, LTBP2,* and *TEK* for PCG; *MYOC*, *TBK1,* and *OPTN* for JOAG; and *FOXC1, PITX2, PAX6,* and *CPAMD8* for glaucoma associated with nonacquired ocular anomalies.[1,4] Variants in these genes are usually associated with strong penetrance but variable expressivity, which contributes to a broad phenotypic spectrum and overlap between clinical entities. For example, biallelic *CYP1B1* variants have been associated with PCG, JOAG, and glaucoma associated with nonacquired ocular anomalies.[4] The genetic heterogeneity of childhood and early onset glaucoma coupled with the difficulty of accurately establishing clinical diagnoses in young individuals highlights the importance of genetic testing in childhood and early onset glaucoma.[5]

To the best of our knowledge, the genetic findings of the phenotypes described by the CGRN have not been reported in a large cohort of childhood and early onset glaucoma. In this study, we applied the CGRN classification to the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG) childhood and early onset glaucoma cohort. We report the genetic results and diagnostic yield for childhood and early onset glaucoma and each glaucoma subtype in a large population.

Methods

Participants

Ethics approval was obtained through the Southern Adelaide Clinical Human Research Ethics Committee. The study adhered to the revised Declaration of Helsinki (2013) and the National Health and Medical Research Council statement of ethical conduct in research involving humans (2018). Participants included in this study were sourced from the ANZRAG as previously described.[6] In brief, participants were referred to the registry by their ophthalmologist or via self-referral pathways. Clinical details were obtained from participants' glaucoma specialists. Maximum intraocular pressure (IOP) and age at glaucoma diagnosis were recorded for each individual by the referring clinician. Registry staff ensured consistent data recording and requested information from the specialist or reviewed case notes to complete any missing records where possible. Participants, or their parent or guardian, provided informed written consent. Participants then provided a blood or saliva sample for DNA extraction. Participants, or their parent or guardian, were also specifically asked to self-report their continental ancestry (i.e., European, including European Australian and European New Zealander, or non-European, including Asian, Middle Eastern, African, South or Central American, and Oceanian) and any known family history of any subtype of glaucoma, defined as the presence of a fourth-degree or closer relative affected by glaucoma.

All participants who were referred to the registry since its establishment in 2007 to October 2020 and who had a clinical diagnosis of glaucoma between the ages of 0 and <40 years were included. Individuals with a diagnosis of glaucoma between the ages of 0 to <18 years were assigned a diagnosis of childhood glaucoma, and those diagnosed between age 18 to <40 years were considered to have early onset glaucoma. Glaucoma suspects were not included. Participants in either cohort were subsequently assigned 1 of the following 6 CGRN classifications:[2]

I. Primary Glaucoma:

1. PCG, further classified as neonatal onset (0−1 month of age), infantile onset (1−24 months of age), late onset or late recognition of disease (>2 years of age), or spontaneously arrested PCG. Spontaneously arrested PCG was diagnosed in the presence of buphthalmos and Haab striae, with normal IOP, normal-appearing optic discs, and no corneal edema.[7]
2. JOAG, defined as a diagnosis of open-angle glaucoma between age 4 to <40 years of age, not exhibiting features of PCG (i.e., buphthalmos, Haab striae). Individuals were further reported to have normal-tension glaucoma (NTG, described as maximum recorded IOP ≤ 21 mmHg) or high-tension glaucoma (HTG, maximum recorded IOP > 21 mmHg) in the affected eye/s, where possible.

II. Secondary Glaucoma:

1. Glaucoma associated with acquired conditions (in which glaucoma is secondary to a condition that is not present at birth).
2. Glaucoma associated with nonacquired ocular anomalies (in which glaucoma is secondary to a nonacquired condition that is predominantly ocular).
3. Glaucoma associated with nonacquired systemic disease (in which glaucoma develops in the presence of a disease that is predominantly systemic, with or without ocular manifestations).
4. Glaucoma following cataract surgery (in which cataract surgery precedes glaucoma onset regardless of any coexisting ocular or systemic abnormality).

An "Unclassified" category was additionally assigned to individuals for circumstances in which it could not be determined if their glaucoma was primary or secondary due to an insufficient view of the ocular structures or unavailable medical records.

As per the CGRN classification, individuals were classified as having glaucoma associated with nonacquired ocular anomalies, even in the presence of systemic disease, if the disorder was predominantly ocular. This includes individuals with Peters' anomaly or ARS.[2] Individuals with only posterior embryotoxon and no

systemic features were not considered to have ARS as per the 9th Consensus Report of the World Glaucoma Association.[8] When an individual had anterior segment dysgenesis (ASD) that did not fit a specific phenotype, we used the term "unclassified ASD" as recommended by Idrees et al.[9] Individuals with primary angle-closure glaucoma were classified as having glaucoma associated with nonacquired ocular anomalies because this entity is caused by anatomic disorders of the iris, lens, and retrolenticular structures.[10]

Genetic Testing

Targeted genetic testing and exome or genome sequencing were performed on collected samples as they were received, such that the most recent samples are yet to be tested. Venous blood specimens were collected into EDTA tubes, and DNA was extracted by a QIAcube automated system using QIAamp DNA Blood Mini kit (Qiagen). Saliva specimens were collected into an Oragene DNA Self-Collection kit (DNA Genotek Inc.). DNA was isolated as per manufacturer's instructions. Targeted genetic testing was based on the clinical diagnosis (e.g., *CYP1B1* sequencing for PCG, *MYOC* sequencing for JOAG, *FOXC1*/*PITX2* sequencing and copy number variant analysis for ARS). Additionally, some cohorts previously had targeted genetic testing for specific genes (e.g., 160 individuals with JOAG had *CYP1B1* sequencing,[11] and 86 individuals with JOAG and NTG had *TBK1* copy number variant analysis and *OPTN* sequencing for p.E50K).[12] Exome or genome sequencing was performed as previously described[13] on individuals who did not have a molecular diagnosis via targeted genetic testing. Unlike targeted genetic testing, there were no minimum gene-based coverage thresholds applied to exome and genome sequencing data. Genetic results were validated through the National Association of Testing Authorities–accredited laboratories of SA Pathology at Flinders Medical Centre. A majority of the molecular diagnoses presented have been published and are appropriately identified in the "Results."[11-20]

Statistical Analysis

All calculations were performed using SPSS version 27.0 for Windows (IBM/SPSS Inc.). Data normality was assessed using the Shapiro–Wilk test. Continuous variables were expressed as median (interquartile range). Categorical data were expressed as counts and percentages. Statistical analyses of European ancestry and family history were performed on probands only to provide a more accurate representation of the data among families. The chi-square test with Yates' correction for continuity or Fisher exact test was used for categorical variables as appropriate. Standardized adjusted residuals were used during post hoc analyses to interpret any statistical significance. Gender distribution was assessed using a binomial test with the exact Clopper-Pearson 95% confidence interval (CI), in which the probability of male gender is 0.49 based on Australian and New Zealand census data.[21,22] The median test was applied to nonparametric continuous variables, and post hoc pairwise analyses used the Bonferroni adjustment. A P value < 0.05 was considered statistically significant. Multiple testing adjustments were not used beyond post hoc pairwise analyses because all analyses were exploratory in nature.

Results

Clinical Diagnosis and Classification

A total of 1219 eyes of 660 individuals with childhood or early onset glaucoma were included. Exact clinical phenotypes per classification are reported in Table S1 (available at www.aaojournal.org). Bilateral disease was reported in 86.7% of individuals (566/653), and 55.8% of individuals (368/660) were male, representing a male:female ratio of 1.26:1 (95% CI, 0.519–0.596, $P < 0.001$). A positive family history of glaucoma was reported in 59.9% of probands (344/574) and 76.2% (428/562) with self-reported European ancestry.

Childhood Glaucoma

Of the whole cohort, 533 eyes of 290 individuals (43.9%) were classified as having childhood glaucoma (Table 1). Primary glaucoma was more common than secondary glaucoma (223/290, 76.9% vs. 63/290, 21.7%). Four individuals had unclassified glaucoma (1.4%). Primary congenital glaucoma was the most common subtype (167/290, 57.6%), followed by JOAG (56/290, 19.3%). Infantile PCG was the most common PCG subtype (89/167, 53.3%, Table S1). Of those with JOAG, 80.4% (45/56) had HTG (Table S1). Bilaterality was significantly different across all subtypes ($P < 0.001$), where those with glaucoma associated with nonacquired systemic disease were least likely to have bilateral disease (1/6, 16.7%) compared with JOAG (53/56, 94.6%, $P < 0.001$). Gender distribution did not significantly differ between subgroups ($P = 0.61$), although an overall male:female ratio of 1.28:1 was found in the childhood cohort (95% CI, 0.503–0.620, $P = 0.008$). The PCG cohort recorded a higher male:female ratio of 1.46:1 (95% CI, 0.514–0.668, $P = 0.005$). A positive family history of glaucoma was significantly different across subgroups ($P = 0.004$). It was most commonly reported in probands with JOAG (29/45, 64.4%) and less commonly reported in probands with PCG (50/140, 35.7%, $P = 0.007$). Parental consanguinity was self-reported in 8 individuals with childhood glaucoma, of whom 5 had PCG. Median maximum IOP was highest in those with glaucoma associated with an acquired condition (48 [46–49] mmHg), but differences in IOP across subgroups did not reach statistical significance ($P = 0.07$). However, there was a statistically significant difference in the median age at disease diagnosis across subtypes ($P < 0.001$). The median age at diagnosis of those with glaucoma associated with nonacquired ocular anomalies (3 [0.2–8] years) was significantly different in both PCG (0.25 [0–0.6] years, $P < 0.001$) and JOAG cohorts (14 [12–16] years, $P < 0.001$).

Early Onset Glaucoma

A total of 686 eyes of 370 individuals (56.1%) were diagnosed with early onset glaucoma (Table 2). Juvenile open-angle glaucoma was the most prevalent subtype (271/370, 73.2%). Of these, 78.6% (213/271) had HTG, and 8.1% (22/271) had NTG (Table S1). Bilaterality was significantly different across all subtypes ($P < 0.001$), with those with JOAG more likely to have bilateral involvement (247/266, 92.9%) compared with individuals with glaucoma associated with an acquired condition (33/49, 67.3%, $P < 0.001$). An overall male:female ratio of 1.24:1 was found across all early onset glaucoma cases (95% CI, 0.502–0.605, $P = 0.008$). The distribution of gender was significantly different between groups ($P = 0.002$); glaucoma associated with acquired conditions was more common in male individuals (36/49, 73.5%), compared with JOAG (147/271, 54.2%, $P = 0.04$). Family history was significantly different between groups ($P = 0.007$); probands with glaucoma associated with an acquired condition were least likely to report a family history of glaucoma (25/45, 55.6%) compared with those with JOAG (187/246, 76.0%, $P = 0.03$). Differences in IOP between subgroups reached statistical significance ($P = 0.002$). Those with JOAG had a lower median maximum recorded IOP (29 [23-38] mmHg) compared with those with an associated acquired condition (36 [30–48] mmHg, $P = 0.03$) and nonacquired ocular anomalies (39 [28–45] mmHg, $P = 0.02$). The median maximum IOP recorded

Table 1. Childhood Glaucoma (diagnosed between age 0–<18 years)

	PCG	JOAG	Acquired Condition	Nonacquired Ocular Anomalies	Nonacquired Systemic Disease	Following Cataract Surgery	Unclassified	Total	P Value
All cases, n (%)	167 (57.6)	56 (19.3)	3 (1.0)	49 (16.9)	6 (2.1)	5 (1.7)	4 (1.4)	290 (100.0)	-
Eyes, n (%)	303 (56.8)	109 (20.5)	5 (0.9)	93 (17.4)	7 (1.3)	8 (1.5)	8 (1.5)	533 (100.0)	-
Bilateral, n (%)	138/165 (83.6)	53/56 (94.6)	2/3 (66.7)	44/49 (89.8)	1/6 (16.7)	3/5 (60.0)	4/4 (100.0)	245/288 (85.1)	**<0.001***
Male gender, n (%)	99/167 (59.3)	28/56 (50.0)	2/3 (66.7)	28/49 (57.1)	2/6 (33.3)	3/5 (60.0)	1/4 (25.0)	163/290 (56.2)	0.61*
Probands, n (%)	148 (59.2)	45 (18.0)	3 (1.2)	41 (16.4)	6 (2.4)	5 (2.0)	2 (0.8)	250 (100.0)	-
Family history, n (%)	50/140 (35.7)	29/45 (64.4)	0/3 (0.0)	20/39 (51.3)	3/6 (50.0)	2/5 (40.0)	2/2 (100.0)	106/240 (44.2)	**0.004***
European ancestry, n (%)	104/138 (75.4)	30/44 (68.2)	3/3 (100.0)	26/39 (66.7)	4/6 (66.7)	3/4 (75.0)	1/2 (50.0)	171/236 (72.5)	0.72*
Highest recorded IOP (mmHg)	30 (24–40)	40 (27–46)	48 (46–49)	35 (27–45)	31 (30–38)	37 (22–49)	40 (n/a)	32 (25–40)	0.07†
Age at diagnosis (yrs)	0.25 (0–0.6)	14 (12–16)	6 (5–6)	3 (0.2–8)	0 (0–4)	11 (0–15)	4 (3–6)	0.6 (0–7)	**<0.001†**

IOP = intraocular pressure; JOAG = juvenile open-angle glaucoma; n/a = not available; PCG = primary congenital glaucoma.
Totals for each variable may not equal the total number of cases because of missing data. Highest recorded IOP and age at diagnosis are presented as median (interquartile range). All cases include probands and nonprobands. European ancestry and family history were calculated for probands only. Bold values indicate statistical significance ($P < 0.05$).
*Fisher exact test.
†Median test.

was 15 [13–17] mmHg among individuals with NTG compared with 32 [26–40] mmHg among those with HTG (Table S1). The median age at disease diagnosis reached statistical significance between groups ($P = 0.03$). Those with nonacquired systemic disease had the youngest median age at disease diagnosis (23 [21–30] years), but post hoc analyses did not show statistical significance between specific groups.

Differences in Childhood and Early Onset Glaucoma Cohorts

Laterality ($P = 0.34$) and gender ($P = 0.90$) were similarly distributed in childhood and early onset cohorts. Probands with early onset glaucoma showed a higher prevalence of European ancestry than probands with childhood glaucoma, but this did not reach statistical significance (78.8% vs. 72.5%, respectively, $P = 0.10$). A positive family history of glaucoma was more likely to be reported in probands with early onset glaucoma compared with probands with childhood glaucoma (71.3% vs. 44.2%, respectively, $P < 0.001$). The distribution of exact clinical phenotypes per cohort is shown in Table S1. The distribution of age at diagnosis and highest recorded IOP per glaucoma subtype per cohort are shown in Figures S1 and S2 (available at www.aaojournal.org), respectively.

Genetic Results

A total of 506 (506/594, 85.2%) probands underwent genetic testing, of whom 36.8% (186/506) underwent targeted genetic testing and 63.2% (320/506) underwent whole-exome or genome sequencing. A molecular diagnosis was determined in 24.7% (125/506). The diagnostic yield was 37.6% (83/221) in probands with childhood glaucoma and 14.7% (42/285) in probands with early onset glaucoma. Genetic diagnoses were achieved through targeted genetic testing in 75.2% (94/125) of probands and whole-exome or genome sequencing in 24.8% (31/125). Genetic results are presented and discussed in the context of the whole cohort's clinical diagnosis and CGRN classification (Table S2, available at www.aaojournal.org, shows distribution of molecular diagnoses per cohort). Genetic results confirmed the clinical diagnosis in 89.6% (112/125) of probands. The remaining 10.4% (13/125) of probands underwent reexamination and were found to have other ocular or systemic features consistent with their molecular diagnosis and consequently had a change in clinical diagnosis. A molecular diagnosis for glaucoma was not achieved in any individual with glaucoma associated with acquired conditions or glaucoma following cataract surgery. The distribution of associated genes per glaucoma subtype per proband, after reclassification, are presented in Figure 1. Figure S3 (available at www.aaojournal.org) conversely shows the distribution of glaucoma subtypes per associated gene.

Primary Congenital Glaucoma

The majority of probands with PCG (135/148, 91.2%) were genetically tested, and 30.4% were given a molecular diagnosis (41/135). Biallelic variants in *CYP1B1* (n = 21, 15.6%), *CPAMD8* (n = 5, 3.7%), and *COL18A1* (n = 1, 0.7%), and heterozygous variants in *TEK* (n = 8, 5.9%), *FOXC1* (n = 5, 3.7%), and *ANGPT1* (n = 1, 0.7%) were found. One individual with a homozygous variant in *CYP1B1* reported parental consanguinity. Biallelic variants in *CYP1B1* were present in 7 of 80 male and 14 of 55 female probands with PCG who underwent genetic testing ($P = 0.02$).

After genetic diagnosis, 24.4% of PCG probands (10/41) had a reclassification of their clinical diagnosis based on reexamination. All individuals with *FOXC1* variants were consequently found to have features of ARS and were reclassified to have glaucoma associated with nonacquired ocular anomalies. One individual did

Table 2. Early Onset Glaucoma (diagnosed between age 18−<40 years)

	JOAG	Acquired Condition	Nonacquired Ocular Anomalies	Nonacquired Systemic Disease	Following Cataract Surgery	Total	P Value
All cases n (%)	271 (73.2)	49 (13.2)	44 (11.9)	5 (1.4)	1 (0.3)	370 (100.0)	-
Eyes n (%)	513 (74.8)	82 (12.0)	79 (11.5)	10 (1.5)	2 (0.3)	686 (100.0)	-
Bilateral n (%)	247/266 (92.9)	33/49 (67.3)	35/44 (79.5)	5/5 (100.0)	1/1 (100.0)	321/365 (87.9)	**<0.001***
Male gender n (%)	147/271 (54.2)	36/49 (73.5)	22/44 (50.0)	0/5 (0.0)	0/1 (0.0)	205/370 (55.4)	**0.002***
Probands n (%)	250 (72.7)	49 (14.2)	40 (11.6)	4 (1.2)	1 (0.3)	344 (100.0)	-
Family history n (%)	187/246 (76.0)	25/45 (55.6)	24/39 (61.5)	2/3 (66.7)	0/1 (0.0)	238/334 (71.3)	**0.007***
European ancestry n (%)	190/242 (78.5)	37/45 (82.2)	25/34 (73.5)	4/4 (100.0)	1/1 (100.0)	257/326 (78.8)	0.76*
Highest recorded IOP (mmHg)	29 (23−38)	36 (30−48)	39 (28−45)	35 (29−47)	23 (n/a)	30 (24−40)	**0.002†**
Age at diagnosis (yrs)	34 (29−37)	32 (30−37)	31 (25−35)	23 (21−30)	20 (n/a)	33 (28−36)	**0.03†**

IOP = intraocular pressure; JOAG = juvenile open-angle glaucoma; n/a = not available
Totals for each variable may not equal the total number of cases because of missing data. Highest recorded IOP and age at diagnosis are presented as median (interquartile range). All cases include probands and nonprobands. European ancestry and family history were calculated for probands only. Bold values indicate statistical significance ($P < 0.05$).
*Fisher exact test.
†Median test.

not have any evident ocular features of ARS after thorough reexamination, but based on systemic features associated with ARS, was reclassified into this category with other individuals with ARS.[5] Four of the 5 probands with biallelic *CPAMD8* variants were found to have features consistent with unclassified ASD,[13] and the individual with biallelic *COL18A1* variants was subsequently discovered to have Knobloch syndrome. These individuals were reclassified to have glaucoma associated with nonacquired ocular anomalies.

Juvenile Open-Angle Glaucoma

Collectively, 15.5% of tested JOAG probands across both cohorts (39/252) received a molecular diagnosis, including 30.8% (12/39) of those with childhood-onset and 12.7% (27/213) of those with early onset glaucoma.

The results consisted of biallelic variants in *CYP1B1* (n = 8, 3.2%) and *CPAMD8* (n = 1, 0.4%), heterozygous variants in *MYOC* (n = 24, 9.5%), *FOXC1* (n = 2, 0.8%), *OPTN* (n = 1, 0.4%), and *COL2A1* (n = 1, 0.4%), and copy number variants of

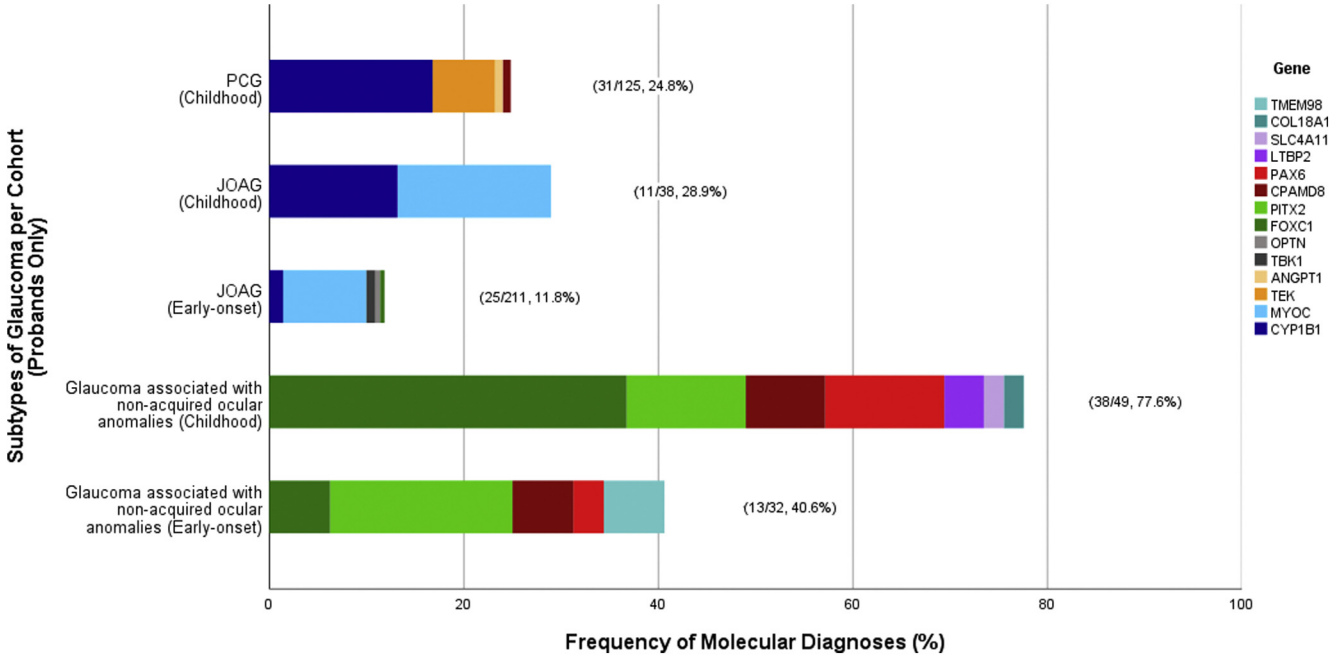

Figure 1. Frequencies of molecular diagnoses in tested probands stratified by Childhood Glaucoma Research Network (CGRN) classification (after reclassification postgenetic diagnosis). The number of genetically tested probands who had a molecular diagnosis within each cohort is included after each respective bar. Glaucoma associated with acquired conditions and glaucoma following cataract surgery were excluded because no molecular diagnoses were determined. Glaucoma associated with nonacquired systemic disease and unclassified glaucoma classifications were also excluded because of the small number of probands. JOAG = juvenile open-angle glaucoma; PCG = primary congenital glaucoma.

TBK1 (n = 2, 0.8%). Upon reexamination, the individual with biallelic *CPAMD8* variants was found to have unclassified ASD,[13] and the individual with a *COL2A1* variant had a history of retinal detachment and joint problems consistent with Stickler syndrome. One of the 2 individuals with a *FOXC1* variant,[5] who was from the childhood cohort, had a revised diagnosis of ARS upon reexamination, and the other individual did not have any ocular anomalies or systemic features consistent with ARS.

Glaucoma Associated with Nonacquired Ocular Anomalies

A molecular diagnosis was determined in 56.5% of probands (39/69) with glaucoma associated with nonacquired ocular anomalies. The most frequent variants found included heterozygous variants in *FOXC1* (n = 14, 20.3%), *PITX2* (n = 12, 17.4%), and *PAX6* (n = 7, 10.1%). Less frequent findings included biallelic variants in *LTBP2* (n = 2, 2.9%), *TMEM98* (n = 2, 2.9%), *SLC4A11* (n = 1, 1.4%), and *CPAMD8* (n = 1, 1.4%).

All individuals with an original clinical diagnosis of glaucoma associated with nonacquired ocular anomalies and *FOXC1* variants had ARS,[14] and all individuals with *PAX6* variants had phenotypes consistent with aniridia.[15] All individuals with *PITX2* variants except 1 had ARS, whereas the remaining individual had Peters' anomaly.[14] Meanwhile, biallelic *TMEM98* variants were found in individuals with nanophthalmos,[16] and biallelic *SLC4A11* variants were found in an individual with congenital hereditary endothelial dystrophy. Biallelic *LTBP2* variants were associated with microspherophakia in 1 individual who reported parental consanguinity and widespread peripheral anterior synechiae, high myopia, and phacodonesis in another. The latter individual was determined to have unclassified ASD. The individual with biallelic variants in *CPAMD8* also had unclassified ASD.[13]

Glaucoma Associated with Nonacquired Systemic Disease

A total of 71.4% of probands (5/7) with an original clinical diagnosis of glaucoma associated with a nonacquired systemic disease were given a molecular diagnosis. Two individuals with neurofibromatosis type 1 were found to have heterozygous variants in *NF1*, 1 of whom reported parental consanguinity. Genetic results also confirmed the clinical diagnoses of Weill-Marchesani syndrome (*ADAMTS17*), Nail Patella syndrome (*LMX1B*), and Stickler syndrome (*COL2A1*) in 1 individual each. No molecular diagnosis was determined for the other 2 probands who had a clinical diagnosis of Sturge-Weber syndrome.

Unclassified Glaucoma

One of 2 probands (50%) with this classification underwent genetic testing. This individual was found to have biallelic *LTBP2* variants with a history of aphakia after removal of congenital cataracts and glaucoma onset by 1 year of age. It could not be ascertained whether glaucoma preceded or followed cataract surgery.

Genotype-Phenotype Correlations

The demographic and clinical characteristics of cases associated with each gene are presented in Table 3. Biallelic variants in *CYP1B1* (n = 29, 23.2%) and heterozygous variants in *MYOC* (n = 24, 19.2%) and *FOXC1* (n = 21, 16.8%) were the most common in all probands with a molecular diagnosis (n = 125). Bilateral glaucoma was least common in individuals with *TEK* (9/12, 75.0%) and *NF1* variants (1/2, 50%). Overall, a molecular diagnosis was less prevalent in male than female individuals (73/168, 43.5% vs. 95/168, 56.5%). The number of female individuals with biallelic *CYP1B1* variants was twice the number of male individuals (24/36, 66.7% vs. 12/36, 33.3%, 95% CI, 0.490−0.814, P = 0.03). Biallelic *LTBP2* variants were exclusively found in individuals of self-reported Middle Eastern ancestry in our dataset, and 28.6% of *CYP1B1* variants were identified in probands of non-European descent.

The median age at glaucoma diagnosis was lowest in individuals with *CPAMD8* (0 [0−17] years), *TEK* (0.17 [0−0.25] years), and *CYP1B1* variants (0.14 [0−8] years), which is consistent with those who were clinically diagnosed with PCG. The median age at glaucoma diagnosis was highest in individuals with heterozygous variants in *OPTN* (33 [30−35] years) and *MYOC* (29 [15−35] years), and *TBK1* copy number variants (30 [25−38] years). These variants were found exclusively in individuals with JOAG, with the exception of 1 nonproband with a *MYOC* variant and PCG. The lowest median maximum IOP was recorded in individuals with *TBK1* copy number variants (13 [13−14] mmHg) and *OPTN* variants (18 [17−18] mmHg), consistent with their diagnoses of NTG. *TBK1* copy number variants and *OPTN* variants were found in 14.3% (2/14) and 7.1% (1/14) of probands with NTG, respectively, all of whom had European ancestry.

Discussion

The CGRN classification system for childhood glaucoma offers well-defined guidelines and enables reproducible and transparent categorization of individuals with the disease.[2] It has since been adopted by several studies,[23−28] enabling thorough and accurate comparisons of childhood glaucoma phenotypes in other populations. In this study, we did not include individuals who were glaucoma suspects because it is not the primary aim of our registry to recruit these individuals. To the best of our knowledge, this cohort represents the largest childhood glaucoma cohort of European ancestry and one of the largest international cohorts reported (290 cases). Previous published studies that used the CGRN classification include cohorts from Akron, Ohio (108 cases),[23] Miami, Florida (205 cases),[24] Egypt (207 cases),[25] South India (275 cases),[26] and Brazil (496 cases),[27] and an international study (441 cases) that included cohorts from India, United States, United Kingdom, Saudi Arabia, Ghana, Singapore, Israel, and Germany, comprising just 89 cases of European ancestry.[28]

The spectrum of childhood glaucoma diagnoses in a given study depends on the composition of the population studied and potential recruitment biases. In this study, PCG was the most prevalent subtype of childhood glaucoma (57.6%), whereas glaucoma associated with acquired conditions was the least common (1.0%). Given that a major goal of our glaucoma registry (ANZRAG) is to identify the genetic causes of glaucoma, individuals with acquired childhood glaucoma were not actively recruited (e.g., traumatic and uveitic glaucoma), explaining the lower representation of cases in this group. Individuals with glaucoma associated with nonacquired systemic disease or glaucoma after cataract surgery were similarly not actively recruited. Other studies applying the CGRN classifications reported PCG among 32% to 55% of their childhood

Table 3. Demographic and Clinical Associations of Genes Associated with Childhood and Early Onset Glaucoma Cases with a Molecular Diagnosis

Gene (Inheritance)	All Cases, n (%)	Eyes, n (%)	Bilateral, n (%)	Male Gender, n (%)	Probands, n (%)	European Ancestry, n (%)	Family History, n (%)	Highest Recorded IOP (mmHg)	Age at Diagnosis (yrs)	Cases Described Elsewhere
CYP1B1 (AR)	36 (21.4)	70 (21.7)	35/35 (100.0)	12/36 (33.3)	29 (23.2)	20/28 (71.4)	15/28 (53.6)	38 (30–40)	0.14 (0–8)	[11]
MYOC (AD)	36 (21.4)	71 (22.0)	35/36 (97.2)	16/36 (44.4)	24 (19.2)	21/24 (87.5)	24/24 (100.0)	40 (29–45)	29 (15–35)	[17]
FOXC1 (AD)	28 (16.7)	53 (16.4)	25/28 (89.3)	13/28 (46.4)	21 (16.8)	13/20 (65.0)	14/21 (66.7)	32 (24–41)	3 (0.11–14)	[5,14]
PITX2 (AD)	15 (8.9)	29 (9.0)	14/15 (93.3)	8/15 (53.3)	12 (9.6)	12/12 (100.0)	8/12 (66.7)	40 (28–52)	14 (8–21)	[14]
TEK (AD)	12 (7.1)	21 (6.5)	9/12 (75.0)	7/12 (58.3)	8 (6.4)	8/8 (100.0)	2/8 (25.0)	27 (22–37)	0.17 (0.0–0.25)	[18,19]
CPAMD8 (AR)	9 (5.4)	18 (5.6)	9/9 (100.0)	4/9 (44.4)	7 (5.6)	6/7 (85.7)	5/7 (71.4)	40 (38–44)	0 (0–17)	[13]
PAX6 (AD)	7 (4.2)	13 (4.0)	6/7 (85.7)	4/7 (57.1)	7 (5.6)	7/7 (100.0)	4/6 (66.7)	42 (39–48)	8 (4–14)	[15]
TBK1 (AD)	6 (3.6)	11 (3.4)	5/6 (83.3)	3/6 (50.0)	2 (1.6)	2/2 (100.0)	2/2 (100.0)	13 (13–14)	30 (25–38)	[12]
LTBP2 (AR)	5 (3.0)	10 (3.1)	5/5 (100.0)	2/5 (40.0)	3 (2.4)	0/3 (0.0)	3/3 (100.0)	40 (40–43)	4 (1–4)	-
OPTN (AD)	2 (1.2)	4 (1.2)	2/2 (100.0)	1/2 (50.0)	1 (0.8)	1/1 (100.0)	1/1 (100.0)	18 (17–18)	33 (30–35)	-
COL2A1 (AD)	2 (1.2)	4 (1.2)	2/2 (100.0)	1/2 (50.0)	2 (1.6)	2/2 (100.0)	2/2 (100.0)	29 (23–35)	25 (21–28)	-
TMEM98 (AD)	2 (1.2)	4 (1.2)	2/2 (100.0)	1/2 (50.0)	2 (1.6)	1/1 (100.0)	2/2 (100.0)	41 (40–42)	26 (21–31)	[16]
LMX1B (AD)	2 (1.2)	4 (1.2)	2/2 (100.0)	0/2 (0.0)	1 (0.8)	1/1 (100.0)	1/1 (100.0)	45 (29–60)	30 (30–30)	-
NF1 (AD)	2 (1.2)	3 (0.9)	1/2 (50.0)	0/2 (0.0)	2 (1.6)	1/2 (50.0)	1/2 (50.0)	35 (32–38)	0.0 (0–0)	-
ADAMTS17 (AR)	1 (0.6)	2 (0.6)	1/1 (100.0)	0/1 (0.0)	1 (0.8)	1/1 (100.0)	0/0 (0.0)	47 (n/a)	18 (n/a)	-
ANGPT1 (AD)	1 (0.6)	2 (0.6)	1/1 (100.0)	0/1 (0.0)	1 (0.8)	1/1 (100.0)	0/1 (0.0)	29 (n/a)	0.30 (n/a)	[20]
COL18A1 (AR)	1 (0.6)	2 (0.6)	1/1 (100.0)	1/1 (100.0)	1 (0.8)	0/1 (0.0)	1/1 (100.0)	22 (n/a)	11 (n/a)	-
SLC4A11 (AR)	1 (0.6)	2 (0.6)	1/1 (100.0)	0/1 (0.0)	1 (0.8)	0/1 (0.0)	0/1 (0.0)	41 (n/a)	0.20 (n/a)	-
Total	168 (100.0)	323 (100.0)	156/167 (93.4)	73/168 (43.5)	125 (100.0)	97/122 (79.5)	85/122 (69.7)	35 (27–44)	9 (0.17–25)	

AD = autosomal dominant; AR = autosomal recessive; IOP = intraocular pressure; n/a = not available.
Totals for each variable may not equal the total number of cases because of missing data. Highest recorded IOP and age at diagnosis are presented as median (interquartile range). All cases include probands and nonprobands. European ancestry and family history were calculated for probands only, and all other variables were calculated using data from all cases (where available).

glaucoma cohort,[24–28] similar to this study. The estimated incidence of PCG in Australia is 1:30 000 births,[29] but incidence figures increase up to 9-fold in populations with higher rates of parental consanguinity.[30] The high proportion of PCG cases in our cohort may be explained by a recruitment bias or may reflect the diverse ethnic background of the population studied, with 24.6% of PCG cases self-reporting non-European ancestry. The population of Australia and New Zealand is just over 30 million, thus a rate of PCG affecting 1/30 000 would suggest there would be 1000 cases of PCG (all ages) in the 2 countries, of whom we have recruited 167 (16.7% of the predicted total).

The lack of classification systems for individuals with early onset glaucoma (defined here as disease diagnosis between age 18 to <40 years) makes it difficult to understand the underlying causes in this heterogeneous group. Moreover, global prevalence rates appear to discount this age group, with reports typically including only individuals aged 40 years and above.[31] We therefore opted to apply the CGRN classifications to early onset glaucoma cases in this study. This process was simple, and individuals were assigned a diagnostic category without overlap. With this classification, JOAG was the most common diagnosis (73.2%), followed by glaucoma associated with acquired conditions (13.2%). Birla et al[32] emphasized that individuals diagnosed with glaucoma before the age of 40 years require a more formalized phenotypic classification system. Using a cluster analysis based on iris and angle morphology, they reported angle abnormalities in two-thirds of individuals with JOAG.[32] Such features may represent an otherwise distinct ocular phenotype that may be crucial for genetic analyses. We support the use of a unified classification system to group phenotypically diverse early onset glaucomas, which is offered by the CGRN classification system.[2] Further subtyping of ocular anomalies is encouraged under each CGRN classification and enables a better understanding of disease phenotypes and genetic diagnoses in this age group.

Previous studies have reported on the contribution of specific genes in childhood glaucoma (e.g., *CYP1B1*)[33] or the diagnostic yield using exome sequencing in some glaucoma subtypes (e.g., anterior segment disorders).[34] However, no studies have reported the diagnostic yield in a comprehensive cohort of heterogeneous childhood or early onset glaucoma. In total, pathogenic variants in 18 genes were reported across the entire cohort. Targeted genetic testing was successful in identifying a variant in 75.2% of probands with a molecular diagnosis, whereas whole-exome or genome sequencing was required to identify variants in the remaining probands. Similar to inherited retinal diseases and congenital cataracts, the genetic heterogeneity in our cohort supports the use of a comprehensive gene panel testing approach inclusive of all genes with evidence of association to childhood and early onset glaucoma. Additional screening for variants in the *CHRDL1* gene may be indicated where an individual has megalocornea and a diagnosis of PCG is under consideration.

Biallelic *CYP1B1* variants were the most common genetic diagnosis in PCG (15.6%). This is similar to the prevalence reported by other studies on populations of European ancestry (15%–22%),[35,36] yet lower than other populations with high consanguinity, as expected for variants associated with an autosomal recessive trait.[37] We found a significant gender difference in those with *CYP1B1* variants and PCG, with a male:female ratio of 1:2, whereas in the whole PCG cohort the male:female ratio was 1.46:1. Previous studies have reported the same trend of male preponderance in PCG,[24,25,27] whereas 2 studies reported a higher proportion of female individuals with *CYP1B1* variants and PCG.[38,39] This raises the possibility that 1 or more unidentified genes causing PCG in male individuals may be sex linked. Additionally, the higher proportion of female individuals with *CYP1B1* variants may be related to the fact that *CYP1B1* variants have been found to reduce the metabolism of 17β-estradiol, an estrogen steroid hormone that is found within the trabecular meshwork.[40] Its role in PCG pathogenesis, however, is not yet fully understood, and additional studies are needed to understand the sex bias observed in this study. Meanwhile, one-third of PCG probands had a family history of glaucoma. This reflects the current genetic landscape of PCG caused by variants in genes inherited in an autosomal recessive manner (e.g., *CYP1B1*) or an autosomal dominant manner with incomplete penetrance (e.g., *TEK*).

Heterozygous variants in *MYOC* were the major genetic cause of JOAG (9.5%). Our group previously reported *MYOC* variants in 17% of 103 individuals with JOAG with advanced visual field loss, highlighting that *MYOC* variants are associated with more severe disease in primary glaucoma.[17] *MYOC* is otherwise reported in 8% to 36% of JOAG cases and variants are typically associated with HTG,[41,42] whereas *TBK1* copy number variants and *OPTN* variants are typically associated with NTG, consistent with our study results.[12,43] Biallelic variants in *CYP1B1* were implicated in 3.2% of probands with JOAG, similar to previously reported results by our group.[11]

The highest diagnostic yield was achieved in probands with glaucoma associated with nonacquired ocular anomalies (56.5%). This is not surprising considering that the majority of this cohort comprises individuals with ARS, which has a reported diagnostic yield of 40% to 63%,[34,44,45] mainly accounted for by variants in *FOXC1* and *PITX2*. The diagnostic yield improved once probands were reclassified into this category, most of whom had an initial clinical diagnosis of PCG. We have previously reported the challenges associated with ASD diagnoses with subtle features that can be clinically diagnosed as PCG in individuals with variants in *FOXC1*.[5] The difficulty of examining the anterior segment to diagnose ASD in infants and the absence of some associated systemic features (e.g., dental anomalies) in infants can make clinical diagnoses of PCG and ASD challenging and highlight the importance of genetic testing in reaching an accurate diagnosis. This is illustrated by 1 individual in this study diagnosed with

PCG and a heterozygous variant in *FOXC1* with systemic features consistent with ARS (hearing loss, congenital heart defect) yet no ocular features of Axenfeld-Rieger anomaly found on detailed examinations under anesthesia. Despite the absence of ocular features, this individual was reclassified as having glaucoma associated with nonacquired ocular anomalies based on the presence of systemic features and genetic results consistent with ARS. A heterozygous variant in *FOXC1* was also found in an individual with JOAG who had no ocular or systemic features consistent with ARS. This phenomenon has been reported before in 2 other cases of JOAG,[46] although both individuals were reported as having posterior embryotoxon. Although posterior embryotoxon is not considered as a diagnostic feature in ARS,[8] it may represent a subtle ocular phenotype in such individuals. Individuals with PCG and biallelic *CPAMD8* variants were reclassified as having glaucoma associated with nonacquired ocular anomalies and the subtype of unclassified ASD, as described before.[13] Biallelic variants in *CPAMD8* have been reported in individuals with unclassified ASD[47] and PCG.[47,48] Currently, ASD is the more common found ocular phenotype in individuals with biallelic *CPAMD8* variants.

The number of individuals with glaucoma associated with nonacquired systemic disease in our cohort was low, most likely explained by the fact that the ANZRAG did not initially aim to recruit these individuals. However, this cohort may represent an underdiagnosed group as illustrated by the individual with a clinical diagnosis of JOAG but a genetic diagnosis indicative of Stickler syndrome (heterozygous *COL2A1* variant). This is supported by a recent study reporting systemic abnormalities in 12.9% of individuals with childhood glaucoma[49] and emphasizes the importance of referring individuals to a genetic service for a thorough medical examination to refine clinical diagnosis. Our cohort otherwise reported a molecular diagnosis in 71.4% of individuals with the remaining 2 genetically undiagnosed probands having Sturge-Weber syndrome. Sturge-Weber syndrome is caused by somatic variants in *GNAQ*, and consequently individuals require a biopsy of an affected tissue (typically skin) for molecular diagnosis.[50]

Reaching a molecular diagnosis has several benefits for affected individuals and their families. In this study, 10.4% of individuals had a change of clinical diagnosis based on genetic results. These individuals and their family members can now be accurately counseled about the mode of inheritance and the risks for relatives. At-risk family members can benefit from predictive genetic testing, and parents of affected individuals can consider reproductive options. Individuals with syndromic glaucoma can benefit from appropriate referrals for the management of associated systemic features that require specialized care. Finally, future therapeutic approaches may be gene-specific, similar to inherited retinal diseases, highlighting the importance of molecular diagnosis in precision medicine.

Study Limitations

Study limitations include missing clinical and demographic information for some participants. Furthermore, clinical diagnoses of participants were obtained by the treating specialists, which may have introduced some variation or bias. However, this reflects a genuine representation of the clinical diagnostic landscape of childhood and early onset glaucoma across Australasia. Genetic testing is an ongoing process of the ANZRAG and is therefore not complete for all individuals included in this study who may have been recruited but full genetic analyses were not yet available. Furthermore, a known limitation of exome and genome sequencing is the insufficient coverage of some exons or gene regions.[5] Therefore, it is possible that some disease-causing variants in known or novel glaucoma genes were not sufficiently covered or interrogated, including deep intronic variants, copy number variants, and structural variants. This may lead to an underestimated diagnostic rate in this cohort. Additionally, our recruitment is somewhat biased toward individuals with glaucoma suspected to be genetic in origin because this was the original design of the ANZRAG. Consequently, those with acquired glaucoma, including those with glaucoma after ocular trauma or cataract surgery, may be underrepresented, and we expect the prevalence of these conditions to be higher in the wider population. Finally, the genetic architecture of a cohort depends on its ancestry. Our cohort is predominantly of European ancestry, although 23.8% of the cohort reported a different ancestry, which reflects the diverse ancestral lineage of individuals in Australasia. The prevalence of different glaucoma subtypes and diagnostic yield in populations of non-European ancestry should be reported in future studies.

In conclusion, the present study reported the glaucoma phenotypes in the largest Australasian cohort with disease onset before the age of 40 years, according to the CGRN classification system. It is also the largest study to ascribe genetic findings according to these criteria. We have identified a diagnostic yield of 37.6% in probands with childhood glaucoma and 14.7% in probands with early onset glaucoma. These findings contribute to our understanding of childhood and early onset glaucoma phenotypes and their genetic basis. The diagnostic yield in this rare and heterogeneous disease supports the need for international collaborative efforts to identify new genetic associations. Our results emphasize the importance of accurate clinical diagnosis and the genetic heterogeneity of the disease, and support the development of a childhood and early onset genetic testing panel that will ultimately become critical in the age of gene therapy for glaucoma.

Acknowledgments

The authors thank the referring specialists.

Footnotes and Disclosures

Originally received: January 13, 2021.
Final revision: March 12, 2021.
Accepted: April 12, 2021.
Available online: ■■■. Manuscript no. D-21-00089.

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Disclosure(s):

All authors have completed and submitted the ICMJE disclosures form.

The author(s) have made the following disclosure(s): O.M.S.: Grants — National Health and Medical Research Council, the Channel 7 Children's Research Foundation, and the Rebecca L. Cooper Foundation, during the conduct of the study.

J.E.C. and D.A.M.: Supported by the National Health and Medical Research Council Practitioner Fellowships.

E.S.: Supported by an Early Career Fellowship from the Hospital Research Foundation.

L.S.W.K.: Funded by Flinders Health and Medical Research Institute and Flinders Foundation scholarships. The sponsor or funding organization had no role in the design or conduct of this research.

This project was supported by the Australian National Health and Medical Research Council Centres of Research Excellence Grant (APP1116360) and Flinders Foundation Health Seed Research Grant. The Centre for Eye Research Australia receives Operational Infrastructure Support from the Victorian Government.

HUMAN SUBJECTS: Human subjects were included in this study. Ethics approval was obtained through the Southern Adelaide Clinical Human Research Ethics Committee. All research adhered to the tenets of the Declaration of Helsinki and the National Health and Medical Research Council statement of ethical conduct in research involving humans. All participants provided informed consent.

No animal subjects were used in this study.

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Abbreviations and Acronyms:
ANZRAG = Australian and New Zealand Registry of Advanced Glaucoma; **ARS** = Axenfeld-Rieger spectrum; **ASD** = anterior segment dysgenesis; **CGRN** = Childhood Glaucoma Research Network; **CI** = confidence interval; **HTG** = high-tension glaucoma; **IOP** = intraocular pressure; **JOAG** = juvenile open-angle glaucoma; **NTG** = normal-tension glaucoma; **PCG** = primary congenital glaucoma.

Keywords:
Childhood glaucoma, Early onset glaucoma, Genetic testing, Glaucoma, Glaucoma genetics.

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