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ARTICLE

Exome-based mutation screening in South African children with primary congenital glaucoma

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OBJECTIVES: To identify pathogenic variants in a cohort of 23 black South African children with sporadic primary congenital glaucoma (PCG) using an exome-based approach.
METHODS: Children with PCG were recruited from two Paediatric Ophthalmology Clinics in Johannesburg, South Africa. Whole exome sequencing was performed on genomic DNA. Of the 23 children, 19 were male and 19 had bilateral PCG. A variant prioritization strategy was employed whereby variants in known PCG genes (*CYP1B1*, *LTBP2* and *TEK*) were evaluated first, followed by the identification of putative disease-causing variants in other genes related to eye diseases and phenotypes.
RESULTS: Validated pathogenic variants in the *CYP1B1* gene (c.1169 G>A; p.Arg390His) and *TEK* gene (c.922 G>A; p.Gly308Arg) were identified in one child each. No *LTBP2* mutations were identified in this cohort. *In silico* predictions identified potentially damaging rare variants in genes previously associated with eye development phenotypes or glaucoma in a further 12 children.
CONCLUSIONS: This study demonstrates the value of whole exome sequencing in identifying disease-causing variants in African children with PCG. It is the first report of a *TEK* disease-causing variant in an African PCG patient. Potential causative variants detected in PCG candidate genes warrant further investigation.

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INTRODUCTION

Primary congenital glaucoma (PCG) is a heritable ocular disorder characterised by trabecular meshwork dysgenesis. This isolated developmental anomaly causes an elevated intraocular pressure and patients typically present with the clinical triad of epiphora, photophobia and blepharospasm. Ocular enlargement involving the cornea or the entire globe (buphthalmos) occurs in infants due to the elasticity of immature collagen fibres in the cornea and sclera. The corneal enlargement is associated with curvilinear breaks in Descemet's membrane (Haab's striae), corneal oedema and opacification. The final feature, the hallmark of all glaucomatous disease, is optic nerve damage. PCG is more common in males and approximately two thirds of cases are bilateral. It most commonly occurs after 1 month and up to 2 years of age.

PCG is the most common form of childhood glaucoma and untreated cases are a major cause of early onset blindness worldwide [1]. PCG occurs in both sporadic and familial patterns and inheritance is usually autosomal recessive in familial cases. PCG is characterised by both phenotypic and genetic variability that complicate genetic enquiry [2–4].

Five genetic loci for PCG (GLC3A–E) have been identified to date. The cytochrome P450 gene (*CYP1B1*; OMIM #231300) is responsible for the linkage signal at the GLC3A locus [5]. Akarsu et al. mapped the GLC3B locus to chromosome 1p36.2-p36.1 in 1996, but the disease-causing gene at this locus remains unidentified to date [6]. Locus GLC3C was mapped to chromosome 14q24.3 in 2002 [7]. Subsequent studies localised another linkage signal to 14q24.2–24.3, and closer inspection showed that this locus is immediately adjacent to but does not overlap GLC3C [8, 9], leading to the designation of the fourth PCG locus, GLC3D. The gene responsible for GLC3D has been identified as the latent transforming growth factor beta binding protein 2 genes (*LTBP2*; OMIM #613086) [10], while the GLC3C gene remains unknown. The GLC3E locus was assigned to the tunica interna endothelial cell kinase (*TEK*) gene (GLC3E; OMIM #617272) after Souma et al. identified heterozygous loss-of-function mutations in the gene in a PCG cohort of 189 families that do not carry mutations in the known PCG causal genes [11].

The diagnosis of PCG is usually made on the basis of a clinical examination under anaesthesia in an infant with suggestive symptoms. However, mutations in *CYP1B1* or *LTBP2* as autosomal recessive traits (homozygous and compound heterozygous mutations in either gene) or in *TEK* as an autosomal dominant trait (heterozygous mutation) with variable expressivity would confirm the diagnosis if clinical features are inconclusive [12]. The prevalence of *CYP1B1* or *LTBP2* mutations varies considerably across different populations [13–19]. The probability of identifying pathogenic variants in *CYP1B1* increases with the presence of a positive family

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history, parental consanguinity, and bilateral and severe disease [12]. Lower diagnostic yields are, however, expected in cohorts with lower consanguinity rates and in sporadic cases. In these cohorts, an expanded gene panel or exome/genome-based mutation screening approach may therefore be an appropriate strategy to increase the likelihood of identifying the disease-causing mutation in patients presenting with clinical features of PCG.

Data on the genetic aetiology of PCG in African populations remain limited [20, 21]. We describe a cohort of 23 unrelated black South African children with sporadic PCG. We used an exome-based approach to screen for pathogenic mutations in known PCG genes and other genes with putative disease-causing variants that could possibly explain the clinical phenotypes.

METHODS

Patient recruitment

The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, South Africa (protocol number M131125), and followed the tenets of the Declaration of Helsinki. The children were recruited from the Paediatric Ophthalmology Clinics at St John Eye Hospital (the eye department of Chris Hani Baragwanath Hospital) and Charlotte Maxeke Johannesburg Academic Hospital. These two public, academic hospitals together serve the greater Johannesburg metropolitan region. Written informed consent was obtained from all patients (and/or their parents/legal guardian) after the nature and possible consequences of the study were explained to them. The diagnosis of PCG required an increased corneal diameter (greater than 11 mm) associated with corneal oedema or Haab striae and an elevated IOP (greater than 21 mmHg or greater than 16 mmHg if measured under general anaesthesia) and/or a vertical cup-to-disc ratio of greater than 0.4. Patients with other ocular abnormalities (like Axenfeld Reiger syndrome, Peters anomaly, aniridia and congenital cataract) or systemic conditions were not included in the study. Detailed information was obtained from the parents and/or a record review was performed on all the participants recording the following details: self-reported ethnicity (home language), presence of consanguinity, family history, medical history, age at onset and diagnosis, laterality, and treatment. Each participant had undergone a complete ophthalmological examination, including an examination under anaesthesia. The following details from ocular examinations performed under anaesthesia were recorded: corneal diameters, intraocular pressures, corneal clarity, pupillary reactions, and vertical cup-to-disc ratio.

None of the children had previously had genetic studies. Genomic DNA was extracted from whole blood using a modified version of the salting out method [22] or from saliva using Oragene DNA Saliva kits (DNA Genotek) and the accompanying prepIT extraction reagents as per the manufacturer's instructions.

Whole exome sequencing, assembly and variant calling

Targeted exome capture was performed by preparing sequencing libraries from genomic DNA using the NuGEN Ovation Ultralow DR Multiplex protocol followed by a SureSelectXT Human All Exon V5+UTRs (70MB) target enrichment (Agilent). Captured libraries were sequenced on the Illumina HiSeq 2500 (Illumina Inc.) with 76-bp paired-end reads. Reads were mapped to the human hg19 genome assembly using the Burrows-Wheeler Aligner (BWA) [23] and GATK base quality score recalibration, indel realignment, and duplicate removal was applied according to GATK Best Practises [24]. Variants were called with the GATK Unified Genotyper and annotated with the Ensembl Variant Effect Predictor [25]. Variants with a VQSLOD score below 2 and/or a base coverage below 5x were considered low quality calls and consequently not included in the analysis.

Variant prioritization strategy

We excluded the following: (1) non-coding or synonymous variants; (2) variants with a minor allele frequency of more than 2% in the gnomAD database [26]; (3) variants that were reported as benign or likely benign in ClinVar. Variants were evaluated for possible functional impact using the full range of *in silico* pathogenicity prediction- and conservation scores annotated by the Ensembl Variant Effect Predictor [25].

We then explored the remaining variants, using a model-free approach, for biological relevance. Putative disease-causing variants and variants of uncertain significance (VUSs) were identified using the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines for variant interpretation [27]. For these variants, frequencies in black South African control populations were computed from two data sources: 100 isiZulu individuals as part of the African Genome Variation Project (AGVP) ($n = 100$) [28]; and 100 South African south-eastern Bantu-speakers who are part of the AWI-Gen study [29] [personal communication MR]. Frequencies were extracted and merged using vcftools [30]. If at least one putative pathogenic allele was observed among the 200 individuals, then that variant is considered less likely to be a PCG causing variant.

Lastly, we analysed all samples with Moon (http://www.diploid.com/moon) to look for variants plausibly linked to individual patients' clinical phenotypes that were not otherwise identified. Moon is an automated genome interpretation platform that uses artificial intelligence to automatically filter and rank possible pathogenic variants that are associated with patient phenotypes.

Sanger sequencing validations

Validation for the two variants, rs752184169 (*TEK*) and rs56010818 (*CYP1B1*) was performed using Sanger sequencing, with primer pairs, 5′-AGTTGGCATG ATAGGAGCTCA-3′ and 5′-TGCTGTGCTTTAGGATTTAGGA-3′, and 5′-CCCT GAAATCGCACTGGTG-3′ and 5′-GCTCACTTGCTTTTCTCTCTCC-3′, respectively. The amplicons were sequenced using the BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3–100/1000 (Nimagen) on the ABI 3500XL Genetic Analyser (Applied Biosystems), according to the manufacturer's instructions. The AB1 files were analysed using Geneious Prime 2020.1.1 (https://www.geneious.com).

RESULTS

Clinical evaluation

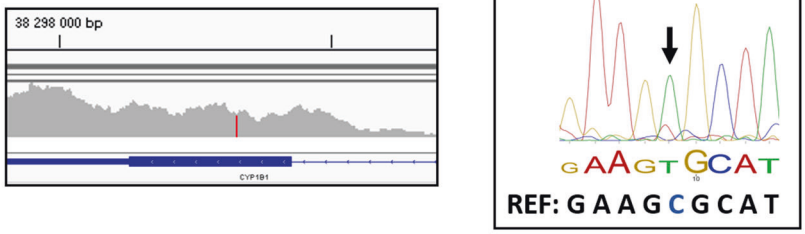
Twenty-three unrelated black South African children were enrolled in the study. Nineteen (83%) were male. Only one child came from a consanguineous union. Another child had a possible (unverified) family history of an affected cousin. The age at diagnosis ranged from a diagnosis at birth to 8 years of age with a median of 6 months of age. Only two of the children were over the age of 3 years at the time of diagnosis. Both had unilateral diseases that had been identified by the parents much earlier but no treatment had been sought. In total, four (17%) of the children presented with unilateral PCG, the remainder had bilateral disease. The corneal diameter of affected eyes ranged from 11.5 mm to 16 mm with a median of 13 mm. The intraocular pressure at the first examination under anaesthesia in affected eyes ranged from 13 mmHg to 41 mmHg with a median of 26.5 mmHg. The cornea was oedematous in all but two of the patients, however, the clear corneas demonstrated Haab's striae. Four of the patients had an afferent pupillary defect in one eye at the time of presentation. The vertical cup to disc ratio ranged from 0.1 to 1.0 in affected eyes with a median of 0.9. The majority of patients were managed surgically, with trabeculotomy being the most commonly performed procedure. Two patients required multiple surgical procedures including glaucoma drainage devices and cyclophotocoagulation to control the intraocular pressure.

Whole exome sequencing coverage and variant filtering

We performed whole exome sequencing on all 23 individuals to an average depth of 43x. On average, 98% and 95% of bases were covered to 5x and 10x within the targeted regions, respectively. The three known PCG genes (*CYP1B1*, *LTBP2* and *TEK*) were covered adequately in all samples. We identified two variants in known PCG genes (Fig. 1). We identified a pathogenic homozygous c.1169 G>A (p.Arg390His) *CYP1B1* variant in patient P009 that had been reported in PCG patients before (rs56010818; ClinVar ID 592512). Patient P017 had a heterozygous c.922 G>A (p.Gly308Arg) variant in *TEK* that is classified as likely pathogenic. Neither variant was identified in the AWI-Gen or AGVP South African control datasets.

We identified VUSs in 12 patients. These were rare variants with damaging *in silico* predictions in genes that have been associated with eye development phenotypes or glaucoma in the literature

Gene	Chr	Position	Ref	Var	Zygosity	Variant type	RS ID	HGVS
CYP1B1	2	38298328	C	T	Homo	SNV	rs56010818	NM_000104.3: c.1169G>A



Gene	Chr	Position	Ref	Var	Zygosity	Variant type	RS ID	HGVS
TEK	9	27180258	G	A	Het	SNV	rs752184169	NM_000459.5: c.922G>A

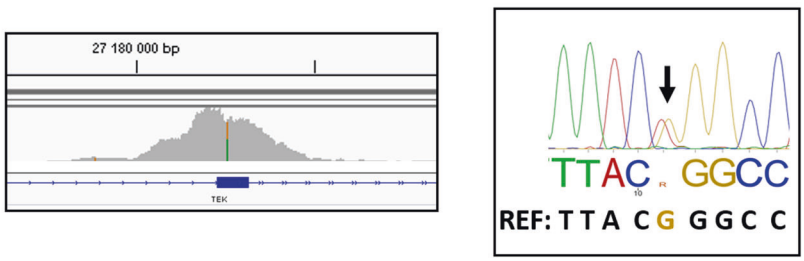


Fig. 1 Confirmation of the disease-causing mutations, rs56010818 and rs752184169; observed in IGV (Integrative Genomics Viewer) and validated using Sanger sequencing. *The sequence is in the reverse direction, therefore, the complementary bases are observed.

without a definitive link to PCG pathogenesis (Table 1). Eight of the variants were identified in at least one individual of either or both of the AWI-Gen or AGVP control datasets suggesting that they are less likely to be pathogenic. The remaining variants were not observed in the South African control datasets, and when present in gnomAD, had low frequencies.

Monoallelic *WDR36* and *ASB10* variants have been linked to POAG [31, 32]. Patient P004 had rare variants in both *WDR36* and *ASB10*. His diagnosis of PCG was made at 3 months of age. At diagnosis, he had oedematous corneas with diameters of 16 mm in both eyes and had fully cupped discs. His right eye was treated with a trabeculotomy, but he required multiple surgical procedures to control the intraocular pressure in the left eye.

HSPG2 (encoding perlecan; patient P022) and *FBN2* (encoding fibrillin-2; patient P023) are microfibril-associated genes and linked to the current theory that microfibril deficiencies cause glaucoma [33, 34]. Fibrillin-2 is the dominant fibrillin in microfibrils of the developing lens [35], while mice deficient in Fbn2 were found to have eye development anomalies, particularly anterior segment dysgenesis [36].

BMP4 (patient P012) is a strong candidate to contribute to anterior segment dysgenesis and other developmental conditions associated with glaucoma as heterozygous deficiency of *BMP4* results in anterior segment dysgenesis and elevated IOP in a mouse model [37, 38].

SLC16A12, with a variant identified in patient P027, a 3-year old boy diagnosed with bilateral advanced PCG, is a gene that has previously been associated with anterior segment dysgenesis but it is mostly associated with cataract phenotypes and its association with glaucoma is unclear [39, 40].

VUSs in *PIEZO1* were identified in a further two affected boys, patients P018 and P026. *PIEZO1* is thought to play a role in optic nerve head astrocyte mechanotransduction [41], and it is hypothesised that this gene might be involved in IOP-mediated glaucomatous neurodegeneration [42, 43].

A 3-month old boy (patient P016) had two heterozygous VUSs in the *GALNS* gene, neither of which was observed in the Southern African datasets. The *GALNS* gene (OMIM #253000) is associated with autosomal recessive mucopolysaccharidosis type IVA, also known as Morquio syndrome A. Glaucoma is known to occur in Morquio syndrome, although it is considered rare [44]. The patient in question has, unfortunately, been lost to follow-up and we were unable to perform retrospective phenotyping on him to explore a diagnosis of Morquio syndrome.

The VUSs identified were not validated with Sanger sequencing.

DISCUSSION

In this genetic enquiry into PCG in a South African cohort of unrelated patients, we were able to identify causative mutations in only two of the twenty-three children enrolled in the study. This is, to our knowledge, the first study of its kind in sub-Saharan Africa. Similar studies from around the world suggest that the yield of pathogenic variants in unrelated, sporadic PCG is quite low, with the exception of populations with high levels of consanguinity. In most populations, there is a 10 to 40% chance of identifying a causative mutation [39]. Identifying genes involved in PCG is complicated by incomplete penetrance and variable expressivity of the genetic variants as well as genetic interactions. Since our cohort is relatively small, the detection of 2/23 cases with mutations is not unexpected, and the list of potentially damaging variants may increase the yield if they are validated.

CYP1B1 mutations are the most commonly described in patients with PCG. *CYP1B1* is the largest known enzyme of the cytochrome P450 pathway enzymes. This enzyme family is responsible for the metabolism of drugs and dietary compounds and the synthesis of steroid hormones. *CYP1B1* is highly expressed in human and murine ocular tissues during development. *Cyp1b1* deficient mice exhibit ocular drainage structure abnormalities, which include trabecular meshwork defects and a small or absent Schlemm's canal, features similar to those reported in human PCG patients [45]. However, the underlying molecular mechanism of *CYP1B1*'s contribution to the development of the anterior chamber of the eye remains largely unknown [46]. The proportion of patients with

Table 1. Variants of uncertain significance in black South African patients with PCG.

	Gene	Gene allelic requirement	pLI[a]	Variant[b]	Transcript interpretation	Impact	GNOMAD		Variant allele frequency in South African Datasets[c]	Glaucoma association
							Total variant allele frequency	African/African American variant allele frequency		
P004	*WDR36*	Monoallelic	0.00	c.2120 C>G (rs113635239)	ENST00000506538.2:c.2120 C>G (p.Ala707Gly)	Missense	NA	NA	0	POAG
	HSPG2	Biallelic	0.00	c.4519 A>T (rs557789671)	HSPG2(NM_005529.7): c.4519 A>T (p.Ser1507Cys)	Missense	$<10^{-5}$	$<10^{-4}$	0.015	Microfibril
	ASB10	Monoallelic	0.00	c.16 A>C	ASB10(ENST00000422024.1): c.16 A>C (p.Ser6Arg)	Missense	$<10^{-4}$	$<10^{-3}$	NA	POAG
P023	*FBN2*	Monoallelic	1.00	c.4417 C>T (rs1233430118)	FBN2(NM_001999.4):c.4417 C>T (p.Arg1473Cys)	Missense	$<10^{-5}$	0	NA	Microfibril
P012	*BMP4*	Monoallelic	0.96	c.416 G>A (rs773804981)	BMP4(NM_130851.3):c.416 G>A (p.Arg139His)	Missense	$<10^{-4}$	$<10^{-4}$	NA	Eye development and Glaucoma (mouse model)
P011	*UNC45B*	Monoallelic	0.00	c.761 G>T (rs1053157449)	UNC45B(NM_173167.3): c.761 G>T (p.Gly254Val)	Missense	NA	NA	0.005	Eye development (model organisms)
P024	*PXDNL*	Unknown	0.00	c.694 C>T (rs77835065)	PXDNL(NM_144651.5):c.694 C>G (p.Gln232Glu)	Frameshift	$<10^{-2}$	<0.05	0.0025	Homologue of *PXDN* (linked to PCG)
	SERPING1	Mono/biallelic	0.96	c.65 C>T (rs952441370)	SERPING1(NM_000062.3): c.65 C>T (p.Ser22Leu)	Missense	$<10^{-5}$	NA	0.0075	POAG risk
P027	*SLC16A12*	Monoallelic	0.49	c.1472 C>A (rs189046619)	UNC45B(NM_173167.3): c.761 G>T (p.Gly254Val)	Missense	$<10^{-4}$	$<10^{-3}$	0	Anterior segment dysgenesis and glaucoma
P018	*PIEZO1*	Monoallelic	0.00	c.6150delC	PIEZO1(NM_001142864.4): c.6150delC	Frameshift	$<10^{-3}$	0	0	Optic nerve head mechanotransduction
P022	*HSPG2*	Biallelic	0.00	c.11518 C>T	HSPG2(NM_005529.7): c.11518 C>T (p.His3840Tyr)	Missense	NA	NA	NA	Microfibril
	COL2A1	Monoallelic	1.00	c.4348 A>G (rs113238468)	COL2A1(NM_001844.5): c.4348 A>G	Missense	$<10^{-5}$	0	0.005	Glaucoma in Stickler Syndrome
P002	*OPTN*	Monoallelic	0.00	c.275 A>T (rs202044898)	OPTN(NM_021980.4):c.275 A>T	Missense	$<10^{-4}$	0	0.01	POAG
P003	*HSPG2*	Biallelic	0.00	c.10084 C>T (rs368151242)	HSPG2(NM_005529.7): c.10084 C>T	Missense	$<10^{-4}$	$<10^{-3}$	0.005	Microfibril
	HSPG2	Biallelic	0.00	c.2549 G>A (rs140392638)	HSPG2(NM_005529.7): c.2549 G>A	Missense	$<10^{-3}$	$<10^{-2}$	0.02	Microfibril
PO16	*GALNS*	Biallelic	0.00	c.137 C>T (rs34745339)	GALNS(NM_001323543.2): c.137 C>T	Missense	$<10^{-5}$	$<10^{-4}$	NA	Morquio syndrome (MPSIVA)
	GALNS	Biallelic	0.00	c.299 G>A (rs727503946)	GALNS(NM_001323544.2): c.299 G>A	Missense	$<10^{-4}$	0	NA	Morquio syndrome (MPSIVA)
P026	*PIEZO1*	Monoallelic	0.00	c.5882 A>T	PIEZO1(NM_001142864.4): c.5882 A>T	Missense	NA	NA	NA	Optic nerve head mechanotransduction

[a]The probability that the gene is intolerant to loss-of-function (LOF) mutations based on the gene's observed depletion of LOF variants in Genome Aggregation Database (gnomAD).
[b]All variants were observed in the heterozygous state.
[c]South African frequencies computed from two sources: 100 isiZulu individuals sequenced at low (2x to 4x) coverage as part of the African Genome Variation Project (AGVP) [27]; and 100 South African south-eastern Bantu-speakers sequenced at 30x who are part of the AWI-Gen study [28].
NA: variant not present; no frequency information available.

CYP1B1 mutations among PCG patients varies in different populations. In an American cohort 15% had pathogenic *CYP1B1* mutations [13], in an Australian study 22% [47] and in a European study 35% [48], whereas in a Saudi Arabian study 92% had *CYP1B1* mutations [49]. A similar cohort to ours (also from Gauteng province in South Africa) of eleven African children with PCG was investigated for *CYP1B1* mutations in 2003 [50] and no mutations were identified.

In this South African study, one infant was identified as being homozygous for a damaging mutation (c.1169 G>A (p.Arg390His)) in the *CYP1B1* gene. The mutation is in an important structural core region of the protein and has been classified as a null mutation [51]. This mutation has been reported in PCG patients in India, Pakistan and France [52–54], and one patient was a compound heterozygote with a deletion in *CYP1B1* as the second mutation [54]. Arg390His has also been reported in the heterozygous state in a 26-year old diagnosed with juvenile-onset primary open-angle glaucoma in France [55]. In our study, the affected infant with this homozygous *CYP1B1* mutation (a boy) was born in Johannesburg to first cousins (mother's father and father's mother are siblings). The child was the only one in our cohort from a consanguineous union. PCG in this infant was severe and bilateral and was diagnosed before he reached 2 months of age. He was treated with bilateral trabeculotomies. *CYP1B1* mutations are generally associated with more severe PCG and with earlier onset. Lopez-Garrido et al., in a large European cohort of PCG patients, found that those with *CYP1B1* mutations were younger (most diagnosed at birth) and required more surgical procedures to control their disease [48]. They further demonstrated that recessive *CYP1B1* POAG with absent CYP1B1 activity had a worse prognosis.

LTBP2 mutations were first identified in Pakistani and Roma families [10]. Some of these families were found to have the same mutation and also to share a haplotype suggesting a distant common ancestor [10]. LTBP2 is the largest member of the family of latent transforming growth factor (TGF)-beta binding proteins. It has been located to the extracellular matrix, where it performs a regulatory role during elastic fibre assembly [9] and a structural role as an essential component of fibrillin-rich microfibrils in connective tissues [56]. The involvement of *LOXL1* in exfoliation glaucoma susceptibility [57] and the recent identification of a mutation in another microfibril-associated gene, *ADAMTS10*, in a dog model of primary glaucoma [58] led researchers to speculate that defective microfibrils may be an underlying cause of glaucoma [33]. Microfibril-associated genes are therefore plausible candidate glaucoma genes. *LTBP2* mutations account for nearly 40% of PCG cases in the Roma ethnic group. In that population, there is a common founder mutation, p.R299X [59]. *LTBP2* mutations have also been identified in Indian studies but seem to be uncommon in other populations. None were identified in a Han Chinese study [60] nor in a Turkish and British study [61], nor did we find any in our cohort.

Heterozygous *TEK* mutations have recently been described in association with PCG [11]. *TEK* is an angiopoietin receptor that is highly expressed in the Schlemm's canal endothelium and regulates its development through its interaction with angiopoietin 1 and 2. *TEK* appears to be essential for the development of the canal of Schlemm. *Tek* knockout mice do not develop Schlemm's canal but develop rapidly progressive ocular hypertension, buphthalmos and severe glaucoma. Mice heterozygous for the *Tek* knockout mutation develop abnormalities and symptoms similar to PCG [11]. The parents of infants with *TEK* mutations are typically asymptomatic. It is not certain whether this is the result of variable expressivity (some mutation carriers develop glaucoma later on), or reduced penetrance or whether the *TEK* variants co-occur with heterozygous variants in other glaucoma genes. Kabra et al. subsequently identified heterozygous *TEK* mutations in 8% of unrelated PCG cases without homozygous mutations in *CYP1B1, LTBP2, FOXC1* or *MYOC* [62]. Some of these *TEK* mutations, however, co-occurred with heterozygous *CYP1B1* mutations. Those cases had a poor visual prognosis, but their parents (with either *TEK* or *CYP1B1* heterozygous mutations) were asymptomatic. In most respects, the presentation of individuals with *TEK* mutations is usually that of typical PCG with presentation before 3 years, elevated intraocular pressure and enlarged corneal diameters. However, approximately 50% of PCG patients with *TEK* mutations have unilateral disease. This is in keeping with mutations that demonstrate variable expressivity. Our study identified one child (a boy) with a heterozygous c.922 G>A (p.Gly308Arg) *TEK* mutation. Multiple *in silico* tools predict this missense change to be deleterious, and it has only been observed in a heterozygous state in two individuals within the gnomAD dataset [26]. Interestingly, this variant is located 1 bp away from the p.Y307* nonsense variant reported in the Souma et al. study in the EGF-like three domain of the Tek protein [11]. The child in this study was born in Johannesburg to asymptomatic parents who were, unfortunately, not genotyped, so we cannot exclude a de novo mutation. He had bilateral disease. His diagnosis was made at 4 months of age at which stage he had oedematous, enlarged corneas, but minimal optic nerve damage with vertical cup-to-disc ratios of 0.2 in both eyes. He was treated with bilateral trabeculotomies. To our knowledge, this is the first African PCG patient to be reported with a *TEK* variant.

In eight of the patients in whom we were unable to identify pathogenic mutations in *CYP1B1, LTBP2* or *TEK*, we identified VUSs in genes previously linked to eye disorders, that are rare in gnomAD and were not identified in Southern African control datasets. One of the participants harboured two rare variants in different genes, one of which could potentially be a modifier allele for the other. Another participant had mutations in a gene associated with Morquio Syndrome A. A number of congenital ocular conditions can mimic PCG, therefore, a model-free approach to gene identification using next generation techniques may identify unexpected associations and assist with the diagnosis. It is not clear how, and to what extent, any of the VUSs identified in this study could be linked to glaucoma phenotypes in the absence of validation and segregation information from parental genotypes.

It is evident from population genetics studies that African populations are genetically heterogeneous [63, 64] and, therefore, our findings on the mutation spectrum for PCG among black South Africans is not representative of other African populations.

This study is limited by a small sample size with limited phenotypic information on the parents as well as no parental genotypes. Furthermore, the VUSs identified, while interesting, have not been validated.

In conclusion, this study demonstrates the value of a whole exome sequencing approach to gene and mutation identification in PCG and -related phenotypes. Using this technique, we were able to identify pathogenic mutations in *CYP1B1* and *TEK* in this group of sub-Saharan Africans with PCG. The study also identified VUSs in PCG candidate genes which contribute to the discussion and investigation of mechanisms underlying PCG.

Summary Table

What was known before

- Primary congenital glaucoma (PCG) is characterised by both phenotypic and genetic variability that complicate genetic enquiry.
- The yield of pathogenic variants in unrelated, sporadic PCG is quite low, with the exception of populations with high levels of consanguinity.
- PCG genetics have not been studied in Sub-Saharan Africa.

What this study adds

- Exome-based mutation screening in this cohort of South African children with PCG successfully identified disease-causing variants in the TEK and CYP1B1 genes.
- It is the first report of a TEK disease-causing variant in an African PCG patient.
- Potential causative variants detected in PCG candidate genes warrant further investigation.

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AUTHOR CONTRIBUTIONS

The contributing authors (NC, SG, MH, J-TB, MR and SW) meet all of the following criteria: Conceived and/or designed the work that led to the submission, acquired data, and/or played an important role in interpreting the results. Drafted or revised the manuscript. Approved the final version. Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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