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Exome sequencing identifies novel genes underlying primary congenital glaucoma in the National Birth Defects Prevention Study

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Abstract

Background: Primary congenital glaucoma (PCG) affects approximately 1 in 10,000 live born infants in the United States (US). PCG has a autosomal recessive inheritance pattern, and variable expressivity and reduced penetrance have been reported. Likely causal variants in the most commonly mutated gene, *CYP1B1,* are less prevalent in the US, suggesting that alternative genes may contribute to the condition. This study utilized exome sequencing to investigate the genetic architecture of PCG in the US and to identify novel genes and variants.

Methods: We studied 37 family trios where infants had PCG and were part of the National Birth Defects Prevention Study (births 1997–2011), a US multicenter study of birth defects. Samples underwent exome sequencing and sequence reads were aligned to the human reference sample (NCBI build 37/hg19). Variant filtration was conducted under *de novo* and Mendelian inheritance models using GEMINI.

Results: Among candidate variants, *CYP1B1* was most represented (5 trios, 13.5%). Twelve probands (32%) had potentially pathogenic variants in other genes not previously linked to PCG but important in eye development and/or to underlie Mendelian conditions with potential phenotypic overlap (e.g., *CRYBB2, RXRA, GLI2*).

Conclusion: Variation in the genes identified in this population-based study may help to further explain the genetics of PCG.

Keywords

CYP1B1; birth defects; congenital glaucoma; newborn eye abnormalities; genetics; mutations

Introduction

Primary congenital glaucoma (PCG) is most often diagnosed in the first 12 months of life and often occurs without overt structural eye defects(Lewis, Hedberg-Buenz, DeLuca, Stone, Alward, & Fingert, 2017). Children present with raised intraocular pressure (IOP), loss of corneal transparency, photophobia, and enlargement of the eye(deLuise & Anderson, 1983). Raised IOP in this context is thought to be a result of reduced flow of aqueous humor out of the anterior chamber of the eye through the trabecular meshwork, a porous structure of tissues(Abu-Hassan, Acott, & Kelley, 2014).

The prevalence of PCG is approximately 1 in 10,000 live births in the United States (US)(Alanazi, Song, Mousa, Morales, Al Shahwan, Alodhayb, Al Jadaan, Al-Turkmani,

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& Edward, 2013; deLuise & Anderson, 1983), but has been reported to be as high as 1 in 1,250 in communities with high consanguinity[4],(AK & PA, 2006). Historically, PCG was considered to be inherited as an autosomal recessive trait; however, this model of inheritance has been recently challenged by reports of transmission in successive generations, unequal sex distribution among affected individuals, and lower-than-expected numbers of affected siblings in familial cases(Demenais, Bonaiti, Briard, Feingold, & Frezal, 1979; Elder, 1969; Francois, 1980; François, 1961; Gencik, Gencikova, & Gerinec, 1980; M. & N., 1978).

Pathogenic variants in several genes underlie some cases of PCG. The most commonly mutated gene reported in the literature is Cytochrome P450 Family 1 Subfamily B Member 1 (*CYP1B1)*. Frequencies of specific pathogenic variants in *CYP1B1* causing PCG vary, with reports of founder alleles explaining 90–100% of cases among Slovakian Roma Gypsies, 50% of cases from Brazil, 44% of cases from India, and 20% of cases from Japan(Lim, Tran-Viet, Yanovitch, Freedman, Klemm, Call, Powell, Ravichandran, Metlapally, Nading, Rozen, & Young, 2013; Mashima, Suzuki, Sergeev, Ohtake, Tanino, Kimura, Miyata, Aihara, Tanihara, Inatani, Azuma, Iwata, & Araie, 2001; Plasilova, Ferakova, Kadasi, Polakova, Gerinec, Ott, & Ferak, 1998). Despite the high prevalence of PCG in some populations, pathogenic variants have only been found in 16–25% of families from the US(Lim, Tran-Viet, Yanovitch, Freedman, Klemm, Call, Powell, Ravichandran, Metlapally, Nading, Rozen, & Young, 2013; Reis, Tyler, Weh, Hendee, Kariminejad, Abdul-Rahman, Ben-Omran, Manning, Yesilyurt, McCarty, Kitchner, Costakos, & Semina, 2016). Variants in other genes, such as Latent-Transforming Growth Factor Beta-Binding Protein 2 (*LTBP2)* and Myocilin (*MYOC),* have been found in consanguineous families affected by PCG, but studies in families from the US failed to find pathogenic variants in these genes(Lim, Tran-Viet, Yanovitch, Freedman, Klemm, Call, Powell, Ravichandran, Metlapally, Nading, Rozen, & Young, 2013). Together, these findings suggest that additional genes underlying PCG remain to be found.

Herein, we describe *CYP1B1* variants identified using whole-exome sequencing (WES) data of complete trios (unaffected mother, unaffected father, and PCG-affected child) from the National Birth Defects Prevention Study (NBDPS), a population-based case-control study that has been shown to be representative of the US population(Cogswell, Bitsko, Anderka, Caton, Feldkamp, Hockett Sherlock, Meyer, Ramadhani, Robbins, Shaw, Mathews, Royle, Reefhuis, & National Birth Defects Prevention, 2009). In addition, we sought to identify candidate genes for PCG based on WES analyses in families without a *CYP1B1* variant.

Methods

Study Population

The NBDPS is a large multicenter population-based case-control study of over 30 major structural birth defects in the US. Detailed methods have been reported previously(Reefhuis, Gilboa, Anderka, Browne, Feldkamp, Hobbs, Jenkins, Langlois, Newsome, Olshan, Romitti, Shapira, Shaw, Tinker, Honein, & National Birth Defects Prevention, 2015). Briefly, case infants were identified through birth defects surveillance systems in 10 states and were diagnosed up to two years after delivery. Clinical geneticists at each study center reviewed abstracted medical records to confirm that identified cases met eligibility criteria (ICD-9

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codes 743.200–743.204). Cases were then systematically reviewed again by a study-wide clinician to perform further classification according to study protocols(Rasmussen, Olney, Holmes, Lin, Keppler-Noreuil, Moore, & National Birth Defects Prevention, 2003). The centralized review process employed a stepwise approach that included: 1) confirmation of case definition criteria; 2) examination of indications for a single-gene condition or chromosomal abnormality previously diagnosed; 3) presence of multiple major defects and pathogenic relationship; and 5) suspicion of a syndrome of known etiology. The NC center clinical geneticist re-reviewed the records for cases that harbored variants in genes that are known to be important in eye development or are known to cause other Mendelian conditions with phenotypic overlap, multiple defects, or evidence of the syndromes linked to our candidate genes. Cases with defects of known etiology, including single-gene disorders and chromosomal abnormalities were excluded. Other exclusions included: isolated microcornea with normal ocular size; iris coloboma and choroid or optic coloboma, without any other anterior chamber defect; cases with anencephaly, holoprosencephaly or anterior encephaloceles; and cases with amniotic bands.

This analysis included WES data from PCG cases born during 1997–2011 from Arkansas, California, Georgia, Iowa, Massachusetts, New York, North Carolina, Texas and Utah. Infants conceived using donor egg or donor sperm were excluded, resulting in 38 complete trios (mother-father-child). The NBDPS was approved by the Institutional Review Boards at the Centers for Disease Control and Prevention and each study center.

Buccal Cell Collection and DNA Isolation

Mothers completed a telephone interview between six weeks and 24 months after delivery and were subsequently mailed a buccal cell collection kit, which included documents for informed consent, six cytobrushes (two per eligible family member), instructions for use, and a postage-paid return mailer. DNA was extracted from one of the cytobrushes using either a phenol-chloroform method or Gentra Puregene® (Qiagen®)(Gallagher, Sturchio, Smith, Koontz, Jenkins, Honein, & Rasmussen, 2011). DNA quantity was assessed by quantitative real-time polymerase chain reaction (PCR) targeting the human RNaseP gene and short tandem repeat (STR) markers. Quality control standards included ≥0.1 ng/uL DNA, successful PCR amplification after at least two attempts with at least one STR marker, and genotypes consistent with the reported family relationship(Reefhuis, Gilboa, Anderka, Browne, Feldkamp, Hobbs, Jenkins, Langlois, Newsome, Olshan, Romitti, Shapira, Shaw, Tinker, Honein, & National Birth Defects Prevention, 2015).

WES and sequence alignment

Samples were prepared for sequencing at the National Institutes of Health Intramural Sequencing Center using 100 ng of genomic DNA and the Accel-NGS 2S Plus DNA Library Kit. Modifications to the library kit protocol included modified PCR cycling conditions to improve recovery of GC-rich regions and swapping a polymerase at the post-capture library amplification step. The Nimblegen SeqCap EZ Exome + UTR Library (version 3.0) was used for WES. Samples were then pooled and sequenced on an Illumina HiSeq 2500 instrument. Image analysis and base calling were performed using Illumina Genome Analyzer Pipeline software (version 1.18.64.0) with default parameters. Sequence reads

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were aligned to the human reference sequence (NCBI build 37/hg19) using the Illumina aligner “ELAND” (Efficient Large-scale Alignment of Nucleotide Databases). When ≥ 1 read in a pair mapped to a unique location in the genome, the pair of reads were then subjected to more accurate alignment with Novoalign v3.02.07. The aligned lane BAM files were merged, sorted, and indexed. Sequenced bases with a probability of an incorrect base call of 1 in 100(“Illumina. Quality Scores for Next-Generation Sequencing,”), or a phred quality score less than 20, were excluded from analysis.

Candidate variant and gene prioritization

The University of Washington Center for Mendelian Genomics reviewed the WES data and pedigree files and confirmed the sex of all participants and the relationships reported within trios. Detailed methods are published elsewhere(Jenkins, Almli, Pangilinan, Chong, Blue, Shapira, White, McGoldrick, Smith, Mullikin, Bean, Nembhard, Lou, Shaw, Romitti, Keppler-Noreuil, Yazdy, Kay, Carter, Olshan, Moore, Nascone-Yoder, Finnell, Lupo, Feldkamp, Program, University of Washington Center for Mendelian, Nickerson, Bamshad, Brody, Reefhuis, & National Birth Defects Prevention, 2019). Briefly, variant filtration was conducted under *de novo* and Mendelian inheritance models using GEMINI 0.20.2(Paila, Chapman, Kirchner, & Quinlan, 2013). Variant filters included FILTER flags of either PASS or SBFilter to allow for mosaic variation, depth ≥ 6, genotype quality of ≥ 20, alternative allele ≤ 0.005 across any single population within reference databases (ExAC v0.3(Lek, Karczewski, Minikel, Samocha, Banks, Fennell, O’Donnell-Luria, Ware, Hill, Cummings, Tukiainen, Birnbaum, Kosmicki, Duncan, Estrada, Zhao, Zou, Pierce-Hoffman, Berghout, Cooper, Deflaux, DePristo, Do, Flannick, Fromer, Gauthier, Goldstein, Gupta, Howrigan, Kiezun, Kurki, Moonshine, Natarajan, Orozco, Peloso, Poplin, Rivas, Ruano-Rubio, Rose, Ruderfer, Shakir, Stenson, Stevens, Thomas, Tiao, Tusie-Luna, Weisburd, Won, Yu, Altshuler, Ardissino, Boehnke, Danesh, Donnelly, Elosua, Florez, Gabriel, Getz, Glatt, Hultman, Kathiresan, Laakso, McCarroll, McCarthy, McGovern, McPherson, Neale, Palotie, Purcell, Saleheen, Scharf, Sklar, Sullivan, Tuomilehto, Tsuang, Watkins, Wilson, Daly, MacArthur, & Exome Aggregation, 2016), Exome Sequencing Project 6200 (v2), 1000 Genomes (phase 3), or the UK10K 2016-02-15 release(Consortium, Walter, Min, Huang, Crooks, Memari, McCarthy, Perry, Xu, Futema, Lawson, Iotchkova, Schiffels, Hendricks, Danecek, Li, Floyd, Wain, Barroso, Humphries, Hurles, Zeggini, Barrett, Plagnol, Richards, Greenwood, Timpson, Durbin, & Soranzo, 2015)), and a predicted medium to high impact on the gene/protein. Three approaches were undertaken to further narrow focus on gene variants which could result in PCG phenotypes. The first was to prioritize variants in genes underlying PCG and related disorders: *CYP1B1*, *FOXC1*, *LTBP2*, and *MYOC* (Ling, Zhang, Zhang, Sun, Du, & Li, 2020). The second approach prioritized variants cosegregating with PCG with evidence of conservation or potential pathogenicity, defined as phred-scaled Combined Annotation Dependent Depletion (CADD)(Rentzsch, Witten, Cooper, Shendure, & Kircher, 2019) scores >20 and Genomic Evolutionary Rate Profiling (GERP) scores >5(Cooper, Stone, Asimenos, Program, Green, Batzoglou, & Sidow, 2005). The third approach taken was a biologic plausibility approach in which we focused on those genes in which variants are known to underlie other Mendelian conditions with phenotypic overlap, genes with roles in eye development, or those which are in the same pathways as genes in

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which variants have been reported in PCG cases or other anterior segment defects of the eye(Chakrabarti, Kaur, Rao, Mandal, Kaur, Parikh, & Thomas, 2009).

Results

WES was performed on samples from 38 complete trios with a PCG-affected proband. The 38 probands were generally demographically similar to the entire set of PCG cases in the NBDPS (n=136), though a few differences were apparent (Table 1). The PCG trios with WES included fewer mothers who identified as Hispanic, and both maternal and paternal age at delivery were slightly higher relative to other NBDPS participants, along with slight differences in the proportion of samples from each NBDPS center. There was one discrepancy on the sex of the affected child in one of the families. Due to our inability to resolve this through a review of collected clinical and interview data, we excluded that family from further analysis, leaving a total sample size of 37 trios. Two families had high levels of contamination (freemix(Jun, Flickinger, Hetrick, Romm, Doheny, Abecasis, Boehnke, & Kang, 2012)=0.035 and 0.09) and are included but identified as such.

Variants in two genes previously linked to congenital glaucoma may explain the phenotype for six of the 37 families within our study (Table 2, Supplemental Table 1). Five of 37 families (13.5%) carried known *CYP1B1* variants and one family carried a known *FOXC1* variant. Each *CYP1B1* case had compound heterozygous genotypes which included variants that have been reported in children with PCG previously and were annotated as pathogenic or likely pathogenic in ClinVar. There were four frameshift changes, four missense variants, and two stop gains. Two variants were identified in more than one family: a 13-bp frameshift deletion (p.Arg355fs) was identified in families B1_10 and B1_33, while the missense variant p.Glu387Lys was identified in one non-Hispanic White and one non-Hispanic Black family, (families B1_12 and B1_13). In one family with no suspected pathogenic *CYP1B1* variants, the affected child had a *de novo* frameshift variant (c.1141dupG) in Forkhead Box C1 (*FOXC1)* which is predicted to lead to an early termination of the protein, removing 173 of 553 amino acids. No rare coding change in *LTBP2* or *MYOC* cosegregated with PCG in our sample.

Nine candidate variants in nine genes cosegregated with PCG in families without *CYP1B1* or *FOXC1* variants and were prioritized for their evidence of conservation or potential pathogenicity (Table 3, Supplemental Table 2). All were missense variants, except for a splice donor variant in *RELN*. When assessing inheritance patterns, eight variants were identified through a *de novo* inheritance pattern and one was identified through an autosomal recessive inheritance pattern. Most of the genes identified using this approach, while known to be important in cellular and developmental processes, have not been previously linked to eye development, with the exception of *CRYBB2* and *POMT2*(Driessen, Herbrink, Bloemendal, & de Jong, 1980; van Reeuwijk, Janssen, van den Elzen, Beltran-Valero de Bernabe, Sabatelli, Merlini, Boon, Scheffer, Brockington, Muntoni, Huynen, Verrips, Walsh, Barth, Brunner, & van Bokhoven, 2005).

Probands in 12 families (32%) harbored variants in genes that are known to be important in eye development or are known to cause other Mendelian conditions with phenotypic

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overlap. Information about functional changes, whether each variant has been previously identified in public databases, CADD, PolyPhen, and GERP scores of variants within these genes can be found in Table 4, while variant quality metrics are provided in Supplemental Table 3. *De novo* variants thought to result in changes to the protein structure were found in *CRYBB2, RXRA, GLI2, POU4F1, PIEZO2,* and *B3GALT6*. Among these, *POU4F1* p.Ala237del, was flagged for relatively low genotype quality and excluded after variant review(Thorvaldsdottir, Robinson, & Mesirov, 2013). Under a recessive model, one homozygous *POMT2* variant met our inclusion criteria, along with compound heterozygous changes in five probands, implicating *ALDH3A2, CREBBP, USH2A, UGT1A8,* and *SLC4A11* in PCG.

Discussion

We showed that *CYP1B1* is likely the main gene underlying PCG in families in the NBDPS. We also showed that *CYP1B1* variants are less common among cases from the US (13.5% this study, Lim et al., 14.9%(Lim, Tran-Viet, Yanovitch, Freedman, Klemm, Call, Powell, Ravichandran, Metlapally, Nading, Rozen, & Young, 2013)) than has been previously reported in Japanese patients (20%)(Mashima, Suzuki, Sergeev, Ohtake, Tanino, Kimura, Miyata, Aihara, Tanihara, Inatani, Azuma, Iwata, & Araie, 2001). We identified one family with a pathogenic variant in *FOXC1*, which has previously been implicated in PCG[26]. It has previously been suggested that there may be other novel variants in genes which may underly PCG in more ethnically heterogeneous populations, such as our multiethnic sample. We described candidate variants in 12 genes which have not previously been investigated in association with PCG, but which are known to be important in eye development or are known to cause Mendelian conditions with phenotypic overlap, including *CRYBB2, CREBBP, SLC4A11, POMT2, B3GALT6, PIEZO2, RXRA, GLI2, POU4F1, ALDH3A2, USH2A,* and *UGT1A8*. Of note, there are some families (7, 20, 30) that had multiple candidate variants detected, which could be unrelated to phenotype or could suggest a digenic inheritance or complex interactions that have not been previously reported.

Variant in Crystallin Beta B2 (CRYBB2)

Beta-crystallins are major proteins of the vertebrate lens and are important for maintaining the transparency and refractory index of the lens(Ganguly, Favor, Neuhauser-Klaus, Sandulache, Puk, Beckers, Horsch, Schadler, Vogt Weisenhorn, Wurst, & Graw, 2008). The specific *CRYBB2* variant (p.Trp59Arg) that we found has not been previously described, though it is predicted to be damaging by PolyPhen and CADD. *CRYBB2* p.Trp59Arg occurs in exon 4 near the conversion of a β-sheet to an α-helix, an important region of the protein influencing protein folding(Zhao, Xu, Chen, Liu, Yao, & Yan, 2017). A similar variant (p.Trp59Cys) has been described in congenital cataracts, a condition where the lens becomes opaque(Santhiya, Kumar, Sudhakar, Gupta, Klopp, Illig, Soker, Groth, Platzer, Gopinath, & Graw, 2010).

Variant in CREB Binding Protein (CREBBP)

During eye development, *CREBBP* is upregulated along with αA-crystallins as the primary lens fibers differentiate(Yang, Wolf, & Cvekl, 2007). The *CREBBP* protein has

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intrinsic histone acetyltransferase activity allowing for transcriptional activation(Q. Chen, Dowhan, Liang, Moore, & Overbeek, 2002), and it can also act as a sort of scaffold to stabilize proteins of the transcription complex(Goodman & Smolik, 2000). Variants in *CREBBP* underlie Rubinstein-Taybi syndrome (RSTS) which is characterized by distinctive dysmorphic features, short stature, and moderate to severe intellectual disability(Hennekam, 2006; Menke, van Belzen, Alders, Cristofoli, Study, Ehmke, Fergelot, Foster, Gerkes, Hoffer, Horn, Kant, Lacombe, Leon, Maas, Melis, Muto, Park, Peeters, Peters, Pfundt, van Ravenswaaij-Arts, Tartaglia, & Hennekam, 2016). In the last 50 years, there have been two reports of glaucoma in children diagnosed with RSTS; one with juvenile glaucoma[36] and one with congenital glaucoma(McKusick, 1968).

Variant in Solute Carrier Family 4 Member 11 (SLC4A11)

There are multiple molecular actions proposed for the *SLC4A11* protein, including effects on ion channels and potential aquaporin activities(Patel & Parker, 2015). While *SLC4A11* is present in the corneal endothelium, there are no reports of expression in the trabecular meshwork or aqueous outflow pathways(Patel & Parker, 2015). Dysfunction in this gene has been strongly linked to congenital hereditary endothelial dystrophy (CHED), a rare disorder of the corneal endothelium in which opacification of the cornea may be present at birth(Patel & Parker, 2015). There are only a few published case reports of glaucoma in patients diagnosed with CHED(Mullaney, Risco, Teichmann, & Millar, 1995; Patel & Parker, 2015). Alsaif *et al.*(Alsaif, Khan, Patel, Alkuraya, Hashem, Abdulwahab, Ibrahim, Aldahmesh, & Alkuraya, 2019) recently published WES analyses of *CYP1B1*-variant negative children with CHED from Saudi Arabia where they reported one family with a *SLC4A11* variant which they determined had been misdiagnosed as primary congenital glaucoma. The homozygous variant (*SLC4A11* c.748G>T) reported resulted in an amino acid change in a different region of the protein than the variants we observed (*SLC4A11* p.Met856Lys/p.Arg730*). A case study of a patient with Harboyan syndrome who received an initial diagnosis of congenital glaucoma reported the presence of the p.Arg730* variant in *SLC4A11*(Liskova, Dudakova, Tesar, Bednarova, Kidorova, Jirsova, Davidson, & Hardcastle, 2015). However, no changes in endothelial cell morphology or density were observed in the heterozygous daughter of the reported case.

Variant in Protein O-mannosyltransferase 2 (POMT2)

The protein encoded by *POMT2* is part of the O-mannosyltransferase (POMT) enzyme complex, which plays a role in glycosylation of α-dystroglycan(“MedlinePlus Genetics: POMT2 gene.,”). This enzyme complex is present in many tissues, but is especially high in the skeletal muscles, testes, and fetal brain[41]. The homozygous variant found in one family from our sample (*POMT2* p.Arg659Gln) has been predicted to be likely pathogenic based on two reports of muscular dystrophy-dystroglycanopathy (congenital brain and eye anomalies), type A2. Variants in *POMT2* have been reported in individuals affected by Walker-Warburg syndrome, or congenital muscular dystrophies that affect the muscles, brain, and anterior portion of the eye(Bouchet, Gonzales, Vuillaumier-Barrot, Devisme, Lebizec, Alanio, Bazin, Bessieres-Grattagliano, Bigi, Blanchet, Bonneau, Bonnieres, Carles, Delahaye, Fallet-Bianco, Figarella-Branger, Gaillard, Gasser, Guimiot, Joubert, Laurent, Liprandi, Loget, Marcorelles, Martinovic, Menez, Patrier, Pelluard-Nehme, Perez, Rouleau-

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Dubois, Triau, Laquerriere, Encha-Razavi, & Seta, 2007; van Reeuwijk, Janssen, van den Elzen, Beltran-Valero de Bernabe, Sabatelli, Merlini, Boon, Scheffer, Brockington, Muntoni, Huynen, Verrips, Walsh, Barth, Brunner, & van Bokhoven, 2005). Eye abnormalities include microphthalmia (small eyes), buphthalmos (enlarged eyes), cataracts, and problems with the optic nerve(Vajsar & Schachter, 2006).

Variant in Beta-1,3-Galactosyltransferase 6 (B3GALT6)

B3GALT6 encodes for an enzyme involved in biosynthesis of glycosaminoglycans (GAG), which are long chains of unbranched polysaccharides with a repeating disaccharide unit that are the most abundant heteropolysaccharide in the human eye(Pacella, Pacella, De Paolis, Parisella, Turchetti, Anello, & Cavallotti, 2015). GAGs are important for their ability to fill in space, including in the extracellular matrix between cells and to organize water molecules(Mattson, Turcotte, & Zhang, 2017). They also make up a portion of the cornea, where the transparency is due to uniform distribution of collagen fibrils which are regulated by proteoglycans(Maurice, 1967; Millodot, 2008). Human studies have demonstrated that GAGs play an important role in age-related diseases of the cornea(Pacella, Pacella, De Paolis, Parisella, Turchetti, Anello, & Cavallotti, 2015). *B3GALT6* variants have been reported in spondyloepimetaphyseal dysplasia (SEMD), a group of skeletal disorders, some of which show mild craniofacial dysmorphism including prominent eyes(Nakajima, Mizumoto, Miyake, Kogawa, Iida, Ito, Kitoh, Hirayama, Mitsubuchi, Miyazaki, Kosaki, Horikawa, Lai, Mendoza-Londono, Dupuis, Chitayat, Howard, Leal, Cavalcanti, Tsurusaki, Saitsu, Watanabe, Lausch, Unger, Bonafe, Ohashi, Superti-Furga, Matsumoto, Sugahara, Nishimura, & Ikegawa, 2013). There have not been any reports of variants in *B3GALT6* in congenital glaucoma cases. The missense variant identified in our study (*B3GALT6* p.Thr266Phe) is not among those that have been reported in patients with SEMD.[50]

Variant in Piezo Type Mechanosensitive Ion Channel Component 2 (PIEZO2)

PIEZO2 encodes a large mechanically-activated cation channel with more than thirty transmembrane domains(“Gene Cards: PIEZO2 Gene. Human Gene Database.,” 2019). Variants have been reported in patients with Distal Arthrogryposis Type 5, Marden-Walker syndrome, and Distal Arthrogryposis Type 3, all of which may present with ocular abnormalities(McMillin, Beck, Chong, Shively, Buckingham, Gildersleeve, Aracena, Aylsworth, Bitoun, Carey, Clericuzio, Crow, Curry, Devriendt, Everman, Fryer, Gibson, Giovannucci Uzielli, Graham, Hall, Hecht, Heidenreich, Hurst, Irani, Krapels, Leroy, Mowat, Plant, Robertson, Schorry, Scott, Seaver, Sherr, Splitt, Stewart, Stumpel, Temel, Weaver, Whiteford, Williams, Tabor, Smith, Shendure, Nickerson, University of Washington Center for Mendelian, & Bamshad, 2014). The majority of cases reported to date with pathogenic *PIEZO* variants have Distal Arthrogryposis Type 5 with dominant (Coste, Houge, Murray, Stitziel, Bandell, Giovanni, Philippakis, Hoischen, Riemer, Steen, Steen, Mathur, Cox, Lebo, Rehm, Weiss, Wood, Maas, Sunyaev, & Patapoutian, 2013), (McMillin, Beck, Chong, Shively, Buckingham, Gildersleeve, Aracena, Aylsworth, Bitoun, Carey, Clericuzio, Crow, Curry, Devriendt, Everman, Fryer, Gibson, Giovannucci Uzielli, Graham, Hall, Hecht, Heidenreich, Hurst, Irani, Krapels, Leroy, Mowat, Plant, Robertson, Schorry, Scott, Seaver, Sherr, Splitt, Stewart, Stumpel, Temel, Weaver, Whiteford, Williams, Tabor, Smith, Shendure, Nickerson, & Bamshad, 2014) and recessive (Delle Vedove et al., 2016)

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forms reported. A recent study of 14 patients with microphthalmia and/or anophthalmia identified one patient with a heterozygous missense variant in *PIEZO2* (p.Ser234Leu) that was predicted to be deleterious using PolyPhen-1, PolyPhen-2, and SIFT, but predicted to be neutral by MAPP, PhD-SNP and SNAP(Matias-Perez, Garcia-Montano, Cruz-Aguilar, Garcia-Montalvo, Nava-Valdez, Barragan-Arevalo, Villanueva-Mendoza, Villarroel, Guadarrama-Vallejo, la Cruz, Chacon-Camacho, & Zenteno, 2018). We identified a *de novo* missense variant resulting in an amino acid change (*PIEZO2* p.Ile1441Val) that is predicted to be damaging based on the PolyPhen2 score.

Variant in Retinoid X Receptor Alpha (RXRA)

Retinoic acid, a metabolite of vitamin A, serves as a signaling molecule during embryonic development of a number of tissues including the eye(Cvekl & Wang, 2009). Retinoic acid receptor alpha (RXRA*)* is a receptor located in the nucleus that can mediate the biological effects of retinoids[55]. Exposure to retinoids, like isotretinoin, early in pregnancy has been implicated with a spectrum of neural crest-related phenotypes including craniofacial malformations and malformations of the central nervous system (CNS)(Mondal, S, & Mishra, 2017). A number of variants near *RXRA* have been associated with central corneal thickness, which has been shown to be thinner in glaucoma patients than the general population(Sng, Ang, & Barton, 2017), suggesting that variants near *RXRA* can cause ocular phenotypes. The variant found in our study includes an insertion in exon 8 of 10 that results in an early termination and a truncation of the RXRA protein from 462 amino acids to 368.

Variant in GLI Family Zinc Finger 2 (GLI2)

GLI2 encodes a C2H2-type zinc finger that has the ability to act as a mediator of Sonic hedgehog (shh) signaling which is important in embryonic development(Roessler, Ermilov, Grange, Wang, Grachtchouk, Dlugosz, & Muenke, 2005). Variants in this gene have been found in patients with holoprosencephaly-like features(Roessler, Du, Mullor, Casas, Allen, Gillessen-Kaesbach, Roeder, Ming, Ruiz i Altaba, & Muenke, 2003). Holoprosencephaly is the most common forebrain defect in humans, which can manifest in a failure of separation of the eye and forebrain(Roessler, Du, Mullor, Casas, Allen, Gillessen-Kaesbach, Roeder, Ming, Ruiz i Altaba, & Muenke, 2003; Roessler & Muenke, 2001). The variant found in this study resulted in the change from a hydrophobic to an uncharged amino acid toward the end of this protein. *GLI2* variants in the same region of the protein that have been reported in ClinVar among cases with holoprosencephaly have been reported as benign or likely benign(Landrum, Lee, Benson, Brown, Chao, Chitipiralla, Gu, Hart, Hoffman, Jang, Karapetyan, Katz, Liu, Maddipatla, Malheiro, McDaniel, Ovetsky, Riley, Zhou, Holmes, Kattman, & Maglott, 2018).

Variant in Aldehyde Dehydrogenase 3 Family Member A2 (ALDH3A2)

ALDH3A2 is a member of the aldehyde dehydrogenase family which includes proteins that catalyze the conversion of fatty aldehydes with medium- to very-long-chain fatty acids(Amr, El-Bassyouni, Ismail, Youness, El-Daly, Ebrahim, & El-Kamah, 2019). According to the Human Protein Atlas(“ALDH3A2. The Human Protein Atlas,”), *ALDH3A2* is not expressed in the eye but is present in a number of other tissues. This gene is frequently mutated

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in Sjogren-Larsson Syndrome, another Mendelian condition in which patients present with intellectual disability, spastic di- or tetraplegia, and ichthyosis(Naganuma, Takagi, Kanetake, Kitamura, Hattori, Miyakawa, Sassa, & Kihara, 2016). The variants discovered in one of our PCG trios were both heterozygous missense variants, with one variant predicted to be benign by PolyPhen2 and the other in exon 7 predicted to be possibly damaging. Approximately one-third of Sjogren-Larsson syndrome-causing variants in *ALDH3A2* are missense variants, including some in exon 7(Weustenfeld, Eidelpes, Schmuth, Rizzo, Zschocke, & Keller, 2019).

Variant in Usherin (USH2A)

USH2A encodes for a basement membrane protein called usherin which is present in the inner ear and retina(Huang, Mao, Yang, Li, Li, & Yang, 2018). Among other genes, *USH2A* has been implicated in Usher syndrome which can include retinitis pigmentosa (RP), an eye condition that can result in gradual vision loss(Huang, Mao, Yang, Li, Li, & Yang, 2018; Seyedahmadi, Rivolta, Keene, Berson, & Dryja, 2004). Although vision loss is reported in both PCG and patients with RP, the age at onset for visual loss in RP patients is typically after age 10 years("Usher syndrome. Rare Disease Database," 2018). The *USH2A* p.Asp778Tyr variant has been identified in patients with Usher syndrome. There are conflicting reports of the pathogenicity of the *USH2A* p.Asp778Tyr variant in ClinVar with one report of it being pathogenic for retinal dystrophy(Lenassi, Vincent, Li, Saihan, Coffey, Steele-Stallard, Moore, Steel, Luxon, Heon, Bitner-Glindzicz, & Webster, 2015; Santos, Molina Thurin, Gustavo Vargas, Izquierdo, & Oliver, 2022).

Variant in UDP Glucuronosyltranferase Family 1 Member A8 (UGT1A8)

UGT1A8 encodes an enzyme that is part of the glucuronidation pathway that transforms small lipophilic molecules like steroids, hormones, and water-soluble, excretable metabolites[73]. *UGT1A8* is associated with drug metabolism pathways that include cytochrome P450 enzymes (in which *CYP1B1* belongs) and is mostly expressed in hepatic cells("UGT1A8. Human Gene Database," ; Xiao, Nunome, Yahara, Inoue, Nabeshima, Tsuchida, Hamaue, & Aoki, 2014). Most of the conditions associated with genes in this family include conditions of the gastrointestinal tract and the liver, such as pericholangitis("UGT1A8. Human Gene Database,") and Gilbert syndrome(Ehmer, Kalthoff, Fakundiny, Pabst, Freiberg, Naumann, Manns, & Strassburg, 2012) with unknown modes of inheritance.

Conclusions

We identified several potentially pathogenic variants which may offer insight into the etiology of PCG in the US. Similar to previous work, we found that *CYP1B1* was the gene most commonly affected. The frequency of variants within this gene in our sample (13.5%) was lower than reported in a Moroccan study,(Hilal, Boutayeb, Serrou, Refass-Buret, Shisseh, Bencherifa, El Mzibri, Benazzouz, & Berraho, 2010) in other studies of heterogeneous populations with primarily European (22–48%)(Campos-Mollo, Lopez-Garrido, Blanco-Marchite, Garcia-Feijoo, Peralta, Belmonte-Martinez, Ayuso, & Escribano, 2009; Colomb, Kaplan, & Garchon, 2003; Dimasi, Hewitt, Straga, Pater, MacKinnon, Elder, Casey, Mackey, & Craig, 2007) or Asian (17–22%)(Y. Chen, Jiang, Yu, Katz, Zhang,

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Wan, & Sun, 2008; Kakiuchi-Matsumoto, Isashiki, Ohba, Kimura, Sonoda, & Unoki, 2001; Sitorus, Ardjo, Lorenz, & Preising, 2003) ancestry, or relatively homogeneous populations with higher rates of endogamy and consanguinity (70–100%)(Alfadhli, Behbehani, Elshafey, Abdelmoaty, & Al-Awadi, 2006; Bejjani, Stockton, Lewis, Tomey, Dueker, Jabak, Astle, & Lupski, 2000; Chitsazian, Tusi, Elahi, Saroei, Sanati, Yazdani, Pakravan, Nilforooshan, Eslami, Mehrjerdi, Zareei, Jabbarvand, Abdolahi, Lasheyee, Etemadi, Bayat, Sadeghi, Banoei, Ghafarzadeh, Rohani, Rismanchian, Thorstenson, & Sarfarazi, 2007; Plasilova, Stoilov, Sarfarazi, Kadasi, Ferakova, & Ferak, 1999); however, it is similar to a previous estimate near 15% in a US-based population(Lim, Tran-Viet, Yanovitch, Freedman, Klemm, Call, Powell, Ravichandran, Metlapally, Nading, Rozen, & Young, 2013). In addition, we identified a family with a variant in *FOXC1* which has already been linked to PCG, as reviewed elsewhere(Ling, Zhang, Zhang, Sun, Du, & Li, 2020).

A major strength of this study is the design of the NBDPS, a multi-year, population-based study which includes participants from 10 different states. These participants reflect race/ethnic, geographic, and socioeconomic diversity generally representative of the US population(Cogswell, Bitsko, Anderka, Caton, Feldkamp, Hockett Sherlock, Meyer, Ramadhani, Robbins, Shaw, Mathews, Royle, Reefhuis, & National Birth Defects Prevention, 2009). Furthermore, ascertainment and classification methods were rigorous and standardized across centers ensuring thorough and uniform data collection. Our examination of those included WES trios compared to all PCG cases showed few differences. More broadly, individuals who chose to participate in NBDPS were not substantially different than those who chose not to be interviewed, which further provides support of the representativeness of our sample(Forestieri, Desrosiers, Freedman, Aylsworth, Voltzke, Olshan, Meyer, & National Birth Defects Prevention, 2019). Clinical review and classification of probands infants provides a rigorously defined case sample. This study also benefited from the availability of WES which facilitated the analysis of genes/variants beyond sequencing or arrays focused on candidate genes. Much like the strengths of genome-wide association studies(Hirschhorn & Daly, 2005), WES allows for a comprehensive scan of protein-coding genes in a less biased way to identify important and novel factors. Further, in this analysis we had WES available from complete trios which allows for high confidence in the inheritance patterns of each of these variants and likely fewer false positive results.

There are some limitations of this study. NBDPS is one of the largest studies of birth defects in the US, though cases are limited to infants diagnosed within the first year of life. Although close to 80% of infants with PCG are diagnosed within the first year of life(Allingham, Damji, Freedman, Mori, & Shafranov, 2005), this means that our case sample might miss approximately 20% of cases. Those missing may include milder cases that are not diagnosed until after one year of age or those who are diagnosed later for other reasons (e.g., reduced access to healthcare). Despite a few reports(Cascella, Strafella, Germani, Novelli, Ricci, Zampatti, & Giardina, 2015; Souma, Tompson, Thomson, Siggs, Kizhatil, Yamaguchi, Feng, Limviphuvadh, Whisenhunt, Maurer-Stroh, Yanovitch, Kalaydjieva, Azmanov, Finzi, Mauri, Javadiyan, Souzeau, Zhou, Hewitt, Kloss, Burdon, Mackey, Allen, Ruddle, Lim, Rozen, Tran-Viet, Liu, John, Wiggs, Pasutto, Craig, Jin, Quaggin, & Young, 2016) in the literature of PCG cases being inherited in an autosomal

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dominant manner, none of the parents in our sample were affected, and it is difficult to conclusively assign pathogenicity to variants with reduced penetrance. While a strength of the study was the use of WES, this method limits detection of changes outside of the exome that might influence gene expression. We also did not investigate copy number or structural variants or Sanger validate candidate variants due to limited DNA, although genotype-specific variant quality metrics provided in supplemental tables indicate we have reported high quality genotypes. Replication studies in an independent population would strengthen the evidence for association of these genes with PCG and lead to a higher priority for validation and/or animal studies. It is possible that these rare variants may be specific to individual families and thus findings will be difficult to replicate without sequencing exomes to identify these and possibly other rare variants in the genes in other large populations, of which there are few in the US.

In conclusion, this study confirms previous findings that although *CYP1B1* is the most frequently mutated gene in PCG, *CYP1B1* variants are less common in the US compared to other populations. We identified candidate variants in 19 genes in 16 families. These variants all lie within genes which have not previously been investigated in association with PCG, including some which are known to underlie other Mendelian conditions with phenotypic overlap, are important in eye development, and have variants that appear to have functional consequences. Future studies to replicate these findings in cohorts of patients without variants in known PCG genes could be informative. Experimental studies would inform the mechanisms associated with the variants reported.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Abu-Hassan DW, Acott TS, & Kelley MJ (2014). The Trabecular Meshwork: A Basic Review of Form and Function. J Ocul Biol, 2(1). doi:10.13188/2334-2838.1000017

AK M, & PA N (2006). The Pediatric Glaucomas: Elsevier Inc.

Alanazi FF, Song JC, Mousa A, Morales J, Al Shahwan S, Alodhayb S, Al Jadaan I, Al-Turkmani S, & Edward DP (2013). Primary and secondary congenital glaucoma: baseline features from a registry at King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia. Am J Ophthalmol, 155(5), 882–889. doi:10.1016/j.ajo.2012.12.006 [PubMed: 23394909]

ALDH3A2. The Human Protein Atlas. Retrieved from https://www.proteinatlas.org/ENSG00000072210-ALDH3A2/tissue

Alfadhli S, Behbehani A, Elshafey A, Abdelmoaty S, & Al-Awadi S (2006). Molecular and clinical evaluation of primary congenital glaucoma in Kuwait. Am J Ophthalmol, 141(3), 512–516. doi:10.1016/j.ajo.2005.11.001 [PubMed: 16490498]

Allingham R, Damji K, Freedman S, Mori S, & Shafranov G (2005). Shield's Textbook of Glaucoma. Philadelphia: Lippincott Williams and Wilkins.

Alsaif HS, Khan AO, Patel N, Alkuraya H, Hashem M, Abdulwahab F, Ibrahim N, Aldahmesh MA, & Alkuraya FS (2019). Congenital glaucoma and CYP1B1: an old story revisited. Hum Genet, 138(8–9), 1043–1049. doi:10.1007/s00439-018-1878-z [PubMed: 29556725]

Amr K, El-Bassyouni HT, Ismail S, Youness E, El-Daly SM, Ebrahim AY, & El-Kamah G (2019). Genetic assessment of ten Egyptian patients with Sjogren-Larsson syndrome: expanding the clinical spectrum and reporting a novel ALDH3A2 mutation. Arch Dermatol Res, 311(9), 721–730. doi:10.1007/s00403-019-01953-6 [PubMed: 31388754]

Bejjani BA, Stockton DW, Lewis RA, Tomey KF, Dueker DK, Jabak M, Astle WF, & Lupski JR (2000). Multiple CYP1B1 mutations and incomplete penetrance in an inbred population segregating primary congenital glaucoma suggest frequent de novo events and a dominant modifier locus. Hum Mol Genet, 9(3), 367–374. doi:10.1093/hmg/9.3.367 [PubMed: 10655546]

Bouchet C, Gonzales M, Vuillaumier-Barrot S, Devisme L, Lebizec C, Alanio E, Bazin A, Bessieres-Grattagliano B, Bigi N, Blanchet P, Bonneau D, Bonnieres M, Carles D, Delahaye S, Fallet-Bianco C, Figarella-Branger D, Gaillard D, Gasser B, Guimiot F, Joubert M, Laurent N, Liprandi A, Loget P, Marcorelles P, Martinovic J, Menez F, Patrier S, Pelluard-Nehme F, Perez MJ, Rouleau-Dubois C, Triau S, Laquerriere A, Encha-Razavi F, & Seta N (2007). Molecular heterogeneity in fetal forms of type II lissencephaly. Hum Mutat, 28(10), 1020–1027. doi:10.1002/humu.20561 [PubMed: 17559086]

Campos-Mollo E, Lopez-Garrido MP, Blanco-Marchite C, Garcia-Feijoo J, Peralta J, Belmonte-Martinez J, Ayuso C, & Escribano J (2009). CYP1B1 mutations in Spanish patients with primary congenital glaucoma: phenotypic and functional variability. Mol Vis, 15, 417–431. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19234632 [PubMed: 19234632]

Cascella R, Strafella C, Germani C, Novelli G, Ricci F, Zampatti S, & Giardina E (2015). The Genetics and the Genomics of Primary Congenital Glaucoma. Biomed Res Int, 2015, 321291. doi:10.1155/2015/321291 [PubMed: 26451367]

Chakrabarti S, Kaur K, Rao KN, Mandal AK, Kaur I, Parikh RS, & Thomas R (2009). The transcription factor gene FOXC1 exhibits a limited role in primary congenital glaucoma. Invest Ophthalmol Vis Sci, 50(1), 75–83. doi:10.1167/iovs.08-2253 [PubMed: 18708620]

Chen Q, Dowhan DH, Liang D, Moore DD, & Overbeek PA (2002). CREB-binding protein/p300 co-activation of crystallin gene expression. J Biol Chem, 277(27), 24081–24089. doi:10.1074/jbc.M201821200 [PubMed: 11943779]

Chen Y, Jiang D, Yu L, Katz B, Zhang K, Wan B, & Sun X (2008). CYP1B1 and MYOC mutations in 116 Chinese patients with primary congenital glaucoma. Arch Ophthalmol, 126(10), 1443–1447. doi:10.1001/archopht.126.10.1443 [PubMed: 18852424]

Chitsazian F, Tusi BK, Elahi E, Saroei HA, Sanati MH, Yazdani S, Pakravan M, Nilforooshan N, Eslami Y, Mehrjerdi MA, Zareei R, Jabbarvand M, Abdolahi A, Lasheyee AR, Etemadi A, Bayat B, Sadeghi M, Banoei MM, Ghafarzadeh B, Rohani MR, Rismanchian A, Thorstenson Y, & Sarfarazi M (2007). CYP1B1 mutation profile of Iranian primary congenital glaucoma patients and

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associated haplotypes. J Mol Diagn, 9(3), 382–393. doi:10.2353/jmoldx.2007.060157 [PubMed: 17591938]

Cogswell ME, Bitsko RH, Anderka M, Caton AR, Feldkamp ML, Hockett Sherlock SM, Meyer RE, Ramadhani T, Robbins JM, Shaw GM, Mathews TJ, Royle M, Reefhuis J, & National Birth Defects Prevention, S. (2009). Control selection and participation in an ongoing, population-based, case-control study of birth defects: the National Birth Defects Prevention Study. Am J Epidemiol, 170(8), 975–985. doi:10.1093/aje/kwp226 [PubMed: 19736223]

Colomb E, Kaplan J, & Garchon HJ (2003). Novel cytochrome P450 1B1 (CYP1B1) mutations in patients with primary congenital glaucoma in France. Hum Mutat, 22(6), 496. doi:10.1002/humu.9197

Consortium UK, Walter K, Min JL, Huang J, Crooks L, Memari Y, McCarthy S, Perry JR, Xu C, Futema M, Lawson D, Iotchkova V, Schiffels S, Hendricks AE, Danecek P, Li R, Floyd J, Wain LV, Barroso I, Humphries SE, Hurles ME, Zeggini E, Barrett JC, Plagnol V, Richards JB, Greenwood CM, Timpson NJ, Durbin R, & Soranzo N (2015). The UK10K project identifies rare variants in health and disease. Nature, 526(7571), 82–90. doi:10.1038/nature14962 [PubMed: 26367797]

Cooper GM, Stone EA, Asimenos G, Program NCS, Green ED, Batzoglou S, & Sidow A (2005). Distribution and intensity of constraint in mammalian genomic sequence. Genome Res, 15(7), 901–913. doi:10.1101/gr.3577405 [PubMed: 15965027]

Coste B, Houge G, Murray MF, Stitziel N, Bandell M, Giovanni MA, Philippakis A, Hoischen A, Riemer G, Steen U, Steen VM, Mathur J, Cox J, Lebo M, Rehm H, Weiss ST, Wood JN, Maas RL, Sunyaev SR, & Patapoutian A (2013). Gain-of-function mutations in the mechanically activated ion channel PIEZO2 cause a subtype of Distal Arthrogryposis. Proc Natl Acad Sci U S A, 110(12), 4667–4672. doi:10.1073/pnas.1221400110 [PubMed: 23487782]

Cvekl A, & Wang WL (2009). Retinoic acid signaling in mammalian eye development. Exp Eye Res, 89(3), 280–291. doi:10.1016/j.exer.2009.04.012 [PubMed: 19427305]

deLuise VP, & Anderson DR (1983). Primary infantile glaucoma (congenital glaucoma). Surv Ophthalmol, 28(1), 1–19. doi:10.1016/0039-6257(83)90174-1 [PubMed: 6353647]

Demenais F, Bonaiti C, Briard ML, Feingold J, & Frezal J (1979). Congenital glaucoma: genetic models. Hum Genet, 46(3), 305–317. doi:10.1007/BF00273314 [PubMed: 437773]

Dimasi DP, Hewitt AW, Straga T, Pater J, MacKinnon JR, Elder JE, Casey T, Mackey DA, & Craig JE (2007). Prevalence of CYP1B1 mutations in Australian patients with primary congenital glaucoma. Clin Genet, 72(3), 255–260. doi:10.1111/j.1399-0004.2007.00864.x [PubMed: 17718864]

Driessen HP, Herbrink P, Bloemendal H, & de Jong WW (1980). The beta-crystallin Bp chain is internally duplicated and homologous with gamma-crystallin. Exp Eye Res, 31(2), 243–246. doi:10.1016/0014-4835(80)90082-2 [PubMed: 7428846]

Ehmer U, Kalthoff S, Fakundiny B, Pabst B, Freiberg N, Naumann R, Manns MP, & Strassburg CP (2012). Gilbert syndrome redefined: a complex genetic haplotype influences the regulation of glucuronidation. Hepatology, 55(6), 1912–1921. doi:10.1002/hep.25561 [PubMed: 22213127]

Elder SD (1969). Congential Deformities. St. Louis: CV Mosby.

Forestieri NE, Desrosiers TA, Freedman SF, Aylsworth AS, Voltzke K, Olshan AF, Meyer RE, & National Birth Defects Prevention, S. (2019). Risk factors for primary congenital glaucoma in the National Birth Defects Prevention Study. Am J Med Genet A, 179(9), 1846–1856. doi:10.1002/ajmg.a.61296 [PubMed: 31313509]

Francois J (1980). Congenital glaucoma and its inheritance. Ophthalmologica, 181(2), 61–73. doi:10.1159/000309028 [PubMed: 7219964]

François J (1961). Heredity in Ophthalmology: C. V. Mosby Company.

Gallagher ML, Sturchio C, Smith A, Koontz D, Jenkins MM, Honein MA, & Rasmussen SA (2011). Evaluation of mailed pediatric buccal cytobrushes for use in a case-control study of birth defects. Birth Defects Res A Clin Mol Teratol, 91(7), 642–648. doi:10.1002/bdra.20829 [PubMed: 21630425]

Ganguly K, Favor J, Neuhauser-Klaus A, Sandulache R, Puk O, Beckers J, Horsch M, Schadler S, Vogt Weisenhorn D, Wurst W, & Graw J (2008). Novel allele of crybb2 in the mouse and its expression

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in the brain. Invest Ophthalmol Vis Sci, 49(4), 1533–1541. doi:10.1167/iovs.07-0788 [PubMed: 18385073]

Gencik A, Gencikova A, & Gerinec A (1980). Genetic heterogeneity of congenital glaucoma. Clin Genet, 17(4), 241–248. doi:10.1111/j.1399-0004.1980.tb00142.x [PubMed: 7371217]

Gene Cards: PIEZO2 Gene Human Gene Database. (2019). Retrieved from https://www.genecards.org/cgi-bin/carddisp.pl?gene=PIEZO2

Goodman RH, & Smolik S (2000). CBP/p300 in cell growth, transformation, and development. Genes Dev, 14(13), 1553–1577. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10887150 [PubMed: 10887150]

Hennekam RC (2006). Rubinstein-Taybi syndrome. Eur J Hum Genet, 14(9), 981–985. doi:10.1038/sj.ejhg.5201594 [PubMed: 16868563]

Hilal L, Boutayeb S, Serrou A, Refass-Buret L, Shisseh H, Bencherifa F, El Mzibri M, Benazzouz B, & Berraho A (2010). Screening of CYP1B1 and MYOC in Moroccan families with primary congenital glaucoma: three novel mutations in CYP1B1. Mol Vis, 16, 1215–1226. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/20664688 [PubMed: 20664688]

Hirschhorn JN, & Daly MJ (2005). Genome-wide association studies for common diseases and complex traits. Nat Rev Genet, 6(2), 95–108. doi:10.1038/nrg1521 [PubMed: 15716906]

Huang L, Mao Y, Yang J, Li Y, Li Y, & Yang Z (2018). Mutation screening of the USH2A gene in retinitis pigmentosa and USHER patients in a Han Chinese population. Eye (Lond), 32(10), 1608–1614. doi:10.1038/s41433-018-0130-3 [PubMed: 29899460]

Illumina. Quality Scores for Next-Generation Sequencing. Retrieved from https://www.illumina.com/documents/products/technotes/technote_Q-Scores.pdf

Jenkins MM, Almli LM, Pangilinan F, Chong JX, Blue EE, Shapira SK, White J, McGoldrick D, Smith JD, Mullikin JC, Bean CJ, Nembhard WN, Lou XY, Shaw GM, Romitti PA, Keppler-Noreuil K, Yazdy MM, Kay DM, Carter TC, Olshan AF, Moore KJ, Nascone-Yoder N, Finnell RH, Lupo PJ, Feldkamp ML, Program NCS, University of Washington Center for Mendelian, G., Nickerson DA, Bamshad MJ, Brody LC, Reefhuis J, & National Birth Defects Prevention, S. (2019). Exome sequencing of family trios from the National Birth Defects Prevention Study: Tapping into a rich resource of genetic and environmental data. Birth Defects Res, 111(20), 1618–1632. doi:10.1002/bdr2.1554 [PubMed: 31328417]

Jun G, Flickinger M, Hetrick KN, Romm JM, Doheny KF, Abecasis GR, Boehnke M, & Kang HM (2012). Detecting and estimating contamination of human DNA samples in sequencing and array-based genotype data. Am J Hum Genet, 91(5), 839–848. doi:10.1016/j.ajhg.2012.09.004 [PubMed: 23103226]

Kakiuchi-Matsumoto T, Isashiki Y, Ohba N, Kimura K, Sonoda S, & Unoki K (2001). Cytochrome P450 1B1 gene mutations in Japanese patients with primary congenital glaucoma(1). Am J Ophthalmol, 131(3), 345–350. doi:10.1016/s0002-9394(00)00808-4 [PubMed: 11239867]

Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, Chitipiralla S, Gu B, Hart J, Hoffman D, Jang W, Karapetyan K, Katz K, Liu C, Maddipatla Z, Malheiro A, McDaniel K, Ovetsky M, Riley G, Zhou G, Holmes JB, Kattman BL, & Maglott DR (2018). ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res, 46(D1), D1062–D1067. doi:10.1093/nar/gkx1153 [PubMed: 29165669]

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG, & Exome Aggregation C (2016). Analysis of protein-coding genetic variation in 60,706 humans. Nature, 536(7616), 285–291. doi:10.1038/nature19057 [PubMed: 27535533]

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Lenassi E, Vincent A, Li Z, Saihan Z, Coffey AJ, Steele-Stallard HB, Moore AT, Steel KP, Luxon LM, Heon E, Bitner-Glindzicz M, & Webster AR (2015). A detailed clinical and molecular survey of subjects with nonsyndromic USH2A retinopathy reveals an allelic hierarchy of disease-causing variants. Eur J Hum Genet, 23(10), 1318–1327. doi:10.1038/ejhg.2014.283 [PubMed: 25649381]

Lewis CJ, Hedberg-Buenz A, DeLuca AP, Stone EM, Alward WLM, & Fingert JH (2017). Primary congenital and developmental glaucomas. Hum Mol Genet, 26(R1), R28–R36. doi:10.1093/hmg/ddx205 [PubMed: 28549150]

Lim SH, Tran-Viet KN, Yanovitch TL, Freedman SF, Klemm T, Call W, Powell C, Ravichandran A, Metlapally R, Nading EB, Rozen S, & Young TL (2013). CYP1B1, MYOC, and LTBP2 mutations in primary congenital glaucoma patients in the United States. Am J Ophthalmol, 155(3), 508–517 e505. doi:10.1016/j.ajo.2012.09.012 [PubMed: 23218701]

Ling C, Zhang D, Zhang J, Sun H, Du Q, & Li X (2020). Updates on the molecular genetics of primary congenital glaucoma (Review). Exp Ther Med, 20(2), 968–977. doi:10.3892/etm.2020.8767 [PubMed: 32742340]

Liskova P, Dudakova L, Tesar V, Bednarova V, Kidorova J, Jirsova K, Davidson AE, & Hardcastle AJ (2015). Detailed assessment of renal function in a proband with Harboyan syndrome caused by a novel homozygous SLC4A11 nonsense mutation. Ophthalmic Res, 53(1), 30–35. doi:10.1159/000365109 [PubMed: 25500497]

M., J., & N., R. (1978). Genetic implications of congenital glaucoma. Metab Pediatr Ophthalmol, 2, 257–258.

Mashima Y, Suzuki Y, Sergeev Y, Ohtake Y, Tanino T, Kimura I, Miyata H, Aihara M, Tanihara H, Inatani M, Azuma N, Iwata T, & Araie M (2001). Novel cytochrome P4501B1 (CYP1B1) gene mutations in Japanese patients with primary congenital glaucoma. Invest Ophthalmol Vis Sci, 42(10), 2211–2216. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11527932 [PubMed: 11527932]

Matias-Perez D, Garcia-Montano LA, Cruz-Aguilar M, Garcia-Montalvo IA, Nava-Valdez J, Barragan-Arevalo T, Villanueva-Mendoza C, Villarroel CE, Guadarrama-Vallejo C, la Cruz RV, Chacon-Camacho O, & Zenteno JC (2018). Identification of novel pathogenic variants and novel gene-phenotype correlations in Mexican subjects with microphthalmia and/or anophthalmia by next-generation sequencing. J Hum Genet, 63(11), 1169–1180. doi:10.1038/s10038-018-0504-1 [PubMed: 30181649]

Mattson JM, Turcotte R, & Zhang Y (2017). Glycosaminoglycans contribute to extracellular matrix fiber recruitment and arterial wall mechanics. Biomech Model Mechanobiol, 16(1), 213–225. doi:10.1007/s10237-016-0811-4 [PubMed: 27491312]

Maurice DM (1967). Epithelial potential of the cornea. Exp Eye Res, 6(2), 138–140. doi:10.1016/s0014-4835(67)80065-4 [PubMed: 6024067]

McKusick V (1968). Personal Communication. Baltimore: MD.

McMillin MJ, Beck AE, Chong JX, Shively KM, Buckingham KJ, Gildersleeve HI, Aracena MI, Aylsworth AS, Bitoun P, Carey JC, Clericuzio CL, Crow YJ, Curry CJ, Devriendt K, Everman DB, Fryer A, Gibson K, Giovannucci Uzielli ML, Graham JM Jr., Hall JG, Hecht JT, Heidenreich RA, Hurst JA, Irani S, Krapels IP, Leroy JG, Mowat D, Plant GT, Robertson SP, Schorry EK, Scott RH, Seaver LH, Sherr E, Splitt M, Stewart H, Stumpel C, Temel SG, Weaver DD, Whiteford M, Williams MS, Tabor HK, Smith JD, Shendure J, Nickerson DA, & Bamshad MJ (2014). Mutations in PIEZO2 cause Gordon syndrome, Marden-Walker syndrome, and distal arthrogryposis type 5. Am J Hum Genet, 94(5), 734–744. doi:10.1016/j.ajhg.2014.03.015 [PubMed: 24726473]

McMillin MJ, Beck AE, Chong JX, Shively KM, Buckingham KJ, Gildersleeve HI, Aracena MI, Aylsworth AS, Bitoun P, Carey JC, Clericuzio CL, Crow YJ, Curry CJ, Devriendt K, Everman DB, Fryer A, Gibson K, Giovannucci Uzielli ML, Graham JM Jr., Hall JG, Hecht JT, Heidenreich RA, Hurst JA, Irani S, Krapels IP, Leroy JG, Mowat D, Plant GT, Robertson SP, Schorry EK, Scott RH, Seaver LH, Sherr E, Splitt M, Stewart H, Stumpel C, Temel SG, Weaver DD, Whiteford M, Williams MS, Tabor HK, Smith JD, Shendure J, Nickerson DA, University of Washington Center for Mendelian, G., & Bamshad MJ (2014). Mutations in PIEZO2 cause Gordon syndrome, Marden-Walker syndrome, and distal arthrogryposis type 5. Am J Hum Genet, 94(5), 734–744. doi:10.1016/j.ajhg.2014.03.015 [PubMed: 24726473]

MedlinePlus Genetics: POMT2 gene. Retrieved from https://medlineplus.gov/genetics/gene/pomt2/

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Menke LA, van Belzen MJ, Alders M, Cristofoli F, Study DDD, Ehmke N, Fergelot P, Foster A, Gerkes EH, Hoffer MJ, Horn D, Kant SG, Lacombe D, Leon E, Maas SM, Melis D, Muto V, Park SM, Peeters H, Peters DJ, Pfundt R, van Ravenswaaij-Arts CM, Tartaglia M, & Hennekam RC (2016). CREBBP mutations in individuals without Rubinstein-Taybi syndrome phenotype. Am J Med Genet A, 170(10), 2681–2693. doi:10.1002/ajmg.a.37800 [PubMed: 27311832]

Millodot M (2008). Dictionary of Optometry and Visual Science (7th ed.). Oxford: Butterworth-Heinemann.

Mondal D, S, R. S., & Mishra S (2017). Retinoic Acid Embryopathy. Int J Appl Basic Med Res, 7(4), 264–265. doi:10.4103/ijabmr.IJABMR_469_16 [PubMed: 29308367]

Mullaney PB, Risco JM, Teichmann K, & Millar L (1995). Congenital hereditary endothelial dystrophy associated with glaucoma. Ophthalmology, 102(2), 186–192. doi:10.1016/s0161-6420(95)31037-8 [PubMed: 7862405]

Naganuma T, Takagi S, Kanetake T, Kitamura T, Hattori S, Miyakawa T, Sassa T, & Kihara A (2016). Disruption of the Sjogren-Larsson Syndrome Gene Aldh3a2 in Mice Increases Keratinocyte Growth and Retards Skin Barrier Recovery. J Biol Chem, 291(22), 11676–11688. doi:10.1074/jbc.M116.714030 [PubMed: 27053112]

Nakajima M, Mizumoto S, Miyake N, Kogawa R, Iida A, Ito H, Kitoh H, Hirayama A, Mitsubuchi H, Miyazaki O, Kosaki R, Horikawa R, Lai A, Mendoza-Londono R, Dupuis L, Chitayat D, Howard A, Leal GF, Cavalcanti D, Tsurusaki Y, Saitsu H, Watanabe S, Lausch E, Unger S, Bonafe L, Ohashi H, Superti-Furga A, Matsumoto N, Sugahara K, Nishimura G, & Ikegawa S (2013). Mutations in B3GALT6, which encodes a glycosaminoglycan linker region enzyme, cause a spectrum of skeletal and connective tissue disorders. Am J Hum Genet, 92(6), 927–934. doi:10.1016/j.ajhg.2013.04.003 [PubMed: 23664117]

Pacella E, Pacella F, De Paolis G, Parisella FR, Turchetti P, Anello G, & Cavallotti C (2015). Glycosaminoglycans in the human cornea: age-related changes. Ophthalmol Eye Dis, 7, 1–5. doi:10.4137/OED.S17204 [PubMed: 25674020]

Paila U, Chapman BA, Kirchner R, & Quinlan AR (2013). GEMINI: integrative exploration of genetic variation and genome annotations. PLoS Comput Biol, 9(7), e1003153. doi:10.1371/journal.pcbi.1003153 [PubMed: 23874191]

Patel SP, & Parker MD (2015). SLC4A11 and the Pathophysiology of Congenital Hereditary Endothelial Dystrophy. Biomed Res Int, 2015, 475392. doi:10.1155/2015/475392 [PubMed: 26451371]

Pedersen BS, & Quinlan AR (2017). Who's Who? Detecting and Resolving Sample Anomalies in Human DNA Sequencing Studies with Peddy. Am J Hum Genet, 100(3), 406–413. doi:10.1016/j.ajhg.2017.01.017 [PubMed: 28190455]

Plasilova M, Ferakova E, Kadasi L, Polakova H, Gerinec A, Ott J, & Ferak V (1998). Linkage of autosomal recessive primary congenital glaucoma to the GLC3A locus in Roms (Gypsies) from Slovakia. Hum Hered, 48(1), 30–33. doi:10.1159/000022778 [PubMed: 9463798]

Plasilova M, Stoilov I, Sarfarazi M, Kadasi L, Ferakova E, & Ferak V (1999). Identification of a single ancestral CYP1B1 mutation in Slovak Gypsies (Roms) affected with primary congenital glaucoma. J Med Genet, 36(4), 290–294. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10227395 [PubMed: 10227395]

Rasmussen SA, Olney RS, Holmes LB, Lin AE, Keppler-Noreuil KM, Moore CA, & National Birth Defects Prevention, S. (2003). Guidelines for case classification for the National Birth Defects Prevention Study. Birth Defects Res A Clin Mol Teratol, 67(3), 193–201. doi:10.1002/bdra.10012 [PubMed: 12797461]

Reefhuis J, Gilboa SM, Anderka M, Browne ML, Feldkamp ML, Hobbs CA, Jenkins MM, Langlois PH, Newsome KB, Olshan AF, Romitti PA, Shapira SK, Shaw GM, Tinker SC, Honein MA, & National Birth Defects Prevention, S. (2015). The National Birth Defects Prevention Study: A review of the methods. Birth Defects Res A Clin Mol Teratol, 103(8), 656–669. doi:10.1002/bdra.23384 [PubMed: 26033852]

Reis LM, Tyler RC, Weh E, Hendee KE, Kariminejad A, Abdul-Rahman O, Ben-Omran T, Manning MA, Yesilyurt A, McCarty CA, Kitchner TE, Costakos D, & Semina EV (2016). Analysis of CYP1B1 in pediatric and adult glaucoma and other ocular phenotypes. Mol Vis, 22, 1229–1238. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27777502 [PubMed: 27777502]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Rentzsch P, Witten D, Cooper GM, Shendure J, & Kircher M (2019). CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res, 47(D1), D886–D894. doi:10.1093/nar/gky1016 [PubMed: 30371827]

Roessler E, Du YZ, Mullor JL, Casas E, Allen WP, Gillessen-Kaesbach G, Roeder ER, Ming JE, Ruiz i Altaba A, & Muenke M (2003). Loss-of-function mutations in the human GLI2 gene are associated with pituitary anomalies and holoprosencephaly-like features. Proc Natl Acad Sci U S A, 100(23), 13424–13429. doi:10.1073/pnas.2235734100 [PubMed: 14581620]

Roessler E, Ermilov AN, Grange DK, Wang A, Grachtchouk M, Dlugosz AA, & Muenke M (2005). A previously unidentified amino-terminal domain regulates transcriptional activity of wild-type and disease-associated human GLI2. Hum Mol Genet, 14(15), 2181–2188. doi:10.1093/hmg/ddi222 [PubMed: 15994174]

Roessler E, & Muenke M (2001). Midline and laterality defects: left and right meet in the middle. Bioessays, 23(10), 888–900. doi:10.1002/bies.1130 [PubMed: 11598956]

Santhiya ST, Kumar GS, Sudhakar P, Gupta N, Klopp N, Illig T, Soker T, Groth M, Platzer M, Gopinath PM, & Graw J (2010). Molecular analysis of cataract families in India: new mutations in the CRYBB2 and GJA3 genes and rare polymorphisms. Mol Vis, 16, 1837–1847. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21031021 [PubMed: 21031021]

Santos DF, Molina Thurin LJ, Gustavo Vargas J, Izquierdo NJ, & Oliver A (2022). A Genotype-Phenotype Analysis of Usher Syndrome in Puerto Rico: A Case Series. Cureus, 14(8), e28213. doi:10.7759/cureus.28213 [PubMed: 36003347]

Seyedahmadi BJ, Rivolta C, Keene JA, Berson EL, & Dryja TP (2004). Comprehensive screening of the USH2A gene in Usher syndrome type II and non-syndromic recessive retinitis pigmentosa. Exp Eye Res, 79(2), 167–173. doi:10.1016/j.exer.2004.03.005 [PubMed: 15325563]

Sitorus R, Ardjo SM, Lorenz B, & Preising M (2003). CYP1B1 gene analysis in primary congenital glaucoma in Indonesian and European patients. J Med Genet, 40(1), e9. doi:10.1136/jmg.40.1.e9 [PubMed: 12525557]

Sng CC, Ang M, & Barton K (2017). Central corneal thickness in glaucoma. Curr Opin Ophthalmol, 28(2), 120–126. doi:10.1097/ICU.0000000000000335 [PubMed: 27764022]

Souma T, Tompson SW, Thomson BR, Siggs OM, Kizhatil K, Yamaguchi S, Feng L, Limviphuvadh V, Whisenhunt KN, Maurer-Stroh S, Yanovitch TL, Kalaydjieva L, Azmanov DN, Finzi S, Mauri L, Javadiyan S, Souzeau E, Zhou T, Hewitt AW, Kloss B, Burdon KP, Mackey DA, Allen KF, Ruddle JB, Lim SH, Rozen S, Tran-Viet KN, Liu X, John S, Wiggs JL, Pasutto F, Craig JE, Jin J, Quaggin SE, & Young TL (2016). Angiopoietin receptor TEK mutations underlie primary congenital glaucoma with variable expressivity. J Clin Invest, 126(7), 2575–2587. doi:10.1172/JCI85830 [PubMed: 27270174]

Thorvaldsdottir H, Robinson JT, & Mesirov JP (2013). Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Brief Bioinform, 14(2), 178–192. doi:10.1093/bib/bbs017 [PubMed: 22517427]

UGT1A8. Human Gene Database. Retrieved from https://www.genecards.org/cgi-bin/carddisp.pl?gene=UGT1A8

Usher syndrome. Rare Disease Database. (2018). Disorders NO for R Retrieved from https://rarediseases.org/rare-diseases/usher-syndrome/

Vajsar J, & Schachter H (2006). Walker-Warburg syndrome. Orphanet J Rare Dis, 1, 29. doi:10.1186/1750-1172-1-29 [PubMed: 16887026]

van Reeuwijk J, Janssen M, van den Elzen C, Beltran-Valero de Bernabe D, Sabatelli P, Merlini L, Boon M, Scheffer H, Brockington M, Muntoni F, Huynen MA, Verrips A, Walsh CA, Barth PG, Brunner HG, & van Bokhoven H (2005). POMT2 mutations cause alpha-dystroglycan hypoglycosylation and Walker-Warburg syndrome. J Med Genet, 42(12), 907–912. doi:10.1136/jmg.2005.031963 [PubMed: 15894594]

Weustenfeld M, Eidelpes R, Schmuth M, Rizzo WB, Zschocke J, & Keller MA (2019). Genotype and phenotype variability in Sjogren-Larsson syndrome. Hum Mutat, 40(2), 177–186. doi:10.1002/humu.23679 [PubMed: 30372562]

Xiao Z, Nunome K, Yahara T, Inoue E, Nabeshima M, Tsuchida S, Hamaue N, & Aoki T (2014). Comparative studies of human UDP-glucuronosyltransferase 1A8 and 1A9 proximal

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promoters using single base substitutions. Drug Metab Pharmacokinet, 29(1), 90–93. doi:10.2133/dmpk.dmpk-13-nt-037 [PubMed: 23842475]

Yang Y, Wolf LV, & Cvekl A (2007). Distinct embryonic expression and localization of CBP and p300 histone acetyltransferases at the mouse alphaA-crystallin locus in lens. J Mol Biol, 369(4), 917–926. doi:10.1016/j.jmb.2007.04.004 [PubMed: 17467007]

Zhao WJ, Xu J, Chen XJ, Liu HH, Yao K, & Yan YB (2017). Effects of cataract-causing mutations W59C and W151C on betaB2-crystallin structure, stability and folding. Int J Biol Macromol, 103, 764–770. doi:10.1016/j.ijbiomac.2017.05.109 [PubMed: 28528950]

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Table 1.

Comparison of demographic characteristics of whole exome sequencing (WES) samples to all primary congenital glaucoma (PCG) cases within National Birth Defects Prevention Study, 1997–2011. Counts are provided, with percentages presented in parentheses. One male proband with Hispanic ancestry failed WES quality control and was excluded from subsequent analyses.

Demographic Characteristics	All PCG cases (n=136)	WES PCG samples (n=38)
Reported Maternal Race/Ethnicity		
Non-Hispanic White	63 (46.3%)	23 (60.6%)
Non-Hispanic Black	31 (22.8%)	9 (23.7%)
Hispanic	32 (23.5%)	2 (5.3%)
Asian/Pacific Islander	7 (5.2%)	2 (5.3%)
Other	3 (2.2%)	2 (5.3%)
Maternal Age at Delivery (years)		
≤24	53 (39.0%)	9 (23.7%)
25–34	71 (52.2%)	25 (65.8%)
≥35	12 (8.8%)	4 (10.5%)
Paternal Age At Delivery (years)		
≤24	31 (24.0%)	7 (18.9%)
25–34	67 (51.9%)	21 (56.8%)
≥35	31 (24.0%)	9 (24.3%)

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Table 2.

CYP1B1 and *FOXC1* variants in 6 primary congenital glaucoma families in the National Birth Defects Prevention Study, 1997–2011. Genotype details are available in Supplemental Table 1.

Family	Reported Maternal Race/Ethnicity	Gene	Amino Acid Change (cDNA)	Impact	ClinVar Allele ID	ClinVar Interpretation
10	Non-Hispanic White	*CYP1B1*	p.Arg355fs (c.1064_1076del) p.Thr404fs (c.1200_1209dup)	Frameshift Frameshift	266801 79358	Pathogenic Pathogenic
12	Non-Hispanic Black	*CYP1B1*	p.Glu387Lys (c.1159G>A) p.Pro437Leu (c.1310C>T)	Missense Missense	22774 1244562	Pathogenic/Likely Pathogenic Likely Pathogenic
13	Non-Hispanic White	*CYP1B1*	p.Gln19* (c.55C>T) p.Glu387Lys (c.1159G>A)	Stop Gain Missense	1371817 22774	Pathogenic Pathogenic/Likely Pathogenic
16	Non-Hispanic White	*CYP1B1*	p.Arg290fs (c.868dup) p.Trp57* (c.171G>A)	Frameshift Stop Gain	79360 22776	Pathogenic Pathogenic/Likely Pathogenic
33	Non-Hispanic White	*CYP1B1*	p.Asn423Tyr (c.1267A>T) p.Arg355fs (c.1064_1076del)	Missense Frameshift	22782 266801	Likely Pathogenic Pathogenic
22	Non-Hispanic White[†]	*FOXC1*	p.Ala381GlyfsTer147 (c.1141dup)	Frameshift	1055580	Pathogenic

[†]Ancestry inferred by genetic analysis (Pedersen & Quinlan, 2017).

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Table 3.

Identification of novel candidate primary congenital glaucoma genes in families using a filter-based approach in the National Birth Defects Prevention Study, 1997–2011.

Inheritance Model	Family	Gene	Impact	Consequence	dbSNP ID	CADD Score	PolyPhen2	GERP Score
De novo	7	*CRYBB2*	Missense	p.Trp59Arg*	N/A	21.8	Probably Damaging	5.08
	7	*CNTROB*	Missense	p.Arg752Cys	rs186932749	29.7	Probably Damaging	6.17
	8	*COL2A1*	Missense	p.Arg496Cys	rs121912884	22.5	Probably Damaging	5.05
	20	*ABCC5*	Missense	p.Arg1037Gly	N/A	26.5	Probably Damaging	5.81
	20	*PRDM12*	Missense	p.Asp151Asn	rs773763118	31.0	Probably Damaging	5.21
	25	*ROBO2*	Missense	p.Arg25Cys	N/A	20.2	Probably Damaging	5.12
	26≠	*RELN*	Splice Donor Variant	c.5969+1G>A	rs869320767	27.2	N/A	5.77
	30	*CRKL*	Missense	p.Glu158Asn	N/A	36.0	Possibly Damaging	5.57
Recessive	30	*POMT2*	Missense	p.Arg659Gln (homozygous)*	rs770606360	34.0	Probably Damaging	5.68

Abbreviations: CADD, combined annotation dependent depletion; GERP, genomic evolutionary rate profiling; PolyPhen2, polymorphism phenotypic v2, N/A=Not applicable.

*Variants were identified using both the filter-based and biological plausibility-based approaches and are therefore listed in both Tables 3 and 4.

≠One parent in this family had high freemix levels (0.09) indicative of sample contamination.

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Table 4.

Identification of novel candidate primary congenital glaucoma genes in families using a biological plausibility approach in the National Birth Defects Prevention Study, 1997–2011.

Inheritance Model	Family	Gene	Impact	Consequence	dbSNP ID	CADD Score	PolyPhen2	GERP Score
Recessive	1	*ALDH3A2*	Missense/ Missense	p.Ala360Val/ p.Ser505Gly	rs759058578/ rs200501128	15.8/ 9.2	Possibly Damaging/ Benign	5.12/ 0.75
	2	*CREBBP*	Missense Missense	p.Ser893Leu/ p.Thr856Ala	rs142047649/ rs373531233	12.6/ 16.7	Benign/ Possibly Damaging	5.32/ 5.69
	5	*USH2A*	Frameshift/ Missense	p.Thr5135fs/ p.Asp778Tyr	rs770984400/ rs142898216	N/A/ 21.9	Unknown/ Probably Damaging	4.75/ 6.06
	11	*UGT1A8*	Missense/ Missense	p.Asp151Asn/ p.Leu228Ile	rs14739512/ rs45568432	10.2/ 0.7	Possibly Damaging/ Benign	3.06/ −7.92
	21	*SLC4A11*	Missense/ Stop Gain	p.Met856Lys/ p.Arg730*	N/A / rs772409032	23.5/ 37.0	Possibly Damaging/ Unknown	5.37/ 4.06
	30	*POMT2*	Missense	p.Arg659Gln (homozygous)**	rs770606360	34.0	Probably Damaging	5.68
De novo	7	*CRYBB2*	Missense	p.Trp59Arg**	N/A	21.8	Probably Damaging	5.08
	9	*RXRA*	Stop Gain	c.1101_1102ins96	N/A	N/A	Unknown	3.92
	27≠	*GLI2*	Missense	p.Ala1398Thr	rs768730033	10.2	Benign	3.83
	34	*PIEZO2*	Missense	p.Ile1441Val	N/A	14.3	Probably Damaging	5.57
	37	*B3GALT6*	Missense	p.Thr266Phe	N/A	9.8	Possibly Damaging	2.89

Abbreviations: CADD, combined annotation dependent depletion; GERP, genomic evolutionary rate profiling; PolyPhen2, polymorphism phenotypic v2, N/A=Not applicable.

≠One parent in this family had high freemix levels (0.035) indicative of sample contamination.

**Variants were identified using both the filter-based and biological plausibility-based approaches, and are therefore listed in both Tables 3 and 4.