

CARRIER FREQUENCY OF *CYP1B1* MUTATIONS IN THE UNITED STATES (AN AMERICAN OPHTHALMOLOGICAL SOCIETY THESIS)

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ABSTRACT

Purpose: *CYP1B1* mutations cause autosomal recessive congenital glaucoma. Disease risk assessment for families with *CYP1B1* mutations requires knowledge of the population mutation carrier frequency. The purpose of this study is to determine the *CYP1B1* mutation carrier frequency in clinically normal individuals residing in the United States. Because *CYP1B1* mutations can exhibit variable expressivity, we hypothesize that the mutation carrier frequency is higher than expected.

Methods: Two hundred fifty individuals without glaucoma or a family history of glaucoma were enrolled. *CYP1B1* mutations were identified by DNA sequencing, and pathogenicity was estimated by PolyPhen-2 or a previous report of disease causality.

Results: Based on the disease frequency (1 in 10,000) and prevalence of *CYP1B1*-related congenital glaucoma (15% to 20%), the frequency of *CYP1B1*-related congenital glaucoma in the United States is approximately 1 in 50,000. Assuming Hardy-Weinberg equilibrium, the expected *CYP1B1* mutation carrier frequency would be 1 in 112, or 0.89%. Among the 250 study participants, 11 (4.4%) are carriers of a single pathogenic mutation, representing a carrier frequency of 1 in 22, which is 5.1 times the expected frequency. A higher-than-expected carrier frequency (1 in 33, 3.0%) was also observed in 4300 white individuals sequenced by the National Heart Lung and Blood Institute Exome Sequencing Project.

Conclusions: Our results show that the *CYP1B1* mutation carrier frequency in the US population is between 1 in 22 and 1 in 33, which is 5.1 to 3.4 times the expected frequency. These results suggest that more individuals than expected are carriers of a deleterious *CYP1B1* mutation, and that the prevalence of *CYP1B1*-related disease may be higher than expected.

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INTRODUCTION

Congenital or infantile glaucoma is an important cause of childhood blindness that is usually recognized during the first year of life.¹ Inheritance is primarily autosomal recessive, although pedigrees with dominant inheritance have been described.² Two genes responsible for congenital glaucoma, *CYP1B1* and *LTBP2*, have been identified and several other loci have been mapped.³⁻⁷

Mutations in *CYP1B1*, coding for cytochrome P-450 1B1, are the most common cause of autosomal recessive congenital glaucoma worldwide.^{8,9} The incidence of congenital glaucoma varies among ethnic groups, with the highest incidence in Slovakian Gypsies (1 in 1,250) and the lowest in Western nations (1 in 10,000).¹⁰⁻¹² Genetic testing can provide useful information for patients and families with congenital glaucoma; however, accurate risk assessment for recessive disorders requires information about the frequency of mutation carriers in the normal population.¹³⁻¹⁵ While *CYP1B1* mutations are known to be responsible for 15% to 20% of congenital glaucoma cases in the United States,^{16,17} the *CYP1B1* mutation carrier frequency in the US population has not been determined. The purpose of this study is to estimate the carrier frequency of *CYP1B1* mutations in a population of clinically normal individuals residing in the United States. The variable expressivity of disease presentation in some cases manifested by an older age at onset suggests the hypothesis that the mutation carrier frequency is higher than the expected value based on the frequency of *CYP1B1*-related congenital glaucoma, defined as age at onset of 3 years or younger.

Many different disease-causing *CYP1B1* mutations have been found in populations throughout the world, including missense, frameshift, premature stop codons, small insertion/deletions, and large deletions.^{8,9} The gene product, cytochrome P-450 1B1, metabolizes complex molecules such as polycyclic aromatic hydrocarbons and 17- β -estradiol.¹⁸⁻²⁰ The role of the protein in congenital glaucoma is not clear; however, it has been hypothesized that the P-450 1B1 activity is responsible for metabolism of a compound involved in ocular development.^{21,22} Variable expressivity is a well-known feature of glaucoma caused by mutant forms of *CYP1B1* with an age at onset of *CYP1B1*-associated disease ranging from birth through teenaged years.²³⁻²⁵ Genotype-phenotype studies have suggested that mutations causing premature truncation of the protein (frameshifts, deletions, insertion, nonsense mutations) cause more severe disease with earlier onset than disease caused by missense mutations.²⁶⁻²⁸

Congenital glaucoma is one of the most common primary childhood glaucomas in the United States.^{29,30} The disease overall is diagnosed more frequently in males than females; however, at least one study suggests that *CYP1B1*-related disease is more common in females.³¹⁻³³ In the United States the majority of patients affected by congenital glaucoma are white with presumed European ancestry.^{16,17} In ethnically heterogeneous populations such as the United States, *CYP1B1* mutations also appear to be most common in white congenital glaucoma patients and to be less frequent in patients of African descent.^{16,17,34} Disease severity and response to therapy have not been reported to be influenced by race or ethnicity. Environmental risk factors for *CYP1B1*-related congenital glaucoma have not been described; however, a report of variable disease expressivity in identical twins and inter-ocular variability in some patients suggests that environmental exposures and/or epigenetic effects can impact disease development.^{24,35}

Genetic testing is now readily available for many inherited ocular disorders, including congenital glaucoma.^{13,14} Currently, mutations in 6 genes (*MYOC*, *PITX2*, *FOXC1*, *PAX6*, *CYP1B1*, and *LTBP2*) can cause early-onset (either congenital or juvenile) glaucoma.³⁶ The identification of a gene mutation in patients and/or family members can have a significant impact on clinical care. Disease surveillance can be targeted to mutation carriers, making timely initiation of treatment possible and eliminating unnecessary

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surveillance for family members who do not carry mutant alleles. Mutation detection can also define the mode of inheritance, as *CYP1B1* and *LTBP2* cause autosomal recessive disease while *MYOC*, *PITX2*, *FOXC1*, and *PAX6* all cause dominantly inherited glaucoma. Without genetic testing the pattern of disease inheritance may not be readily apparent because of the variable expressivity and phenotypic overlap among the early-onset disorders caused by mutations in this collection of genes.³⁷ Accordingly, we recommend a panel test that simultaneously screens all 6 genes for mutations.

Disease risk assessment and informed genetic counseling are greatly improved by the information obtained from genetic testing. For patients with *CYP1B1* mutations, family members can be counseled about disease risk for themselves and their offspring. In addition to the carrier and mutation status acquired from the genetic test results, accurate risk assessment for autosomal recessive disorders requires knowledge of the population mutation carrier frequency, as carrier frequency determines the risk that a mutation carrier could have an affected child. Because the carrier frequency of *CYP1B1* mutations has not been reported for any US population, the purpose of this study is to measure the *CYP1B1* carrier frequency in a US clinic-based cohort relevant to the patient population that is served by current genetic testing for disease-related mutations in congenital glaucoma patients.

METHODS

STUDY PARTICIPANTS

This prospective cross-sectional genetic study of normal individuals was approved by the institutional review board of the Massachusetts Eye and Ear Infirmary. After obtaining informed consent, all study participants had a comprehensive eye examination performed by a board-certified ophthalmologist, including visual acuity, tonography, anterior segment examination, and fundoscopic evaluation. Individuals with normal optic nerves (cup-disc ratio less than 0.5 and without a difference in cup-disc ratio between the two eyes of greater than 0.2) and normal intraocular pressure (<21 mm Hg using Goldmann tonography) were judged to have no clinical evidence of glaucoma and were enrolled in this study. Patients were asked about a family history of glaucoma, and those with a known family history of congenital glaucoma in first- or second-degree relatives were excluded. All patients were white and were older than 40 years of age.

COLLECTION OF DNA SAMPLES

DNA samples were obtained from peripheral blood or mouthwash samples according to standard protocols.³⁸

DNA SEQUENCING

DNA samples were sequenced in both directions using Sanger sequencing after polymerase chain reaction amplification of the entire coding sequence of the *CYP1B1* gene. A nested primer design was used that required 5 overlapping amplicons to cover exons 2 and 3, as previously described.¹⁷ The first exon of the *CYP1B1* gene is noncoding and was not sequenced as part of this study.

MUTATION PATHOGENICITY

To assess the pathogenicity of identified DNA sequence variants, we used two criteria: (1) the mutation has previously been reported to be disease causing and/or (2) the PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>) score was in the “probably damaging” or “possibly damaging” range. PolyPhen-2 (Polymorphism Phenotyping v2) is a software tool that predicts the potential impact of amino acid substitutions on the structure and function of human proteins using physical and evolutionary constraints.³⁹ Unlike some software pathogenic prediction tools, such as SIFT (<http://sift.jcvi.org/>), PolyPhen-2 uses protein structure information. For a given mutation, PolyPhen-2 calculates naïve Bayes posterior probability that the mutation is damaging and reports estimates of false-positive rate (the chance that the mutation is classified as damaging when it is in fact nondamaging) and true-positive rate (the chance that the mutation is classified as damaging when it is indeed damaging). The PolyPhen-2 score is reported as a range from 1.0 to 0.0 with the threshold for “probably damaging” from 1.0 to 0.85, “possibly damaging” from 0.85 to 0.20, and less than 0.20 is designated as “benign.” PolyPhen-2 is used to assess the pathogenicity of missense changes (nonsynonymous single-nucleotide polymorphisms). Novel deletions/insertions causing frameshifts or novel nonsense mutations creating a truncated protein were assumed to be pathogenic.

EXTRACTION OF DATA FROM THE NHLBI ESP

CYP1B1 DNA sequence variants were identified in whole exome data from 4300 white and 2200 African-American individuals sequenced as part of the National Heart Lung Blood Institute (NHLBI)-sponsored Exome Sequencing Project (ESP) (<https://esp.gs.washington.edu/drupal/>). For this project 6500 samples (4300 whites and 2200 African-Americans) underwent whole exome sequencing and the results were organized in a database (Exome Variant Server) maintained at the University of Washington. The individuals selected for sequencing were part of a group of richly phenotyped cohorts with the primary intent to discover novel genes related to cardiovascular and pulmonary disorders. Ocular phenotype data is not included in the clinical data collection for these patients; therefore, it is not possible to determine if any of these individuals carried a diagnosis of congenital glaucoma or had a family history of congenital glaucoma. However, given the low overall prevalence of congenital glaucoma, we do not expect a substantial confounding effect of preexisting congenital glaucoma in this data set. The variant database is publicly available (<http://evs.gs.washington.edu/EVS/>). For this study the database was queried for “CYP1B1.” All variants that (1) were previously reported to be disease causing, and/or (2) had PolyPhen-2 pathogenicity scores of probably damaging or possibly damaging were selected.

RESULTS**CALCULATION OF THE EXPECTED FREQUENCY OF *CYP1B1* MUTATION CARRIERS IN THE US POPULATION**

The frequency of congenital glaucoma, defined as onset of glaucoma between the ages of birth and 3 years, in the United States is estimated to be similar to other Western populations at approximately 1 in 10,000.¹⁰⁻¹² Studies have shown that between 15% and 25% of congenital glaucoma cases in the United States are due to mutations in *CYP1B1*,^{16,17} so the *CYP1B1*-associated congenital disease frequency in the United States is approximately 1 in 50,000. Hardy-Weinberg equilibrium ($p^2 + 2pq + q^2 = 1$) assumptions (a large population, random mating, an autosomal locus, no effect of recurrent mutation, no selection against the phenotype, and no migration in or out of the population) are appropriate for *CYP1B1* mutations in the US population. Using Hardy-Weinberg equilibrium, the expected carrier frequency based on a disease frequency of 1 in 50,000 (q^2) would be 0.89% ($2pq$) or 1 in 112 individuals in the United States.

***CYP1B1* MUTATIONS IDENTIFIED BY SANGER SEQUENCING IN 250 INDIVIDUALS WITHOUT CLINICAL EVIDENCE OF GLAUCOMA OR A FAMILY HISTORY OF CONGENITAL GLAUCOMA**

To measure the *CYP1B1* carrier frequency in a normal population from the United States, we sequenced the entire coding region of the *CYP1B1* gene in 250 white individuals without evidence of glaucoma and without a family history of congenital glaucoma in first- or second-degree relatives enrolled from Massachusetts Eye and Ear ophthalmology clinics (Massachusetts Eye and Ear Clinic sample). The mean age of the study participants was 65 years (range, 40 to 89), and 56% were female. All study participants were unrelated.

DNA sequencing identified mutations in 11 individuals (4.4%) (Table 1). Nine different missense mutations were identified. Deletions, insertions, or nonsense mutations were not found in this population. Two mutations were found more than once (R368H and E229K), and 3 have not been previously identified in any population (P51S, P118S, and D218V). We determined that the PolyPhen-2 score for each of the novel mutations was in the “probably damaging” (1.0 to 0.85) range using the Web-based program (<http://genetics.bwh.harvard.edu/pph2>) (“probably damaging” is the most severe category for this test). The carrier frequency in this population is 1 in 22 individuals (4.4%), which is 5.1 times that of the expected 1 in 112.

TABLE 1. *CYP1B1* MUTATIONS FOUND IN 250 INDIVIDUALS WITHOUT CLINICAL EVIDENCE OF GLAUCOMA OR A FAMILY HISTORY OF CONGENITAL GLAUCOMA*

VARIANT POSITION (CHR2)	RS ID	MUTATION	N	FUNCTION	PROTEIN	POLYPHEN-2 SCORE	REFERENCE
38302381	Unknown	c.151C>T	1	Missense	P51S	1.0	This study
38302291	rs9282671	c.241T>A	1	Missense	Y81N	1.0	40
38302180	Unknown	c.352C>T	1	Missense	P118S [†]	1.0	This study
38301879	rs72549383	c.653A>T	1	Missense	D218V	0.892	This study
38301847	rs57865060	c.685G>A	2	Missense	E229K	0.781	22,41
38298394	rs28936414	c.1103G>A	4	Missense	R368H	1.0	42,43
38398169	rs4986888	c.1328C>G	1	Missense	A443G	0.04	44,45,46
Total			11				

*For each mutation, the table lists the genomic location (variant position on chromosome 2), the rs ID if known, the number of times the mutation is observed (N), the functional consequence of the mutation, the protein location of the mutation, the PolyPhen-2 score, and reference(s) if previously reported. The mutation is indicated as the basepair change in the cDNA (“complementary” DNA, which is the DNA copy of the messenger RNA). For example, c.151C>T is a mutation that causes the C at cDNA position 151 to be replaced with a T. The mutation effect on the protein is indicated using the single letter code for amino acids and the protein position. For example, P51S indicates that the mutation has caused a change in the amino acid at position 51 from the wild type Proline to the mutant Serine.

[†]P118T has been previously reported in an ethnically mixed white population.⁴⁷

***CYP1B1* MUTATIONS IDENTIFIED BY WHOLE EXOME SEQUENCING IN THE NHLBI ESP DATABASE**

To further investigate the *CYP1B1* mutation carrier frequency in the US population, we identified all the *CYP1B1* mutations listed in the whole exome sequence database for 4300 whites who underwent whole exome sequencing as part of the NHLBI ESP. In this population we found 127 mutations (3.0%) representing a carrier frequency of 1 in 33, which is 3.4 times that of the expected 1 in 112. Of the 127 mutations, all but 9 were missense mutations. Ten of the identified mutations had not been previously reported, and 12 mutations were found more than once (Table 2).

TABLE 2. CYP1B1 MUTATIONS FOUND IN WHOLE EXOME SEQUENCE DATA FROM 4300 CAUCASIAN INDIVIDUALS SEQUENCED AS PART OF THE NATIONAL HEART LUNG AND BLOOD INSTITUTE EXOME SEQUENCING PROJECT*

VARIANT POSITION (Chr 2)	RS ID	MUTATION	N	FUNCTION	PROTEIN	POLYPHEN-2 SCORE	REFERENCE
38302377	rs201824781	c.155C>T	2	Missense	P52L	1.0	40
38302361	rs72549387	c.171G>A	2	Stop-gained	W57**	NA	48
38302350	rs28936700	c.182G>A	1	Missense	G61E	1.0	49
38302306	rs377210850	c.226C>A	4	Missense	R76S	0.963	This study
38302291	rs9282671	c.241T>A	34	Missense	Y81N	1.0	40
38302150	rs200998372	c.382G>A	1	Missense	G128S	0.797	This study
38302075	rs368041729	c.457C>T	1	Missense	R153C	1.0	This study
38301847	rs57865060	c.685G>A	41	Missense	E229K	0.781	22,41
38301831	rs375391843	c.701C>T	2	Missense	T234M	1.0	This study
38301774	rs369088292	c.758C>T	1	Missense	P253L	1.0	This study
38301682	rs368249322	c.850C>T	2	Missense	R284W	0.997	This study
38301633	rs370935810	c.899C>A	1	Missense	A300E	0.999	This study
38301511	rs373339128	c.1021T>C	1	Missense	W341R	1.0	40
38301482	unknown	c.1043+2_1043+6 del5	1	Splice-5	NA	NA	This study
38298420	rs72549380	c.1064_1076 del13	2	Frameshift	R355Hfs**69	NA	3
38298394	rs28936414	c.1103G>A	16	Missense	R368H	1.0	42,43
38298338	rs55989760	c.1159G>A	4	Missense	E387K	1.0	17,50
38298329	rs148542782	c.1168C>T	1	Missense	R390C	1.0	51,52
38298302	rs72481806	c.1195A>G	1	Missense	I399V	0.921	53
38298287	rs72466463	c.1209_1210insTC ATGCCACC	2	Frameshift	T404Sfs**30	NA	3,17
38298164	rs148233007	c.1333T>C	1	Missense	F445L	1.0	54
38298092	rs28936701	c.1405C>T	1	Missense	R469W	1.0	24,55
38298043	rs369311647	c.1454C>G	1	Missense	S485C	0.998	This study
38298002	rs376806358	c.1495G>A	1	Missense	E499K [†]	0.04	56
38297955	NA	c.1536_1541 del6	1	Coding	K512_K514 delK514_P513‡	NA	9
38297867	rs368552668	c.1630T>A	2	Stop-lost	**544Kext9 [§]	NA	This study
Total			127				

NA, not applicable.

*For each mutation, the table lists the genomic location (variant position on chromosome 2), the rs ID if known, the number of times the mutation is observed (N), the functional consequence of the mutation, the protein location of the mutation, the PolyPhen-2 score, and reference(s) if previously reported. The mutation is indicated as the basepair change in the cDNA (“complementary” DNA, which is the DNA copy of the messenger RNA). For example, c.155C>T is a mutation that causes the C at cDNA position 155 to be replaced with a T. The mutation effect on the protein is indicated using the single letter code for amino acids and the protein position. For example, P52L indicates that the mutation has caused a change in the amino acid at position 52 from the wild type Proline to the mutant Leucine. Standard nomenclature is used to indicate insertions, deletions, frameshifts, premature stop codons, and mutation involving the wild type stop codon: **Indicates that the mutation causes a nonsense mutation (stop-gained); splice-5 indicates that the mutation involves the conserved splice donor sequence; fs**N indicates that the mutation causes a frameshift and premature stop codon after N amino acids; coding indicates that the mutation causes an inframe deletion or insertion, and stop-lost indicates that the mutation changes the stop codon and extends the protein by N amino acids.

[†]E499G has been previously reported in the Japanese.⁵⁷

[‡]This mutation causes an inframe deletion of K514 and P513.

[§]This mutation changes the stop codon to a Lysine (K) and extends the protein by 9 more amino acids before encountering the next stop codon.

To investigate the *CYP1B1* carrier frequency in African-Americans from the United States, we identified all the *CYP1B1* mutations listed in the whole exome sequence database for 2200 African-Americans who underwent whole exome sequencing as part of the NHLBI ESP. In this population we found 23 mutations (1%), which is higher than the expected carrier frequency for African-Americans (0.2%) based on the lower incidence of *CYP1B1*-related disease in African-Americans in the United States (approximately 1 in 100,000).^{16,29} Of the 23 mutations in African-Americans, 20 were missense alleles (Table 3).

TABLE 3. *CYP1B1* MUTATIONS FOUND IN WHOLE EXOME SEQUENCE DATA FROM 2200 AFRICAN-AMERICAN INDIVIDUALS SEQUENCED AS PART OF THE NATIONAL HEART LUNG AND BLOOD INSTITUTE EXOME SEQUENCING PROJECT*

VARIANT POSITION (Chr2)	rs ID	MUTATION*	N†	FUNCTION‡	PROTEIN§	POLYPHEN-2	REFERENCE
38302361	rs72549387	c.171G>A	2	Stop-gained	W57**	NA	48
38302291	rs9282671	c.241T>A	3	Missense	Y81N	1.0	40
38301847	rs57865060	c.685G>A	5	Missense	E229K	0.781	22, 41
38301517	rs367713104	c.1015C>A	1	Missense	L339M	0.865	This study
38298394	rs28936414	c.1103G>A	5	Missense	R368H	1.0	42,43
38298226	rs367941075	c.1271A>G	1	Missense	Q424R	1.0	This study
38298187	rs56175199	c.1310C>T	1	Missense	P437L	1.0	55,58
38298170	rs370691828	c.1327 G>C	1	Missense	A443P	0.51	This study
38298167	rs377049098	c.1330 C>T	1	Stop-gained	R444**	NA	55,59
38298085	rs147535955	c.1412 T>C	1	Missense	I471T	1.0	This study
38297955	NA	c.1536_1541 del6	2	Coding	K512_K514 delK514_P513†	NA	9
Total			23				

NA not applicable.

*For each mutation, the table lists the genomic location (variant position on chromosome 2), the rs ID if known, the number of times the mutation is observed (N), the functional consequence of the mutation, the protein location of the mutation, the PolyPhen-2 score, and reference(s) if previously reported. The mutation is indicated as the basepair change in the cDNA (“complementary” DNA, which is the DNA copy of the messenger RNA). For example, c.241T>A is a mutation that causes the T at cDNA position 241 to be replaced with an A. The mutation effect on the protein is indicated using the single letter code for amino acids and the protein position. For example, Y81N indicates that the mutation has caused a change in the amino acid at position 81 from the wild type Tyrosine to the mutant Asparagine. Standard nomenclature is used to indicate insertions, deletions, frameshifts, premature stop codons, and mutation involving the wild type stop codon: **indicates that the mutation causes a nonsense mutation (stop-gained); coding indicates that the mutation causes an inframe deletion or insertion.

†This mutation causes an inframe deletion of K514 and P513.

DISCUSSION

In this study we measured the carrier frequency of *CYP1B1* mutations in two different white populations from the United States: 250 individuals clinically known to be without evidence of glaucoma and without a family history of congenital glaucoma (Mass Eye and Ear clinic sample), and 4300 individuals sequenced as part of the NHLBI ESP. In both populations we found that the *CYP1B1* mutation carrier frequency was higher than expected (1 in 22 and 1 in 33, respectively) and that the majority of mutations identified were missense mutations (100% and 93%, respectively).

In the Mass Eye and Ear clinic sample of 250 participants, we identified 7 different mutations in 11 individuals. Two mutations were found more than once. The R368H mutation was found in 4 individuals. This missense mutation has a PolyPhen-2 score of 1.0 (the highest possible PolyPhen-2 score representing the most severe pathogenic category) and has been found in several other populations and is especially common in India.⁴² The other missense mutation found more than once is the E229K mutation found in 2 individuals. This mutation has a PolyPhen-2 score of 0.781 (possibly damaging), but has been reported in a number of other populations as disease-associated.^{22,40,41,54,59-62} Novel mutations were identified in 3 study participants (P51S, P118S, D218V). While P51S has not been previously reported, P52L has been reported to be a disease-causing mutation in a Spanish cohort.⁴⁰ Similarly, P118S had not been previously identified; however, P118T has been reported in an ethnically mixed white population.⁴⁷ D218V has not been reported in any population, and other changes at this protein position have also not been reported. The Aspartate at position 218 is evolutionarily conserved and the predicted pathogenicity by PolyPhen-2 is in the “probably damaging” range. All three of the novel mutations have PolyPhen-2 scores consistent with significant pathogenicity, yet there is a possibility that these novel changes are not disease-causing. However, if these novel mutations were excluded from the analysis, the carrier frequency would still be 3.6

times the expected carrier frequency of 0.89%.

In the NHLBI ESP white population we found 127 mutations. Twelve of these were found more than once, and three mutations (Y81N, E229K, and R368H) were particularly common. E229K and R368H were found in 41 and 16 individuals, respectively, providing further support for the relatively common occurrence of these two mutations in the US population. Y81N was found in 34 individuals in the NHLBI ESP sample. This mutation has been previously reported in a population of congenital glaucoma patients from Spain, and functional tests indicate that the missense change affects protein function.^{22,40,61,63} Ten of the NHLBI ESP mutations have not been previously reported in any population, but are expected to be pathogenic. If, however, these mutations are not causal, removing them from the analysis would not change our conclusions, as the carrier frequency would remain higher than expected (2.7%).

We also investigated the *CYP1B1* carrier frequency in the African-American samples included in the NHLBI ESP database. Among the 2200 samples with exome sequence data, we identified 23 mutations. Five of these were found more than once, and again E229K and R368H were particularly common. Similar to the white results, all but 3 of the identified mutations were missense alleles. In this population the carrier frequency was also higher than expected (1% observed compared with 0.2% expected) but lower than that measured in the white population due to the lower incidence of *CYP1B1*-related congenital glaucoma in individuals of African descent.^{16,48}

Interestingly, the majority of mutations identified in both our clinic-based study of 250 individuals and the NHLBI ESP data set are missense mutations, which could be considered to be less severe mutations (hypomorph mutations) than the frameshift and premature stop codon mutations that cause protein truncation or complete elimination of the protein. Both missense and frameshift mutations have been described in cases of congenital glaucoma caused by mutant *CYP1B1*^{8,9}; however the variable expressivity noted in many *CYP1B1*-related cases appears to be more common in patients with missense mutations.^{23,24,25,31,40,48,49,55,56,64,65,66} Notably, the G61E mutation is the most common mutation in Saudi Arabia and Kuwait, two populations that are most likely to demonstrate variable expressivity manifested by later onset of disease.^{24,49,67,68}

There are several possible explanations for the unexpectedly high *CYP1B1* carrier frequency observed in our study of normal individuals from the United States. First, it is possible that many *CYP1B1* mutations, and particularly the missense mutations, cause a range of glaucoma phenotypes that include the classically defined congenital glaucoma with onset before the age of 3 as well as later onset forms of childhood glaucoma, such as juvenile glaucoma, early-onset primary open-angle glaucoma, and glaucoma related to anterior segment dysgenesis. The observed variable expressivity of some *CYP1B1* mutations is consistent with this hypothesis, and previous reports suggest that *CYP1B1* can contribute to both juvenile glaucoma and anterior segment dysgenesis.^{47,69-73} Interestingly, the frequency of frameshift and nonsense mutations is much lower than the frequency of missense mutations in both the Mass Eye and Ear clinic sample and the NHLBI ESP sample, suggesting that the disease phenotype associated with the frameshift and nonsense mutations could be much rarer. It is possible that these protein truncating mutations may be more likely to cause more severe forms of glaucoma with earlier onset.

A second possible explanation for higher *CYP1B1* carrier frequency is that the pathogenicity of *CYP1B1* mutations may be influenced by the background *CYP1B1* haplotype. Haplotypes composed of 5 polymorphic markers distributed throughout the *CYP1B1* gene have been shown to influence the enzymatic activity of the cytochrome P-450 gene product.⁶¹ Some *CYP1B1* haplotypes have been shown to have reduced enzymatic activity, while others have higher activity.^{74,75} Because of this variable effect, the baseline activity of the background haplotype may influence the pathogenic potential of *CYP1B1* mutations. This background haplotype effect could have a larger impact on the pathogenicity of “hypomorph” mutations, such as the missense mutations. In this study *CYP1B1* haplotypes cannot be assessed in either data set because at least one parent needs to be included in the analysis to establish the phase of the mutation relative to the haplotype. In families with *CYP1B1* disease and variable expressivity, it would be interesting to investigate *CYP1B1* haplotypes to potentially provide further insight into the background haplotype effects on mutation expressivity.

Finally, it is possible that the frequency of congenital glaucoma in the United States is underestimated and that further evaluation of congenital glaucoma disease incidence in the United States may be warranted.

There are several limitations of this study. First, as described above, we are not able to identify the background haplotype which may be an important feature of *CYP1B1* mutation pathogenicity. Further investigation using families with *CYP1B1* mutations and variable disease expressivity would help address the role of the *CYP1B1* haplotype in disease expressivity. Second, in the NHLBI dataset we cannot determine if a single individual has more than one mutation on the same chromosome (would therefore be one unaffected carrier but the mutation would be counted twice). This is not likely, as the identified mutations are distributed throughout the gene region and none of these variants are recognized to be in linkage disequilibrium. Additionally, if the assumption that each mutation is found in a single individual is erroneous, then the carrier frequency from the NHLBI would be inflated; however, this data set has a lower frequency than the Mass Eye and Ear clinic-based study suggesting that the assumption is valid. In the Mass Eye and Ear study we have individual-level data and can be confident that each mutation carrier only carries one mutation. Finally, in the NHLBI dataset we do not have information on the ocular phenotype, so it is possible that some individuals included in this sample have a family history of glaucoma or have clinical findings of glaucoma. However, inclusion of individuals with clinical features of glaucoma or a family history of congenital glaucoma would also inflate the mutation carrier frequency and the observed lower carrier frequency, compared to the Mass Eye and Ear sample, which suggests that the lack of clinical information is not a significant issue.

In conclusion, this study shows that the carrier frequency of *CYP1B1* mutations is higher than the expected value based on the frequency of congenital glaucoma in the United States and the percentage of US congenital glaucoma cases expected to be caused by mutations in *CYP1B1*. These results suggest that many more individuals than expected are carriers of a deleterious *CYP1B1* mutation,

and that the incidence of *CYP1B1*-related disease may be higher than expected. Currently genetic testing for *CYP1B1* mutations may be reserved for individuals with congenital glaucoma with onset before age 3. Our analysis of *CYP1B1* carrier frequency suggests that testing for *CYP1B1* gene mutations may be useful for other types of childhood glaucomas, including juvenile open-angle glaucoma and glaucoma associated with anterior segment dysgenesis syndromes.

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REFERENCES

- Walton DS. Primary congenital open angle glaucoma: a study of the anterior segment abnormalities. *Trans Am Ophthalmol Soc* 1979;77(1):746-768.
- François J. Congenital glaucoma and its inheritance. *Ophthalmologica* 1980;181(2):61-73.
- Stoilov I, Akarsu AN, Sarfarazi M. Identification of three different truncating mutations in cytochrome P4501B1 (*CYP1B1*) as the principal cause of primary congenital glaucoma (Buphthalmos) in families linked to the *GLC3A* locus on chromosome 2p21. *Hum Mol Genet* 1997;6(4):641-647.
- Bejjani BA, Lewis RA, Tomey KF, et al. Mutations in *CYP1B1*, the gene for cytochrome P4501B1, are the predominant cause of primary congenital glaucoma in Saudi Arabia. *Am J Hum Genet* 1998;62(2):325-333.
- Stoilov I, Akarsu AN, Alozie I, et al. Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P4501B1. *Am J Hum Genet* 1998;62(3):573-584.
- Ali M, McKibbin M, Booth A, et al. Null mutations in *LTBP2* cause primary congenital glaucoma. *Am J Hum Genet* 2009;84(5):664-671.
- Akarsu AN, Turacli ME, Aktan SG, et al. A second locus (*GLC3B*) for primary congenital glaucoma (Buphthalmos) maps to the 1p36 region. *Hum Mol Genet* 1996;5(8):1199-1203.
- Vasiliou V, Gonzalez FJ. Role of *CYP1B1* in glaucoma. *Annu Rev Pharmacol Toxicol* 2008;48:333-358.
- Li N, Zhou Y, Du L, Wei M, Chen X. Overview of Cytochrome P450 1B1 gene mutations in patients with primary congenital glaucoma. *Exp Eye Res* 2011;93(5):572-579.
- Francois J. Congenital glaucoma and its inheritance. *Ophthalmologica* 1980;181:61-73.
- Gencik A, Gencikova A, Ferak V. Population genetical aspects of primary congenital glaucoma. I. Incidence, prevalence, gene frequency, and age of onset. *Hum Genet* 1982;61:193-197.
- Aponte EP, Diehl N, Mohny BG. Incidence and clinical characteristics of childhood glaucoma: a population-based study. *Arch Ophthalmol* 2010;128(4):478-482.
- Wiggs JL, Pierce EA. Genetic testing for inherited eye disease: Who benefits? *JAMA Ophthalmol* 2013;131(10):1265-1266.
- Stone EM, Aldave AJ, Drack AV, et al. Recommendations for genetic testing of inherited eye diseases: report of the American Academy of Ophthalmology task force on genetic testing. *Ophthalmology* 2012;119(11):2408-2410.
- Kaplan F. Tay-Sachs disease carrier screening: a model for prevention of genetic disease. *Genet Test* 1998;2(4):271-292.
- Lim SH, Tran-Viet KN, Yanovitch TL, et al. *CYP1B1*, *MYOC*, and *LTBP2* mutations in primary congenital glaucoma patients in the United States. *Am J Ophthalmol* 2013;155(3):508-517.
- Sena DF, Finzi S, Rodgers K, Del Bono E, Haines JL, Wiggs JL. Founder mutations of *CYP1B1* gene in patients with congenital glaucoma from the United States and Brazil. *J Med Genet* 2004;41(1):e6.
- Sowers MR, Wilson AL, Kardina SR, Chu J, McConnell DS. *CYP1A1* and *CYP1B1* polymorphisms and their association with estradiol and estrogen metabolites in women who are premenopausal and perimenopausal. *Am J Med* 2006;119(9 Suppl 1):S44-51.
- Tokizane T, Shiina H, Igawa M, et al. Cytochrome P450 1B1 is overexpressed and regulated by hypomethylation in prostate cancer. *Clin Cancer Res* 2005;11(16):5793-5801.
- Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. *Cancer Lett* 2005;227:115-124.
- Choudhary D, Jansson I, Schenkman JB. *CYP1B1*, a developmental gene with a potential role in glaucoma therapy. *Xenobiotica* 2009;39(8):606-615.
- Choudhary D, Jansson I, Sarfarazi M, Schenkman JB. Characterization of the biochemical and structural phenotypes of four *CYP1B1* mutations observed in individuals with primary congenital glaucoma. *Pharmacogenet Genomics* 2008;18(8):665-676.
- Khan AO. Genetics of primary glaucoma. *Curr Opin Ophthalmol* 2011;22(5):347-355.

24. Suri F, Yazdani S, Narooie-Nejhad M, et al. Variable expressivity and high penetrance of CYP1B1 mutations associated with primary congenital glaucoma. *Ophthalmology* 2009;116(11):2101-2109.
25. Martin SN, Sutherland J, Levin AV, Klose R, Priston M, Héon E. Molecular characterisation of congenital glaucoma in a consanguineous Canadian community: a step towards preventing glaucoma related blindness. *J Med Genet* 2000;37(6):422-427.
26. López-Garrido MP, Medina-Trillo C, Morales-Fernandez L, et al. Null CYP1B1 genotypes in primary congenital and nondominant juvenile glaucoma. *Ophthalmology* 2013;120(4):716-723.
27. Bejjani BA, Stockton DW, Lewis RA, et al. 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* 2000;9(3):367-374.
28. Bejjani BA, Lewis RA, Tomey KF, et al. Mutations in CYP1B1, the gene for cytochrome P4501B1, are the predominant cause of primary congenital glaucoma in Saudi Arabia. *Am J Hum Genet* 1998;62(2):325-333.
29. Fung DS, Roensch MA, Kooner KS, Cavanagh HD, Whitson JT. Epidemiology and characteristics of childhood glaucoma: results from the Dallas Glaucoma Registry. *Clin Ophthalmol* 2013;7:1739-1746.
30. Aponte EP, Diehl N, Mohny BG. Incidence and clinical characteristics of childhood glaucoma: a population-based study. *Arch Ophthalmol* 2010;128(4):478-482.
31. Alanazi FF, Song JC, Mousa A, et al. Primary and secondary congenital glaucoma: baseline features from a registry at King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia. *Am J Ophthalmol* 2013;155(5):882-889.
32. MacKinnon JR, Giubilato A, Elder JE, Craig JE, Mackey DA. Primary infantile glaucoma in an Australian population. *Clin Experiment Ophthalmol* 2004;32(1):14-18.
33. Suri F, Chitsazian F, Khoramian-Tusi B, et al. Sex bias in primary congenital glaucoma patients with and without CYP1B1 mutations. *J Ophthalmic Vis Res* 2009;4(2):75-78.
34. Stoilov IR, Costa VP, Vasconcellos JP, et al. Molecular genetics of primary congenital glaucoma in Brazil. *Invest Ophthalmol Vis Sci* 2002;43(6):1820-1827.
35. MacKinnon JR, Giubilato A, Elder JE, Craig JE, Mackey DA. Primary infantile glaucoma in an Australian population. *Clin Experiment Ophthalmol* 2004;32(1):14-18.
36. Fan BJ, Wiggs JL. Glaucoma: genes, phenotypes, and new directions for therapy. *J Clin Invest* 2010;120(9):3064-3072.
37. Wang R, Wiggs JL. Common and rare genetic risk factors for glaucoma. *Cold Spring Harb Perspect Med* 2014 Sep 18 [Epub ahead of print].
38. Desronvil T, Logan-Wyatt D, Abdrabou W, et al. Distribution of COL8A2 and COL8A1 gene variants in Caucasian primary open angle glaucoma patients with thin central corneal thickness. *Mol Vis* 2010;16:2185-2191.
39. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7(4):248-249.
40. Campos-Mollo E, López-Garrido MP, Blanco-Marchite C, et al. CYP1B1 mutations in Spanish patients with primary congenital glaucoma: phenotypic and functional variability. *Mol Vis* 2009;15:417-431.
41. Hollander DA, Sarfarazi M, Stoilov I, Wood IS, Fredrick DR, Alvarado JA. Genotype and phenotype correlations in congenital glaucoma: CYP1B1 mutations, goniodysgenesis, and clinical characteristics. *Am J Ophthalmol* 2006;142(6):993-1004.
42. Reddy AB, Panicker SG, Mandal AK, Hasnain SE, Balasubramanian D. Identification of R368H as a predominant CYP1B1 allele causing primary congenital glaucoma in Indian patients. *Invest Ophthalmol Vis Sci* 2003;44(10):4200-4203.
43. Dimasi DP, Hewitt AW, Straga T, et al. Prevalence of CYP1B1 mutations in Australian patients with primary congenital glaucoma. *Clin Genet* 2007;72(3):255-260.
44. Stoilov IR, Costa VP, Vasconcellos JP, et al. Molecular genetics of primary congenital glaucoma in Brazil. *Invest Ophthalmol Vis Sci* 2002;43(6):1820-1827.
45. Melki R, Colomb E, Lefort N, Brezin AP, Garchon HJ. CYP1B1 mutations in French patients with early-onset primary open-angle glaucoma. *J Med Genet* 2004;41(9):647-651.
46. Lopez-Garrido MP, Sanchez-Sanchez F, Lopez-Martinez F, et al. Heterozygous CYP1B1 gene mutations in Spanish patients with primary open-angle glaucoma. *Mol Vis* 2006;12(1):748-755.
47. Vincent A, Billingsley G, Priston M, et al. Further support of the role of CYP1B1 in patients with Peters anomaly. *Mol Vis* 2006;12(1):506-510.
48. Stoilov IR, Costa VP, Vasconcellos JP, et al. Molecular genetics of primary congenital glaucoma in Brazil. *Invest Ophthalmol Vis Sci* 2002;43(6):1820-1827.
49. Khan AO, Al-Abdi L, Mohamed JY, Aldahmesh MA, Alkuraya FS. Familial juvenile glaucoma with underlying homozygous p.G61E CYP1B1 mutations. *J AAPOS* 2011;15(2):198-209.
50. Plásilová M, Stoilov I, Sarfarazi M, Kádasi L, Feráková E, Ferák V. Identification of a single ancestral CYP1B1 mutation in Slovak Gypsies (Roms) affected with primary congenital glaucoma. *J Med Genet* 1999;36(4):290-294.
51. Tanwar M, Dada T, Sihota R, Das TK, Yadav U, Dada R. Mutation spectrum of CYP1B1 in North Indian congenital glaucoma patients. *Mol Vis* 2009;15(1):1200-1209.
52. Azmanov DN, Dimitrova S, Florez L, et al. LTBP2 and CYP1B1 mutations and associated ocular phenotypes in the Roma/Gypsy founder population. *Eur J Hum Genet* 2011;19(3):326-333.
53. Chitsazian F, Tusi BK, Elahi E, et al. CYP1B1 mutation profile of Iranian primary congenital glaucoma patients and associated haplotypes. *J Mol Diagn* 2007;9(3):382-893.

54. Sivadorai P, Cherninkova S, Bouwer S, et al. Genetic heterogeneity and minor CYP1B1 involvement in the molecular basis of primary congenital glaucoma in Gypsies. *Clin Genet* 2008;74(1):82-87.
55. Abu-Amero KK, Osman EA, Mousa A, et al. Screening of CYP1B1 and LTBP2 genes in Saudi families with primary congenital glaucoma: genotype-phenotype correlation. *Mol Vis* 2011;17(1):2911-2919.
56. Sitorus R, Ardjo SM, Lorenz B, Preising M. CYP1B1 gene analysis in primary congenital glaucoma in Indonesian and European patients. *J Med Genet* 2003;40(1):e9.
57. Mashima Y, Suzuki Y, Sergeev Y, et al. Novel cytochrome P4501B1 (CYP1B1) gene mutations in Japanese patients with primary congenital glaucoma. *Invest Ophthalmol Vis Sci* 2001;42(10):2211-2216.
58. Della Paolera M, de Vasconcellos JP, Umbelino CC, et al. CYP1B1 gene analysis in primary congenital glaucoma Brazilian patients: novel mutations and association with poor prognosis. *J Glaucoma* 2010;19(3):176-182.
59. Colomb E, Kaplan J, Garchon HJ. Novel cytochrome P450 1B1 (CYP1B1) mutations in patients with primary congenital glaucoma in France. *Hum Mutat* 2003;22(6):496-497.
60. Acharya M, Mookherjee S, Bhattacharjee A, et al. Primary role of CYP1B1 in Indian juvenile-onset POAG patients. *Mol Vis* 2006;12(1):399-404.
61. Chavarria-Soley G, Sticht H, Aklillu E, et al. Mutations in CYP1B1 cause primary congenital glaucoma by reduction of either activity or abundance of the enzyme. *Hum Mutat* 2008;29(9):1147-1153.
62. El-Gayar S, Ganesh A, Chavarria-Soley G, et al. Molecular analysis of CYP1B1 in Omani patients with primary congenital glaucoma: a pilot study. *Mol Vis* 2009;15(1):1325-1331.
63. Millá E, Mañé B, Duch S, et al. Survey of familial glaucoma shows a high incidence of cytochrome P450, family 1, subfamily B, polypeptide 1 (CYP1B1) mutations in non-consanguineous congenital forms in a Spanish population. *Mol Vis* 2013;19:1707-1722.
64. Geyer O, Wolf A, Levinger E, et al. Genotype/phenotype correlation in primary congenital glaucoma patients from different ethnic groups of the Israeli population. *Am J Ophthalmol* 2011;151(2):263-271.
65. Chen Y, Jiang D, Yu L, et al. CYP1B1 and MYOC mutations in 116 Chinese patients with primary congenital glaucoma. *Arch Ophthalmol* 2008;126(10):1443-1447.
66. Panicker SG, Mandal AK, Reddy AB, Gothwal VK, Hasnain SE. Correlations of genotype with phenotype in Indian patients with primary congenital glaucoma. *Invest Ophthalmol Vis Sci* 2004;45(4):1149-1156.
67. Alfidhli S, Behbehani A, Elshafey A, Abdelmoaty S, Al-Awadi S. Molecular and clinical evaluation of primary congenital glaucoma in Kuwait. *Am J Ophthalmol* 2006;141(3):512-516.
68. Bejjani BA, Stockton DW, Lewis RA, et al. 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* 2000;9(3):367-374.
69. Abu-Amero KK, Morales J, Aljasim LA, Edward DP. CYP1B1 mutations are a major contributor to juvenile-onset open angle glaucoma in Saudi Arabia. *Ophthalmic Genet* 2013 Oct 7 [Epub ahead of print].
70. Su CC, Liu YF, Li SY, Yang JJ, Yen YC. Mutations in the CYP1B1 gene may contribute to juvenile-onset open-angle glaucoma. *Eye (Lond)* 2012;26(10):1369-1377.
71. López-Garrido MP, Medina-Trillo C, Morales-Fernandez L, et al. Null CYP1B1 genotypes in primary congenital and nondominant juvenile glaucoma. *Ophthalmology* 2013;120(4):716-723.
72. Kelberman D, Islam L, Jacques TS, et al. CYP1B1-related anterior segment developmental anomalies novel mutations for infantile glaucoma and von Hippel's ulcer revisited. *Ophthalmology* 2011;118(9):1865-1873.
73. Khan AO, Aldahmesh MA, Al-Abdi L, et al. Molecular characterization of newborn glaucoma including a distinct aniridic phenotype. *Ophthalmic Genet* 2011;32(3):138-142.
74. Chakrabarti S, Kaur K, Kaur I, et al. Globally, CYP1B1 mutations in primary congenital glaucoma are strongly structured by geographic and haplotype backgrounds. *Invest Ophthalmol Vis Sci* 2006;47(1):43-47.
75. Bhattacharjee A, Banerjee D, Mookherjee S, et al. Leu432Val polymorphism in CYP1B1 as a susceptible factor towards predisposition to primary open-angle glaucoma. *Mol Vis* 2008;14(1):841-850.