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Mutations in *CYP1B1,* the Gene for Cytochrome P4501B1, Are the Predominant Cause of Primary Congenital Glaucoma in Saudi Arabia

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Summary

The autosomal recessive disorder primary congenital glaucoma (PCG) is caused by unknown developmental defect(s) of the trabecular meshwork and anterior chamber angle of the eye. Homozygosity mapping with a DNA pooling strategy in three large consanguineous Saudi PCG families identified the *GLC3A* locus on chromosome 2p21 in a region tightly linked to PCG in another population. Formal linkage analysis in 25 Saudi PCG families confirmed both significant linkage to polymorphic markers in this region and incomplete penetrance, but it showed no evidence of genetic heterogeneity. For these 25 families, the maximum combined two-point LOD score was 15.76 at a recombination fraction of .021, with the polymorphic marker *D2S177*. Both haplotype analysis and homozygosity mapping in these families localized *GLC3A* to a 5-cM critical interval delineated by markers *D2S2186* and *D2S1356*. Sequence analysis of the coding exons for cytochrome P4501B1 (*CYP1B1*) in these 25 families revealed three distinctive mutations that segregate with the phenotype in 24 families. Additional clinical and molecular data on some mildly affected relatives showed variable expressivity of PCG in this population. These results should stimulate a study of the genetic and environmental events that modify the effects of *CYP1B1* mutations in ocular development. Furthermore, the small number of PCG mutations identified in this Saudi population makes both neonatal and population screening attractive public health measures.

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Introduction

The glaucomas are a heterogeneous group of insidious disorders that combine elevated intraocular pressure (IOP) and optic nerve damage that culminate in blindness (Allingham 1994). The most common forms, open-angle glaucomas, have onset in midadulthood and relentless slow progression, unless appropriate medical and/or surgical therapies are instituted. The juvenile and infantile glaucomas are more severe clinically and more difficult to manage. Autosomal dominant juvenile-onset open-angle glaucoma (JOAG) (MIM 137750 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?137750]) (McKusick 1994) has a characteristic onset in the 2d or 3d decade, high IOP, poor responsiveness to medical therapy, and frequent dependence on filtering surgery to control both pressure and the attendant optic neuropathy. Recently, several investigators have established linkage for a locus, *GLC1A,* for JOAG to markers on chromosome 1 (Sheffield et al. 1993; Richards et al. 1994; Wiggs et al. 1994; Morissette et al. 1995) and have identified mutations in a gene, *TIGR,* encoding a trabecular meshwork protein (Stone et al. 1997).

Primary congenital glaucoma (PCG) (MIM 231300 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?231300]) (McKusick 1994) is a clinical and genetic entity clearly distinct from the juvenile forms (Lichter 1994; Anderson et al. 1996). This devastating autosomal recessive disorder is caused by unknown developmental defect(s) of the trabecular meshwork and anterior chamber angle and manifests in the neonatal or infantile period (François 1980; Hoskins et al. 1984). PCG occurs when the developmental anomalies of the angle prevent adequate drainage of aqueous humor, so that the IOP is elevated (deLuise and Anderson 1983). The coats of the infantile eye are sufficiently elastic that they stretch with this elevated pressure and yield an enlarged globe (buphthalmos).

Although PCG is the most common form of glaucoma in infancy, nothing certain is known about its specific pathogenesis, despite studies of various animal models (Hanna et al. 1962; Gelatt et al. 1976; deLuise and An-

derson 1983; Dickens and Hoskins 1989; Buyse 1990). However, there is consensus about its classic clinical characteristics (François 1980): (1) elevated IOP; (2) enlargement of the globe, particularly the anterior segment; (3) edema and opacification of the cornea, with rupture of Descemet's membrane; (4) thinning of the anterior sclera and atrophy of the iris; (5) anomalously deep anterior chamber; (6) structurally normal posterior segment, except for progressive optic atrophy; and (7) photophobia, blepharospasm, and excessive tearing (hyperlacrimation). Differentiation of affected from unaffected siblings is not difficult, especially in the Saudi Arabian population, in which the disorder appears to be more severe, and both relative delay in parental recognition and slow access to medical care are frequent events. Nevertheless, PCG is likely to be genetically heterogeneous, on the basis of current clinical and genetic data (Lichter 1994; Sarfarazi et al. 1995).

Recently, a putative PCG locus, *GLC3A,* was linked to markers on the short arm of chromosome 2 in 11 Turkish families (Sarfarazi et al. 1995). Six other Turkish families did not show linkage to this region of chromosome 2, implying locus heterogeneity for this condition (Sarfarazi et al. 1995). An additional PCG locus, *GLC3B,* has been localized to chromosome 1p36 in some families not showing linkage to the chromosome 2 markers (Akarsu et al. 1996). A subset of families not showing linkage to either locus provided evidence for at least a third as yet unmapped locus for this condition (Akarsu et al. 1996). More recently, Stoilov et al. (1997) described three different mutations in the cytochrome P4501B1 gene, *CYP1B1,* in five Turkish families whose disease previously had been linked to the 2p21 locus.

We report here that a DNA pooling strategy (Sheffield et al. 1994) identified a PCG locus on chromosome 2 in three consanguineous Saudi Arabian families. A polymorphic marker, *D2S177,* on chromosome 2p21 demonstrated a reduction to homozygosity in the PCG-affected pools and suggested that *D2S177* reveals an identity-by-descent (IBD) region that should contain *GLC3A* in these families. We confirmed the localization of *GLC3A* by linkage analysis in an additional 22 Saudi Arabian families and characterized, in *CYP1B1,* three point mutations as the cause of PCG in 24 of these 25 families. Our data demonstrate that 18 individuals, each with two mutant alleles, did not show any evidence for disease at the time of examination, suggesting reduced penetrance for selected alleles.

Subjects, Material, and Methods

Patients

Seventy Saudi PCG families have been ascertained and enrolled into a genetic research program, through either the Glaucoma Clinic or the Pediatric Ophthalmology Clinic at the King Khaled Eye Specialist Hospital (KKESH), in Riyadh, Kingdom of Saudi Arabia. KKESH serves as the secondary and tertiary ophthalmic institution for the entire Kingdom. The families evaluated there are a representative set from various tribes, geographic regions, and provinces of the Kingdom. We selected a panel of 25 families informative for the design and objectives of this study. These families have been described in detail elsewhere (Anderson et al. 1996) (fig. 1). Within each kindred, a detailed family history and pedigree were obtained through personal interviews (by R.A.L. and M.J.) with appropriate family members. Each subject, or the responsible adult on behalf of minors or wards, signed a consent for participation in these investigations, which was approved by the Baylor Affiliates Review Board for Human Subject Research and by the comparable committees at KKESH. The anamnestic and ophthalmologic information on each individual was scored as affected or unaffected by one of three investigators (K.F.T., W.F.A., or D.K.D.) and was reviewed independently by another (R.A.L.), none of whom had prior knowledge of any linkage data. Assignment of affected or unaffected status was based on established criteria that include measurement of the cornea and of the IOP and examination of the optic nerve head. We carefully excluded other recognized associations or combinations of infantile glaucoma, such as aniridia, anterior segment dysgenesis, microcornea, Nance-Horan syndrome, Lowe syndrome, congenital hereditary endothelial dystrophy, neurofibromatosis type 1, and Sturge-Weber syndrome.

Controls

Fifty unrelated native Saudi Arabian adults seen at KKESH for nonhereditary eye disease and with no family history of hereditary eye disease served as a control population for mutational studies of *CYP1B1.*

Pooling of DNA for IBD Screening

DNA was isolated from leukocytes by standard procedures (Lewis et al. 1990). Pooled samples from either affected or unaffected individuals within selected consanguineous PCG families (KKECG-122, KKECG-132, and KKECG-146) were aliquoted into separate tubes, to a final concentration of 8 ng/ml, for each individual in the pooled sample (Sheffield et al. 1994). Each "affected" pool consisted of DNA from three, three, and six individuals, and each "unaffected" pool consisted of DNA from five, four, and five individuals, from KKECG-122, KKECG-132, and KKECG-146, respectively.

PCR was performed (Anderson et al. 1995) in 96-well trays in a Perkin-Elmer GeneAmp® 9600 thermal cycler for 30 cycles in 25-μl reactions containing 40–100 ng genomic DNA, 250 nM each primer (100 nM of one primer previously had been labeled with [^{32}P] γATP with

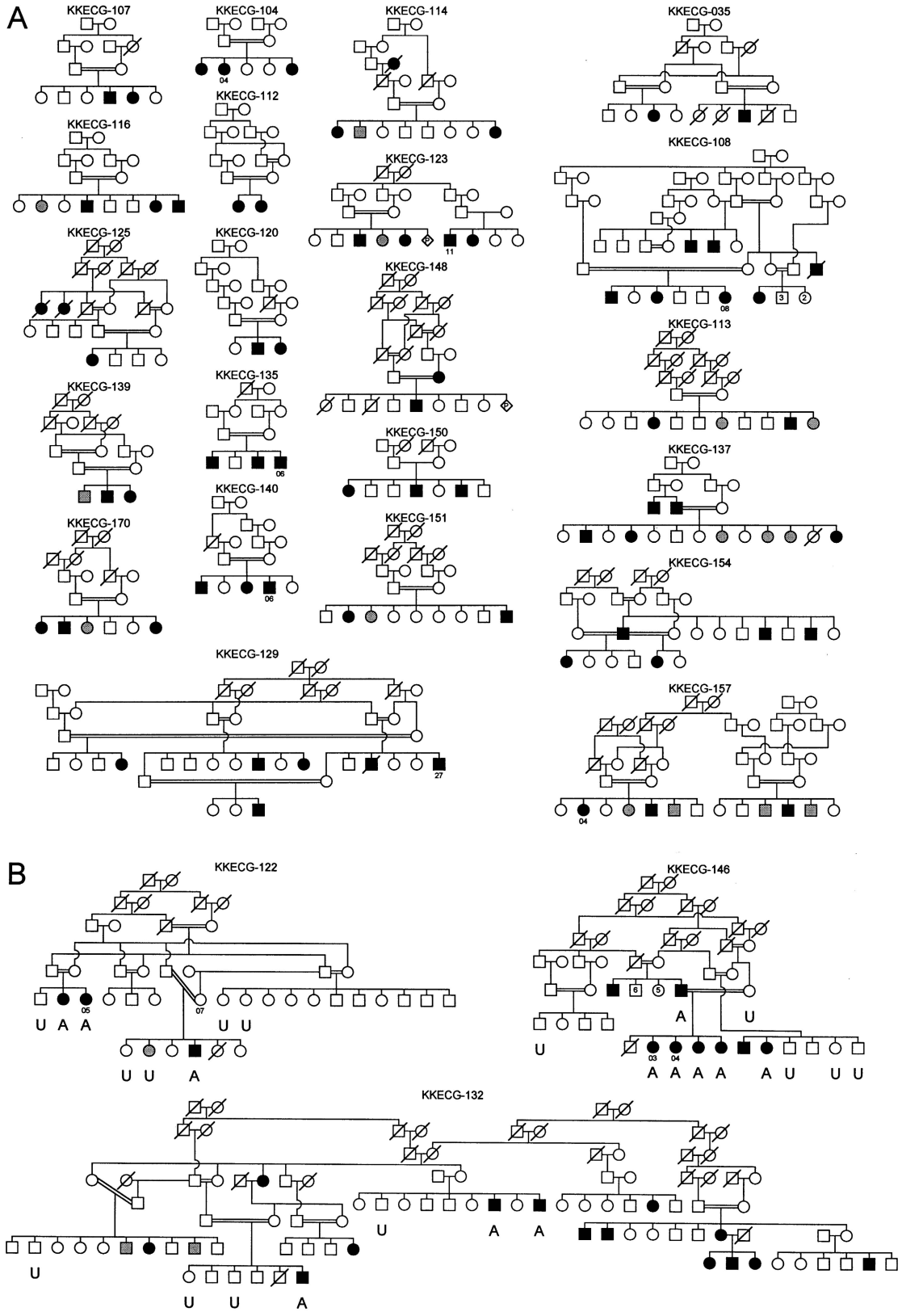

Figure 1 Pedigrees of 25 Saudi Arabian PCG families linked to markers on chromosome 2. Affected individuals are denoted by blackened symbols; individuals showing mild or no expression of the disease yet having haplotypes identical to those of their affected siblings are denoted by gray-shaded symbols; deceased family members are denoted by diagonal slashes; and consanguineous matings are denoted by double lines. Individual identification numbers are included below the symbols of affected individuals with disease chromosomes exhibiting informative crossover events. The three families in *B* were used for IBD analysis. Affected and unaffected individuals used in the IBD screen are labeled "A" and "U," respectively.

Table 1

Primers Used in the Amplification of *CYP1B1* Coding Exons

Exon and Primer	Sequence
2:	
CY2a:	
Forward	5′-AATTAACCCTCACTAAAGGGCGCTCCTGTCTCTGCACCCC-3′
Reverse	5′-GTAATACGACTCACTATAGGGCCGTCGCCGTAGCGCCGCGCC-3′
CY2b:	
Forward	5′-AATTAACCCTCACTAAAGGGGGCTCACCTCTCGTTCGCTC-3′
Reverse	5′-GTAATACGACTCACTATAGGGCGCTGCCGCGCACCAGCAGCG-3′
CY2c:	
Forward	5′-AATTAACCCTCACTAAAGGGGCCACGTGCTGAGCGAGGCG-3′
Reverse	5′-GTAATACGACTCACTATAGGGCTCAAGAACTTGTCCAGGATG-3′
CY2d:	
Forward	5′-AATTAACCCTCACTAAAGGGAATTCGAGCAGCTCAACCGC-3′
Reverse	5′-GTAATACGACTCACTATAGGGCTGGAGCGAAACCCCAAACCC-3′
3:	
CY3:	
Forward	5′-AATTAACCCTCACTAAAGGGTCACTTGCTTTTCTCTCTCC-3′
Reverse	5′-GTAATACGACTCACTATAGGGCAATTTCAGCTTGCCTCTTGC-3′

T4 polynucleotide kinase), 10 mM Tris-HCl (pH 8.4), 40 mM NaCl, 1.5 mM $MgCl_2$, 250 μM spermidine, 5% dimethyl sulfoxide, 200 μM of each dNTP, and 0.25 U AmpliTaq DNA polymerase (Perkin Elmer Cetus). Amplification products were size fractionated in 7% denaturing polyacrylamide gels (5.66 M urea and 32% formamide), and polymorphic alleles were analyzed after placement on Whatman paper and exposure to Amersham-MP film.

DNA microsatellite markers (di-, tri-, and tetranucleotides) of known chromosomal location, which were selected on the basis of available high-resolution index linkage maps (Matise et al. 1994; Utah Marker Development Group 1995) and the Cooperative Human Linkage Center Human Screening Set, version 6 (Research Genetics) (Murray et al. 1994), were used to screen the human genome for areas of homozygosity. Markers were chosen for their high degree of heterozygosity and for their average spacing, of ~10 cM, throughout the genome.

Linkage Analysis

A total of 260 (186 unaffected and 74 affected) individuals in the 25 families were genotyped. Polymorphic alleles were scored by visual inspection (Leppert et al. 1994). Primer sequences and reaction conditions are available from the Genome Data Base (http://gdbwww.gdb.org/gdb/), for the following 11 markers: *D2S1400* (GGAA20G10), *D2S1360* (GATA11H10), *D2S405* (GATA8F07), *D2S2383* (AFMa082xe1), *D2S352* (AFM296vg9), *D2S1325* (GATA22E06), *D2S1788* (GATA86E02), *D2S2186* (AFM163xc11), *D2S177* (AFM267zc9), *D2S1346* (ATA21D11), and *D2S1356* (ATA4F03). Two observers (B.A.B. and K.L.A.), each of whom was masked to affected status, scored the genotypes independently. Two-point linkage analyses were performed with the MLINK subroutine of the LINKAGE program (Lathrop et al. 1985), with an autosomal recessive mode of inheritance, a penetrance of .8, a disease-allele frequency of .02, and equal marker-allele frequencies.

Sequence Analysis of CYP1B1 *Coding Region*

CYP1B1 (GenBank accession number U56438 [http://www.ncbi.nlm.nih.gov/genbank/query_form.html]) coding exons 2 and 3 were each amplified from genomic DNA, under standard conditions (Anderson et al. 1995), with combinations of intron-specific and exon-specific primers tailed with −21 M13 sequences (forward reactions) and M13 reverse sequences (reverse reactions). The primers are listed as table 1. PCR products were then purified with the QIAquick PCR Purification Kit™ according to the manufacturer's recommendations. Sequencing reactions were performed with the ABI Prism™ Dye Primer Cycle Sequencing Ready Reaction Kit, with AmpliTaq DNA polymerase and FS with −21 M13 primers and M13 reverse primer, for the forward and reverse reactions, respectively, as specified by the manufacturer. Products of sequencing reactions were loaded onto 5% Long Ranger™ gels and were run on an Applied Biosystems 377 Automated DNA Sequencer.

Results

IBD Screening

Pooled genomic DNA samples of affected and unaffected individuals from selected pedigrees (KKECG-122, KKECG-132, and KKECG-146) were genotyped with highly polymorphic microsatellite markers. One hun-

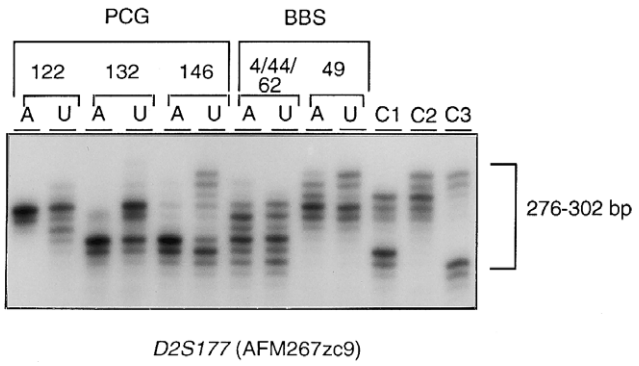

Figure 2 Pooled family samples for IBD screening. DNA from three Saudi PCG families (KKECG-122, KKECG-132, and KKECG-146) and from four Saudi Bardet-Biedl syndrome (BBS) families, used as controls, are combined into affected (lanes A) and unaffected (lanes U) pools and are used as PCR templates for the short-tandem-repeat polymorphic marker *D2S177*. DNA from three unrelated individuals (C1–C3) are also used as controls to assess individual marker "personality." Note the shift in allele pattern to homozygosity in the PCG-affected pools (lanes A), compared with the unaffected pools (lanes U), the BBS pools, and the controls. The reduction to homozygosity in the affected pools suggests that *D2S177* identifies an IBD region linked to a PCG locus in these families.

dred eighty-nine markers spanning chromosomes 1–6, 8, 11, 13, and part of 17, at ~10-cM intervals, were analyzed. One highly polymorphic marker on 2p21 (*D2S177*) showed a reduction to homozygosity in all the PCG affected pools, when compared with the unaffected pools, for all three families tested. These data suggested that *D2S177* reveals an IBD region linked to the putative PCG locus, *GLC3A,* in these families (fig. 2).

Genetic Analysis

Because of both the reduction to homozygosity at *D2S177* in three separate families in the IBD screen and the report, while our screen was in progress, of a PCG gene at the same locus (Sarfarazi et al. 1995), we genotyped 260 individuals from 25 PCG Saudi Arabian families including 74 affected individuals (fig. 1) with 11 highly polymorphic markers that map to this region (table 2). The maximum combined two-point LOD score (Z_{max}) for *D2S177* and the disease locus in all families was 15.76 at a maximum recombination fraction (θ_{max}) of .021 (table 2).

To determine the most likely position of *GLC3A* relative to the 11 linked markers, we analyzed recombinants segregating with *GLC3A,* for the region between *D2S1325* and *D2S1356,* in these 25 families. Ten disease chromosomes showed informative crossover events between the disease locus and flanking loci. These recombinational events narrowed the *GLC3A* locus to a 5-cM interval flanked by *D2S2186* and *D2S1356* (table 3).

Eighteen clinically unaffected individuals in 11 families were noted to have haplotypes identical to those of their affected siblings, which suggested decreased penetrance for this phenotype in our cadre of Saudi families (data not shown). The identification of these individuals allows an accurate calculation of the penetrance in this population (74/[18 + 74] = .8) and confirms our initial estimate of .8. However, subsequent interviews of these families revealed that at least two individuals who previously had been deemed unaffected but who had an "affected" haplotype (i.e., individuals KKECG-123-06 and KKECG-151-05) were diagnosed with glaucoma a few years after their initial enrollment in the study. Thus, PCG shows variable expressivity in this population. More-detailed longitudinal ophthalmologic examination of these families is characterizing further the phenotype of these individuals.

Mutation Analysis

Because of a report of *CYP1B1* mutations in PCG individuals (Stoilov et al. 1997), we screened the *CYP1B1* coding regions and identified three different transition mutations that, by conceptual translation, re-

Table 2

Z Values for *GLC3A* and 11 Markers on 2p21 in 25 Saudi Arabian PCG Families

	LOD Score at θ =								
Locus	.001	.01	.05	.10	.20	.30	.40	Z_{max}	θ_{max}
D2S1400	−2.59	−1.83	−.51	−.11	.45	.33	.11	.45	.207
D2S1360	−11.77	−6.14	−1.67	−.07	.75	.61	.24	.77	.220
D2S405	−3.09	−1.46	.19	.80	.87	.49	.13	.94	.153
D2S2383	−5.09	−1.60	1.49	2.34	2.10	1.14	.30	2.43	.129
D2S352	1.48	2.59	3.39	3.35	2.52	1.40	.44	3.43	.069
D2S1325	1.25	4.05	6.29	6.41	4.89	2.77	.94	6.50	.078
D2S1788	1.91	5.07	7.46	7.71	6.08	3.54	1.20	7.76	.084
D2S2186	8.27	9.71	10.52	9.95	7.39	4.22	1.40	10.52	.047
D2S177	15.09	15.65	15.41	13.99	9.97	5.53	1.77	15.76	.021
D2S1346	14.26	14.16	13.32	11.83	8.26	4.57	1.51	14.27	.000
D2S1356	8.03	9.34	9.84	9.09	6.52	3.62	1.20	9.88	.038

Table 3

Summary of 10 Informative Recombinants Localizing the *GLC3A* Locus on 2p21

	Recombinant Status (Parental Origin of Chromosome) of[a]									
Locus	104-04 (M)	108-08 (P)	122-05 (M)	123-11 (M)	129-27 (M)	135-06 (M)	140-06 (M)	146-03 (P)	146-04 (M)	157-04 (P)
D2S1325	R	…	N	N	N	R	N	…	…	R
D2S1788	…	…	R	N	R	N	R	…	…	…
D2S2186	…	…	…	R	…	…	…	…	…	…
D2S177	…	…	…	…	…	…	…	…	…	…
D2S1346	…	N	…	…	…	…	…	N	N	…
D2S1356	…	R	…	…	…	…	…	R	R	…

[a] P = paternal; M = maternal; R = recombinant; N = not informative; and an ellipsis (…) = nonrecombinant.

sult in missense amino acid substitution in 24 of the 25 families, which are summarized in table 4. The mutant alleles cosegregate with the disease phenotype in each family. As anticipated, in most families the affected individuals were homozygous for the mutant allele. Careful analysis of the haplotypes did not show conclusive evidence either for or against a founder effect for each of the three mutations (data not shown). However, affected individuals in families KKECG-114, KKECG-150, and KKECG-151 were compound heterozygotes. We also confirmed our initial finding of variable expressivity of the phenotype, by sequencing all the clinically unaffected individuals who had haplotypes identical to those of their affected siblings. All 18 individuals were either homozygous or compound heterozygous for mutations in *CYP1B1,* with no apparent correlation with the type of mutation. Interestingly, four families (KKECG-137, KKECG-146, KKECG-148, and KKECG-154) demonstrated a pseudodominant pattern of segregation (fig. 3). Direct sequence analysis (fig. 4) confirmed both the segregation of the mutant alleles and the molecular bases for the pseudodominant transmission. Sequence analysis of the *CYP1B1* coding region in 50 randomly sampled Saudi controls (100 chromosomes) with no known inherited eye disease showed none of these three mutations (data not shown).

Discussion

Both genetic linkage to markers on 2p21 and evidence for genetic heterogeneity have been reported in Turkish PCG families (Sarfarazi et al. 1995). Additionally, three different mutations in *CYP1B1* have been described in five Turkish families, suggesting a potential role for *CYP1B1* in ocular development. These three were homozygous frameshift mutations: a 13-bp deletion in exon 3; an insertion of a single cytosine base in exon 2; and a larger deletion affecting the 5′ end of exon 3 and adjacent intronic sequences. Each of these mutations may result in a functionally null allele (Stoilov et al. 1997).

Our screen of the human genome for regions of IBD by means of a DNA pooling strategy in three consanguineous Saudi Arabian PCG pedigrees identified on 2p21 a locus where a reduction to homozygosity in affected individuals was observed. These data suggested that this locus is linked to a putative PCG gene in these Saudi families. The presence of a PCG gene at this locus was then confirmed by formal linkage analysis in 25 Saudi Arabian families. We found no evidence for genetic heterogeneity in this population. Our data suggest variable expressivity for the PCG phenotype in the Saudi population, since 18 apparently unaffected individuals have haplotypes identical to those of their affected siblings; two of these individuals (KKECG-123-06 and KKECG-151-05) were subsequently diagnosed with glaucoma. Mutation analysis of the coding exons of *CYP1B1* in the 25 families showed either homozygous or compound heterozygous mutations in every affected individual investigated ($N = 30$) in 24 of these families.

Table 4

Summary of Mutations in Affected Members of 24 Saudi Arabian Families

Family/ Families (KKECG Number)	Mutation in Exon 2	Mutations in Exon 3
35, 104, 107, 113, 116, 122 123, 125, 129, 132, 135, 137 139, 140, 148, 154, 157	3987G→A (G61E)[a]	Wild type
114, 151	3987G→A (G61E)	7957G→A (D374N)
150	3987G→A (G61E)	8242C→T (R469W)
108, 112, 170	Wild type	8242C→T (R469W)[a]
146	Wild type	7957G→A (D374N)[a]

[a] Homozygous.

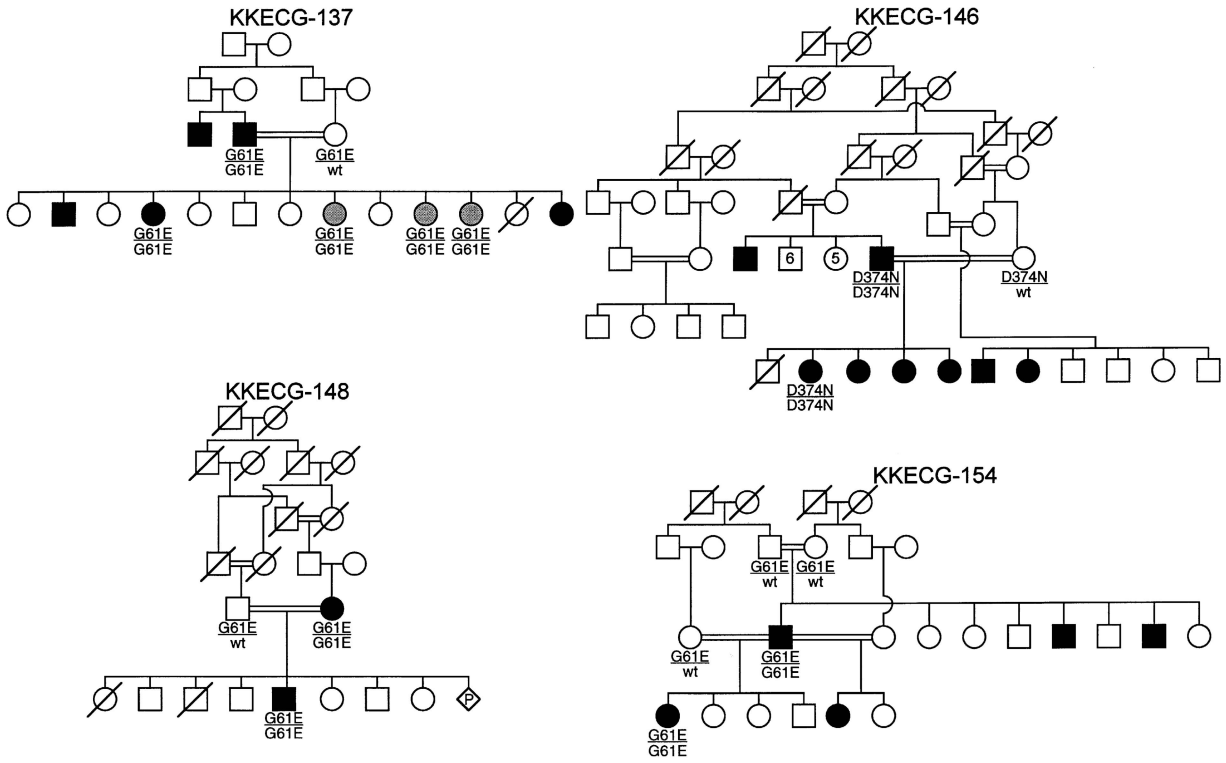

Figure 3 Pedigrees of the four families (KKECG-137, KKECG-146, KKECG-148, KKECG-154), showing pseudodominant inheritance of the PCG phenotype. The homozygous mutations that segregate with the disease phenotype are shown under the affected individuals (pedigree symbols as in fig. 1).

In each family, the mutant alleles cosegregate with the disease phenotype in an autosomal recessive pattern. Intriguingly, four highly consanguineous families show pseudodominant transmission of the phenotype. This finding is likely due to both the presence of multiple consanguinity loops and a high frequency of mutant alleles in this population. Sequence analysis of the affected parents showed homozygous mutations in both *CYP1B1* alleles, thus supporting the molecular basis for this clinical observation (fig. 3).

Three distinctive missense mutations that appear to correlate with tribal ancestry were defined in 24 Saudi Arabian families. The most common mutation, a G→A transition at nucleotide 3987, occurs in 78% of the PCG chromosomes analyzed and results in a missense amino acid substitution of glycine by glutamic acid, at position 61, adjacent to the N-terminal proline-rich region of CYP1B1. The proline-proline-glycine-proline motif may serve to join the membrane-binding N-terminus to the globular region of the P450 protein (Gonzalez 1989). The second mutation, a C→T transition at nucleotide 8242, occurs in 10% of the PCG chromosomes and changes arginine to tryptophan at position 469 of the protein. This switch from a basic to an aromatic amino acid, adjacent to a highly conserved cysteine residue (position 470) observed from bacteria to human (Gonzalez 1989), would be expected to alter substantially the putative enzymatic function of CYP1B1. The third mutation, a G→A transition at nucleotide 7957, in 6% of the PCG chromosomes, results in a change of aspartic acid to asparagine, at position 374 of the protein. All three mutated amino acid residues are highly conserved among different species and across the entire P450 superfamily (http://dot.imgen.bcm.tmc.edu:9331/seq-search/Help/beautypp.html).

The three mutations described in the five Turkish families are presumed to result in null alleles (Stoilov et al. 1997). In contrast, the three mutations identified in our panel of 24 Saudi families do not cause a shift in the reading frame of the gene and are likely to alter the putative enzymatic function of the protein. These may represent hypomorphic alleles whose phenotypic consequences could potentially be altered by levels of expression, modifier genes, or other factors, thus resulting in decreased penetrance and variability of expression.

CYP1B1 is a dioxin-inducible member of the cytochrome P450 gene superfamily (Sutter et al. 1991, 1994; Tang et al. 1996) and has a yet-undefined role in ocular

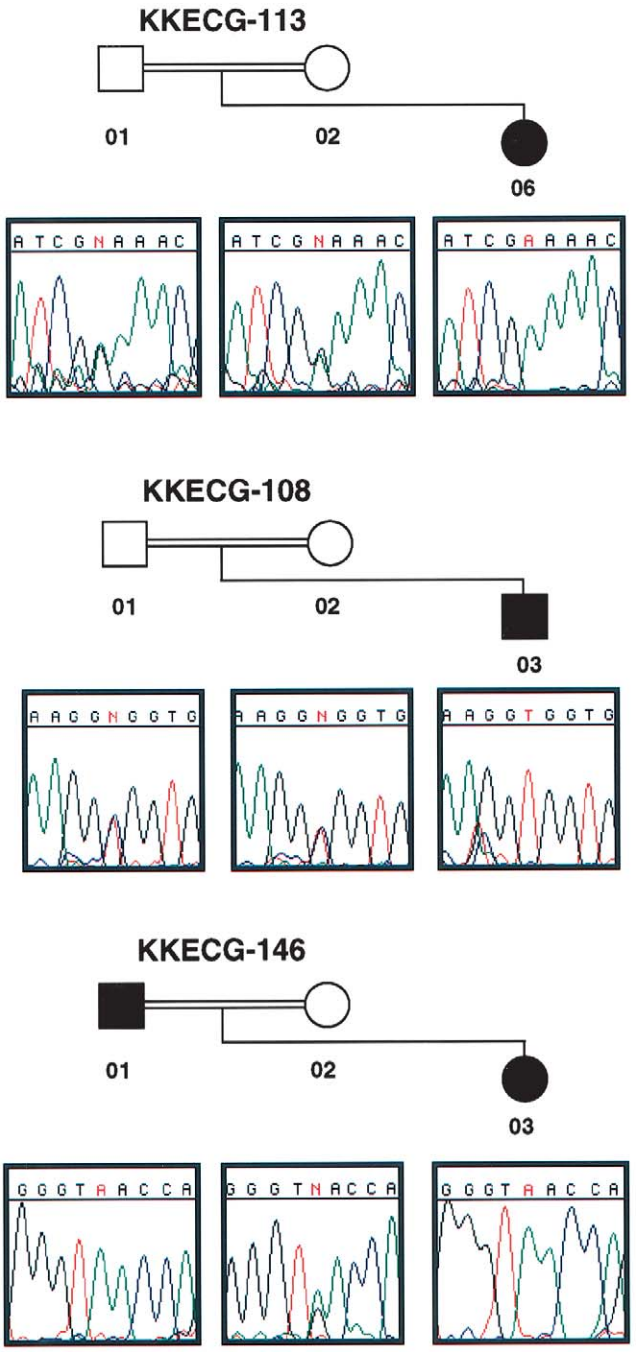

Figure 4 Three missense mutations observed in *CYP1B1* in 24 Saudi families. Direct sequencing of PCR products in homozygous and heterozygous individuals is shown, to demonstrate the segregation of the mutations with disease phenotype. One nuclear family each from KKECG-113, KKECG-108, and KKECG-146 is shown, with the corresponding electropherogram. The nucleotide sequence is N = G/A for KKECG-113, N = C/T for KKECG-108, and N = G/A for KKECG-146.

development. CYP1B1 and other drug-metabolizing enzymes seem to regulate the steady state of ligands that affect growth and differentiation (Nebert 1990, 1991). Mutations that alter the function of *CYP1B1* presumably disrupt that steady state and result in either abnormally high levels of these ligands or prolonged exposure of the developing tissues to these postulated effectors of growth and development. Either event could potentially cause structural aberrations in the developing tissues.

Future studies of CYP1B1, as well as the identification of its putative ligands, will contribute to the understanding of both ocular development and the role of other drug-metabolizing enzymes in organogenesis. Further detailed clinical and molecular examinations of the mildly affected patients described here, as well as of their environmental exposures, may identify additional factors that influence the expression of the PCG phenotype. Such knowledge should provide pharmacological targets for the antenatal modulation or postnatal therapy of PCG and, possibly, other forms of glaucoma. Finally, that only three distinctive mutations are responsible for 94% of the PCG mutations in the Saudi Arabian families that we examined suggests that neonatal or population screening for early therapeutic intervention would be an attractive public health approach to decrease the burden of infantile glaucoma in this population.

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