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DNA sequence variants in the tyrosinase-related protein 1 (TYRP1) gene are not associated with human pigmentary glaucoma

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Purpose: Pigmentary glaucoma is a common form of glaucoma affecting young adults. Previous studies have suggested that multiple factors, including multiple genetic factors, may be responsible for this condition. Recently, a form of glaucoma associated with pigment dispersion and iris atrophy was identified in the DBA/2J mouse. Abnormalities in the mouse *Tyrp1* gene contribute to this condition. The purpose of this study was to determine if DNA sequence variants in the human TYRP1 gene are associated with pigmentary glaucoma in humans.
Methods: The protein coding regions and intron/exon boundaries of the human TYRP1 gene were sequenced using genomic DNA samples from probands from pedigrees affected by pigment dispersion syndrome and pigmentary glaucoma.
Results: Three novel synonymous single nucleotide polymorphisms (SNPs) were found in two affected individuals. However, these polymorphisms did not define a haplotype that segregated with the disease in the families. DNA sequence variants that altered the amino acid sequence of TYRP1 were not found.
Conclusions: Despite the phenotypic similarity between the glaucoma in the DBA/2J mouse and human pigmentary glaucoma, the results of this study suggest that DNA sequence variants in the human TYRP1 gene are not associated with inherited pigmentary glaucoma in humans.

Pigmentary glaucoma is the most common nontraumatic cause of glaucoma in young adults [1]. This form of glaucoma is associated with the release of pigment granules from the iris that subsequently become dispersed throughout the anterior segment including the trabecular meshwork. Approximately 50% of individuals affected with pigment dispersion develop glaucoma [2]. The cause of the glaucoma is not known. Some studies suggest that the accumulation of pigment in the trabecular outflow pathways directly increases the intraocular pressure [3,4]. Other studies suggest that pigmentary glaucoma is the result of a generalized mesodermal dysgenesis of the eye that results in abnormal development of the anterior segment, including the trabecular meshwork and Schlemm's canal [5].

Recently, a form of glaucoma associated with pigment dispersion and iris atrophy has been identified in the DBA/2J mouse. Genetic linkage studies identified two chromosomal regions that contribute to the anterior segment changes and glaucoma: the iris pigment dispersion locus on chromosome 6, and the iris stromal atrophy locus on chromosome 4 [6]. The tryosinase-related protein 1 gene (*Tyrp1*) is located in the chromosome 4 region and is a probable candidate for the iris stromal atrophy phenotype. The DBA/2J mouse has two different missense mutations (Cys110Tyr, Arg326His) in the *Tyrp1* gene [7]. Alteration of the mouse *Tyrp1* gene is likely to cause iris stromal atrophy through a mechanism involving pigment production. Mutations in the human TYRP1 gene have been shown to be associated with Rufus oculocutaneous albinism (ROCA; OCA3), a rare autosomal recessive disease that has been discovered in South African Blacks [8]. Individuals affected by this condition have ocular features of albinism, but not of pigment dispersion or pigmentary glaucoma. Heterozygous carriers of the mutant gene do not have a recognized phenotype. ROCA has not been observed in the Caucasian population, and alterations of the TYRP1 gene have not been identified in Caucasians. It remains a possibility that alteration of the TYRP1 gene in the Caucasian population causes a phenotype that has not yet been recognized. The mutations in the TYRP1 gene identified in patients affected by ROCA all result in truncation of the nascent polypeptide [8]. In humans, missense mutations have not been identified in the TYRP1 gene [9].

We have previously characterized four pedigrees affected by an autosomal dominant form of pigment dispersion syndrome [10]. We have shown that the pigment dispersion trait in these families maps to a region on chromosome 7q35-36. Interestingly, only 30% of the affected members of the pedigrees linked to this region developed glaucoma. This result, and the results of other studies [11], suggest that multiple factors, including other genetic factors, may be necessary for the development of increased intraocular pressure and eventual pigmentary glaucoma in individuals with the pigment dispersion trait. The human TYRP1 gene is an excellent candidate for a secondary genetic factor contributing to glaucoma in patients with pigment dispersion syndrome. In addition to the iris stromal atrophy and pigmentary glaucoma in the DBA/2J

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mouse, alterations of the TYRP1 gene have been shown to influence the phenotypic expression of genes responsible for pigment production. It is possible that mutations in TYRP1 result in a qualitative or quantitative change in iris pigment production that may directly or indirectly result in an increase in intraocular pressure. In a manner similar to the DBA/2J mouse, where the most severe phenotype is evident when abnormalities of the TYRP1 gene and the second gene are present, variants of human TYRP1 may modify the pigment dispersion phenotype to produce the more severe pigmentary glaucoma phenotype. To investigate this hypothesis, we have determined if DNA sequence variants in the human TYRP1 gene are associated with pigmentary glaucoma.

METHODS

Patients: Probands from four families affected by an autosomal dominant form of pigment dispersion syndrome were studied (PDS5-11-2, PDS4-11-2, PDS92-111-1, and PDS221-11-2). Each of these individuals was affected by pigment dispersion syndrome and glaucoma. Clinical details regarding the complete pedigrees were published previously [10]. For the probands included in this study, the diagnosis of pigment dispersion syndrome was made by observation of characteristic iris transillumination defects and increased pigmentation of the trabecular meshwork using slit lamp biomicroscopy and gonioscopy. The diagnosis of glaucoma was established by measurement of intraocular pressure of over 22 mmHg on two occasions, evidence of optic nerve glaucomatous degeneration including increased cup/disc ratio, loss of nerve fiber layer and thinning of the neuroretinal rim, and evidence of visual field defects consistent with the observed damage to the optic nerve.

DNA sequencing: After informed consent was obtained from all participants, peripheral blood samples were collected. DNA was purified from lymphocyte pellets according to standard procedures. Genomic DNA from each proband was used for sequencing. The entire protein-coding regions and intron/exon boundaries of the TYRP1 gene were sequenced in the four pigmentary glaucoma probands as well as a control individual. Each exon of the TYRP1 gene was selectively amplified using flanking oligonucleotide primers. Nested primers end-labeled with ^{32}P were used for cycle sequencing. Bidirectional sequencing was performed for the seven translated and the first untranslated exon. Sequencing of the 5' and 3' regions flanking the gene was not performed.

RESULTS

The human and mouse TYRP1 genes consist of eight exons, seven of which are translated to form the protein product. The first exon is not translated. The seven translated exons, the first untranslated exon, and the intron/exon boundaries of the TYRP1 gene were sequenced in four probands affected by hereditary pigment dispersion syndrome and glaucoma and in one control individual. Three novel synonymous single nucleotide polymorphisms (SNPs) were found in two of the affected individuals (Table 1). Patient PDS 4-11-2 was heterozygous for two of the SNPs, one patient (PDS 92-111-1) was heterozygous for one, and the other two affected individuals and the control individual were homozygous for the common allele. DNA sequence variants that altered the amino acid sequence of TYRP1 were not found. Splice site mutations were also not present in DNA from any of the pigmentary glaucoma probands. Co-segregation of SNP alleles with pigment dispersion or pigmentary glaucoma was not seen in either of the pedigrees with probands who were heterozygous for these polymorphisms (results not shown).

DISCUSSION

Adult onset glaucoma is a complex disorder that is likely to result from alteration of multiple genetic factors [11,12]. Pigment dispersion syndrome appears to be inherited as an autosomal dominant trait and is likely to be the result of an alteration in a single gene. The inheritance of glaucoma in patients with pigment dispersion syndrome is not strictly Mendelian, suggesting that multiple genes may participate in the more severe pigmentary glaucoma phenotype. Proteins involved in pigment production, such as TYRP1, are excellent candidates for genetic factors that could combine with the pigment dispersion trait to produce the pigmentary glaucoma phenotype. Previous studies showed that pigment production is a complex regulatory pathway and that individual gene products involved in pigment production may influence the expression of other pigment related proteins [13].

TYRP1 is known to affect pigment production through a mechanism that involves melanosome stabilization [14]. Mice with missense mutations in the TYRP1 gene have disrupted melanosomes with clumping of pigment in affected tissues, and these abnormalities are likely to lead to the iris atrophy and subsequent glaucoma observed in the DBA/2J mouse. Despite the phenotypic similarity between the glaucoma in the DBA/2J mouse and human pigmentary glaucoma, the results of this study suggest that DNA sequence variants in the human TYRP1 gene are not associated with inherited pigmentary glaucoma in humans.

We have studied one population of patients affected by pigment dispersion and pigmentary glaucoma; it is possible that mutations in the TYRP1 gene may contribute to the disease in patients from other populations. Other proteins that function in the iris pigment cells may also contribute to the human phenotype.

Table 1. Synonymous SNPs in the human TYRP1 gene in patients with pigmentary glaucoma

Individual	Exon	DNA sequence change
PDS4-11-2	2	Arg87Arg (C374A)
PDS4-11-2	4	Ser243Ser (T844C)
PDS92-111-1	5	Asp343Asp (C1144T)

Synonymous single nucleotide polymorphims (SNPs) identified in indivdiuals with pigment dispersion syndrome and pigmentary glaucoma in exons of the human TYRP1 gene.

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