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Mutation screening of the phosducin gene *PDC* in patients with retinitis pigmentosa and allied diseases

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Purpose: To search for a phenotype associated with mutations in the phosducin gene *PDC*.
Methods: We screened 853 patients with retinitis pigmentosa or an allied disease diseases, including groups of 61 to 212 patients, each with dominant retinitis pigmentosa (RP), recessive RP, Leber congenital amaurosis, or cone-rod degeneration, for mutations in the *PDC* gene using direct genomic sequencing of the three coding exons and their flanking intron splice sites.
Results: We found one polymorphism in the 5' untranslated region (minor allele frequency of 0.149) and three rare single-base sequence variants (one missense change, one isocoding change, and one in the 3' untranslated region). The rare variants were found in one heterozygous patient each and none was interpreted as pathogenic.
Conclusions: Phosducin mutations are not a major cause of dominant or recessive RP, Leber congenital amaurosis, or cone-rod degeneration. The human phenotype associated with phosducin defects remains unknown.

Phosducin (*PDC*, OMIM 171490; Locus ID 51320) is a protein abundantly expressed in the retina and is found in both the inner segments and the outer segments of rod and cone photoreceptors [1-3]. While its function is not completely known, it interacts with the βγ complex of transducin [4,5]. In the dark, phosducin is phosphorylated by protein kinase A or calmodulin-dependent protein kinase II, and the phosphorylated form of phosducin more avidly binds to βγ transducin [5-9]. Recently, the binding of phosducin to βγ transducin was shown to inhibit phosducin's degradation by an ubiquitin-proteasome pathway [10]. This function of phosducin may be a potential mechanism for regulating the level of βγ transducin complexes; this, in turn, may modulate the phototransduction cascade.

Mutations in genes encoding many other members of the phototransduction cascade are known causes of retinitis pigmentosa or allied diseases (RetNet). The gene encoding phosducin is located within 1q25-32.1 [11]. There is evidence of a gene causing age-related macular degeneration in this region [12]; however, no mutations were found in the sequence of *PDC* in affected members of families showing linkage to this region [13]. Thus far, no phenotype has yet been associated with mutations in the *PDC* gene. The only published analysis of the *PDC* gene in patients with retinitis pigmentosa or an allied disease found no disease-causing mutations among 83 patients [14]. We report here our extended analysis of this gene in a much larger set of patients.

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METHODS

This study involved human subjects and conformed to the Declaration of Helsinki. Leukocyte DNA was extracted from peripheral blood of 853 unrelated patients with various hereditary retinal diseases. Almost all patients resided in the United States and Canada, and they were of mixed ethnic background comparable to what is found in these countries. Among these patients, 212 patients were diagnosed with autosomal recessive retinitis pigmentosa (RP), 193 with autosomal dominant RP, 93 with autosomal recessive cone-rod degeneration, 61 with Leber congenital amaurosis (LCA), 40 with cone dysfunction syndrome, 37 with achromatopsia, 27 with isolate RP, 26 with macular malfunction, and the remaining 164 with atypical forms of retinal disease, including sector RP [15] and clumped pigmentary retinal degeneration [16] among others, each category of which was composed of small numbers of patients. Patients with autosomal recessive RP included in this study were from families with two or more affected siblings and no previous generations with RP or were the affected offspring of a consanguineous mating of parents without a history of RP; isolate RP patients had no affected relatives and had parents who had no known blood relationship. Patients with autosomal dominant RP came from families with two or more consecutive affected generations and, in most cases, three consecutive affected generations or evidence of father-to-son transmission. Patients with cone-rod degeneration had substantially greater loss of cone function compared to loss of rod function [17]. Patients with LCA came from families exhibiting a recessive inheritance pattern. The diagnosis of cone dysfunction syndrome was given to patients with features of cone malfunction but with no definite indication of a progressive cone degeneration. Patients with macular malfunction had

abnormal foveal ERGs and normal full-field ERGs [18]. None of the relatives of patients with cone dysfunction syndrome or macular malfunction was affected. Patients with identified causative mutations in other genes were excluded from this study except for two patients with recessive RP, 27 with dominant RP, two with LCA, one with cone dysfunction syndrome, 13 with achromatopsia, and three with atypical forms of retinal disease whose causative mutations in genes other than phosducin were discovered during the course of this study.

All three coding exons of the *PDC* gene (GenBank accession number NM_002597) [19,20] and the immediate intronic sequences including splice sites were amplified by the polymerase chain reaction (PCR) and analyzed by direct DNA sequencing using a dye-terminator cycle sequencing kit (version 3.1) and an ABI automated sequencer. The sequences of the primers used for this study are in Table 1.

RESULTS

One missense sequence variant (c.200 G>A; Arg67Gln, CGA>CAA) was observed in a heterozygous patient with macular malfunction; both parents and the only sibling, all unaffected by history, were deceased and thus segregation analysis of this mutation was not possible. An iso-coding sequence variant (c.177 G>A; Arg59Arg, AGG>AGA) was found in a heterozygous isolate patient with RP. Two other patients, one with isolate RP and the other with clumped pigmentary retinal degeneration, were heterozygotes for an intron change in the 3' UTR region of exon 4 (c.*7 A>T) respectively. None of these three changes create or destroy splice donor or acceptor sites based on splice-site prediction software (NN SPLICE, version 0.9) available at Berkeley Drosophila Genome Project [21]. No second change in the phosducin gene was found in any patient.

A polymorphism in the 5' UTR was also identified (c.-9 G>A). The minor allele frequency (the “A” allele) was 14.9% based on an analysis of 820 individuals (1640 chromosomes) in whom this polymorphism could be reliably determined.

DISCUSSION

In this study, mutation screening of 853 patients with RP or an allied retinal disease failed to reveal mutations that convincingly account for any photoreceptor disease. Only three rare sequence variations and a common polymorphism were identified. Among the three rare variants, only one (Arg67Gln) is expected to alter the wild-type amino acid sequence. We were unable to determine whether or not this change was pathogenic. It was found heterozygously in an isolate patient with macular malfunction, and no family members were available for a segregation analysis. While Arg67 is a highly conserved residue among the mammalian phosducins ranging from mouse to man, so are 88-91% of the residues in this protein, so it is not possible to estimate, based on sequence conservation, how important Arg67 is for phosducin’s function. The residue corresponding to position 67 in phosducin is not Arg in the human phosducin-like protein PhLP [22].

It is possible that pathogenic mutations might exist outside of the coding exons and the flanking intron splice sites, or that large deletions or gene rearrangements involving the coding exons might exist that were not detected among the patients evaluated in this study. Nevertheless, based on the numbers of patients evaluated, one can estimate that, with 90% confidence, pathogenic *PDC* mutations in the regions of the gene screened occur in fewer than 1.1% of cases of autosomal recessive RP, 1.2% of autosomal dominant RP, 2.4% of cone-rod degeneration, 3.7% of LCA, and 5.6% of cone dysfunction syndrome. Thus, our results suggest that *PDC* is probably not a major cause of these clinical entities in the population from which the patients are derived.

Table 1. PCR primers used in this study

Primer Name	Nucleotide sequence (5' > 3')	Exon
5106S	CTTATGGAATCTGGCAGCAG	2
5107A	AGCTGGGCAGCAAGAAATTA	2
4926S	TTATGCCAACAGTCCTTTTGAA	3
4927A	TTTTTGCCAGCGTAAGCAAT	3
5084S	AGAAAGGCACATCAGGAGTGA	4
5085A	TCGATTGTTGCATCAGTCATT	4

Primer names terminating in an “S” are in the sense direction; names with an “A” are in the antisense direction.

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REFERENCES

1. Lee RH, Brown BM, Lolly RN. Light-induced dephosphorylation of a 33K protein in rod outer segments of rat retina. Biochemistry 1984; 23:1972-7.
2. Thulin CD, Howes K, Driscoll CD, Savage JR, Rand TA, Baehr W, Willardson BM. The immunolocalization and divergent roles of phosducin and phosducin-like protein in the retina. Mol Vis 1999; 5:40 .
3. Nakano K, Chen J, Tarr GE, Yoshida T, Flynn JM, Bitensky MW. Rethinking the role of phosducin: light-regulated binding of phosducin to 14-3-3 in rod inner segments. Proc Natl Acad Sci U S A 2001; 98:4693-8.
4. Lee RH, Ting TD, Lieberman BS, Tobias DE, Lolley RN, Ho YK. Regulation of retinal cGMP cascade by phosducin in bovine rod photoreceptor cells. Interaction of phosducin and transducin. J Biol Chem 1992; 267:25104-12.
5. Yoshida T, Willardson BM, Wilkins JF, Jensen GJ, Thornton BD, Bitensky MW. The phosphorylation state of phosducin determines its ability to block transducin subunit interactions and inhibit transducin binding to activated rhodopsin. J Biol Chem 1994; 269:24050-7.
6. Lee RH, Brown BM, Lolley RN. Protein kinase A phosphorylates retinal phosducin on serine 73 in situ. J Biol Chem 1990; 265:15860-6.
7. Willardson BM, Wilkins JF, Yoshida T, Bitensky MW. Regulation of phosducin phosphorylation in retinal rods by Ca2+/calmodulin-dependent adenylyl cyclase. Proc Natl Acad Sci U S A 1996; 93:1475-9.

8. Chen F, Lee RH. Phosducin and betagamma-transducin interaction I: effects of post-translational modifications. Biochem Biophys Res Commun 1997; 233:370-4.
9. Thulin CD, Savage JR, McLaughlin JN, Truscott SM, Old WM, Ahn NG, Resing KA, Hamm HE, Bitensky MW, Willardson BM. Modulation of the G protein regulator phosducin by Ca2+/calmodulin-dependent protein kinase II phosphorylation and 14-3-3 protein binding. J Biol Chem 2001; 276:23805-15.
10. Obin M, Lee BY, Meinke G, Bohm A, Lee RH, Gaudet R, Hopp JA, Arshavsky VY, Willardson BM, Taylor A. Ubiquitylation of the transducin betagamma subunit complex. Regulation by phosducin. J Biol Chem 2002; 277:44566-75.
11. Sparkes RS, Lee RH, Shinohara T, Craft CM, Kojis T, Klisak I, Heinzmann C, Bateman JB. Assignment of the phosducin (PDC) gene to human chromosome 1q25-1q32.1 by somatic cell hybridization and in situ hybridization. Genomics 1993; 18:426-8.
12. Klein ML, Schultz DW, Edwards A, Matise TC, Rust K, Berselli CB, Trzupek K, Weleber RG, Ott J, Wirtz MK, Acott TS. Age-related macular degeneration. Clinical features in a large family and linkage to chromosome 1q. Arch Ophthalmol 1998; 116:1082-8.
13. Schultz DW, Klein ML, Humpert AJ, Luzier CW, Persun V, Schain M, Mahan A, Runckel C, Cassera M, Vittal V, Doyle TM, Martin TM, Weleber RG, Francis PJ, Acott TS. Analysis of the ARMD1 locus: evidence that a mutation in HEMICENTIN-1 is associated with age-related macular degeneration in a large family. Hum Mol Genet 2003; 12:3315-23.
14. Ara-Iwata F, Jacobson SG, Gass JD, Hotta Y, Fujiki K, Hayakawa M, Inana G. Analysis of phosducin as a candidate gene for retinopathies. Ophthalmic Genet 1996; 17:3-14.
15. Berson EL, Howard J. Temporal aspects of the electroretinogram in sector retinitis pigmentosa. Arch Ophthalmol 1971; 86:653-65.
16. Sharon D, Sandberg MA, Caruso RC, Berson EL, Dryja TP. Shared mutations in NR2E3 in enhanced S-cone syndrome, Goldmann-Favre syndrome, and many cases of clumped pigmentary retinal degeneration. Arch Ophthalmol 2003; 121:1316-23.
17. Berson EL, Gouras P, Gunkel RD. Progressive cone-rod degeneration. Arch Ophthalmol 1968; 80:68-76.
18. Matthews GP, Sandberg MA, Berson EL. Foveal cone electroretinograms in patients with central visual loss of unexplained etiology. Arch Ophthalmol 1992; 110:1568-70.
19. Abe T, Nakabayashi H, Tamada H, Takagi T, Sakuragi S, Yamaki K, Shinohara T. Analysis of the human, bovine and rat 33-kDa proteins and cDNA in retina and pineal gland. Gene 1990; 91:209-15.
20. Abe T, Kikuchi T, Shinohara T. The sequence of the human phosducin gene (PDC) and its 5'-flanking region. Genomics 1994; 19:369-72.
21. Reese MG, Eeckman FH, Kulp D, Haussler D. Improved splice site detection in Genie. J Comput Biol 1997; 4:311-23.
22. Miles MF, Barhite S, Sganga M, Elliott M. Phosducin-like protein: an ethanol-responsive potential modulator of guanine nucleotide-binding protein function. Proc Natl Acad Sci U S A 1993; 90:10831-5.

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