Molecular Vision 2006; 12:1511-5 <http://www.molvis.org/molvis/v12/a173/>
Received 3 July 2006 | Accepted 29 November 2006 | Published 5 December 2006

Mutation screening of the peropsin gene, a retinal pigment epithelium specific rhodopsin homolog, in patients with retinitis pigmentosa and allied diseases

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Purpose: To investigate the peropsin gene (*RRH*), encoding a retinal pigment epithelium homolog of the rod-expressed opsin (rhodopsin), for the presence of pathogenic mutations causing retinitis pigmentosa (RP) or other retinal degenerations.
Methods: All seven exons composing the RRH open reading frame and the immediate intron sequences were analyzed by direct nucleotide sequencing of 613 patients with forms of retinal degeneration.
Results: One patient with retinitis punctata albescens was a heterozygote with the missense change Cys98Tyr (TGT>TAT, c.293G>A). This change affects the homologous residue that is the target of the rhodopsin mutation Cys110Tyr, a reported cause of dominant RP. Unfortunately, none of the patient's relatives were available for a segregation analysis to determine if this change is unambiguously associated with disease. No definite pathogenic mutation was found in any of the other 612 patients who were evaluated.
Conclusions: The Cys98Tyr is a possible cause of retinitis punctata albescens, although this conclusion is tentative because the change was found in only one patient. Our results indicate that the peropsin gene is not a common cause of RP or some related retinal degenerations, at least in the set of patients we analyzed.

The first step of the visual process occurs in the retinal photoreceptors and is determined by the capture of photons by a class of light-sensing molecules called visual pigments. These conjugated proteins consist of an apoprotein moiety, called an opsin, and a covalently bound chromophore, typically 11-*cis*-retinal in mammals. Upon absorption of light, the 11-*cis* retinal in a visual pigment isomerizes to its all-*trans* form and initiates a cascade of reactions resulting in signaling this photic event to the brain. All-*trans* retinal is subsequently detached from the opsin, reduced to all-*trans* retinol, transported to the retinal pigment epithelium (RPE), and, after a series of transformations, re-converted to 11-*cis* retinal and returned to a photoreceptor cell to regenerate a photosensitive visual pigment [1,2]. Another group of pigments, such as the squid retinochrome [3,4] and the vertebrate retinal pigment epithelium G protein-coupled receptor (RGR) [5] constitute a distinct subset of conjugated proteins with conserved sequence homologies [6]. These pigments, rather than being involved in signaling light absorption, apparently perform the conversion of all-*trans* to 11-*cis* retinal [3,4], at least in vitro (for RGR) [7], possibly contributing to the recycling of the chromophore for the visual pigments.

Peropsin is a visual pigment-like protein expressed exclusively in the RPE. It shares a large degree of homology with the squid retinochrome and RGR [8]. The analysis of the peropsin primary sequence shows the presence of all the necessary elements allowing the binding of a retinoid ligand [8], although the existence of a peropsin chromophore has not yet been demonstrated. Unlike light-sensing pigments, and similar to RGR, peropsin is first expressed early during fetal development; its expression persists into adulthood [8,9]. A putative ortholog of peropsin, the Amphioxus protein Amphiop3, binds to all-*trans* retinal and can catalyze its photoisomerization into 11-*cis* retinal in vitro [6]. Because of its primary sequence, tissue distribution, expression pattern, and similarity with other proteins with known function, peropsin may not be involved in a light-sensing pathway but perhaps instead may aid chromophore recycling for the photoreceptor visual pigments.

Inherited retinal degenerations are a group of diseases that display a high degree of genetic heterogeneity and are globally characterized by the progressive degeneration of the retina in affected patients. Clinically, they are also relatively heterogeneous with regard to the geographic area of the retina primarily affected, and the types of retinal cells that are first affected. Mutations in many genes encoding proteins involved in light sensing and in chromophore regeneration have been found to cause retinal degeneration in humans [10,11]. In addition, mutations in RGR cause retinitis pigmentosa (RP), a form of retinal degeneration [12].

Because of these numerous associations between human retinal degenerations and genes encoding visual pigments, their homologs in the retina, and proteins in the visual cycle, we investigated the peropsin gene for the presence of possible

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mutations in patients affected with hereditary photoreceptor diseases.

METHODS

Patients: This study involved human subjects and was carried out in accordance with the tenets of the Declaration of Helsinki. We recruited index patients with RP and other forms of retinal degeneration (Table 1), along with control individuals who had no visual symptoms and no known blood relatives with hereditary retinal degeneration. Most of the patients were clinically evaluated with an ophthalmologic examination including visual field testing and electroretinography (ERGs). The mode of inheritance of the disease was inferred in most cases from the family history. Patients with autosomal recessive RP had unaffected parents and either had one or more affected siblings or were the offspring of a consanguineous relationship. Patients with dominant inheritance had two or more generations of affected relatives. We also analyzed DNA from patients with diffuse atrophy of the RPE which may or may not have a genetic basis; these patients did not show signs of ocular inflammation and had a negative family history of retinal degeneration. For genetic analyses, patients and relevant members of their families donated 10-50 ml of blood for this research, upon signature of a written informed consent.

Genetic analyses: Genomic DNA was purified from peripheral leukocytes of patients and patients' relatives. PCR primers listed in Table 2 were used to individually amplify each of the seven exons and the immediately flanking intron sequences of the human peropsin gene (*RRH*). For each reaction, 20-100 ng of leukocyte DNA was amplified in a solution containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 1.5 M betaine solution (with the exception of the amplification reactions involving exon 2, for which no betaine was added), 0.2 mM of each dNTP, 0.25 units of AmpliTaq DNA Polymerase (Applied Biosystems, Foster City, CA) and 20 pmoles of each of the two exon-specific oligonucleotide primers. There was an initial denaturing step at 94 °C for 1 min, followed by 35 PCR cycles of 93 °C (30 s), 58 °C (for exons 1, 3-7) or 54 °C (for exon 2; 30 s), 72 °C (30 s). Amplified DNA fragments were treated with 1.5 µl ExoSAP-IT (USB, Cleveland, OH) containing a mix of exonuclease I and shrimp alkaline phosphatase to eliminate residual primer oligomers, single-strand DNA molecules, and unincorporated dNTPs. The fragments were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and a 3100 ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Electropherogram analysis, sequence alignment, and mutation detection were performed with the Staden software package [13].

RESULTS

The complete list of the DNA changes identified in our set of patients is given in Table 3.

A number of single-nucleotide polymorphisms (SNPs) and other polymorphic variants were detected in both exons and introns of the peropsin gene. Exonic SNPs included Leu153Leu (CTG>CTA, c.459G>A), Thr174Thr (ACG>ACA,

Table 1. Diagnoses of 613 patients who were evaluated

Diagnosis	Patients screened	Hypothetical maximum prevalence of peropsin mutations (95% confidence)
Retinitis pigmentosa, autosomal recessive	200	1.50
Retinitis pigmentosa, autosomal dominant	93	3.2
Cone-rod retinal degeneration	86	3.4
Retinitis pigmentosa, atypical	76	3.9
Leber congenital amaurosis	66	4.4
Diffuse atrophy of the retinal pigment epithelium	31	9.2
Generalized choroidal sclerosis	28	10
Sector retinitis pigmentosa	10	26
Cone degeneration	9	28
Clumped pigmentary retinal degeneration	9	28
Retinitis punctata albescens	5	45

The right column indicates the highest portion of hypothetical peropsin-related cases compatible with finding no peropsin mutation in the number of patients evaluated with a 95% confidence interval; it was computed as using a method described in reference [18]. For example, one would have a 95% chance of finding at least one peropsin-related case of recessive RP among a set of 200 unrelated cases if peropsin were responsible for 1.5% or more of all recessive retinitis pigmentosa (RP) cases. Because our analysis excluded some patients who had previously been found to have mutations in other disease genes, the effective number of patients evaluated is higher. This would cause the percentages listed in the right column to be conservative estimates; i.e., the true percentages would be lower.

c.522G>A), and His211Arg (CAT>CGT, c.632A>G). Intronic polymorphisms were IVS4-42G>T (c.552-42G>T, dbSNP: rs4698750), IVS5+8A>G (c.720+8A>G, dbSNP: rs4698795), and the microinsertion IVS4-93insT (c.552-93_552-92insT).

Rare changes that did not affect the *RRH* open reading frame were also detected in both exons and proximal introns of the peropsin gene. All of these changes (as well as the SNPs mentioned in the previous paragraph) were investigated for possible influences on the splicing pattern of the peropsin gene by the use of the splice site prediction software NNSPLICE [14], and in all instances they were predicted to be neutral with respect to the splicing of peropsin RNA. This was the case, specifically, for the heterozygous changes IVS1+20T>A (c.106+20T>A) found in a patient with dominant RP, Ile41Ile (ATC>ATT, c.123C>T) detected in a patient with cone-rod degeneration, and IVS5-5T>C (c.721-5T>C) found in a patient with recessive RP. We considered these changes to be nonpathogenic.

Some rare changes that were predicted to alter the encoded protein were nonetheless interpreted as nonpathogenic. The missense Ser18Leu (TCG>TTG, c.53C>T) was detected in two index patients: one with atypical RP and one with recessive RP. However, this change was absent in the affected sister of the patient with recessive RP (data not shown). The microdeletion Val251fs (GTG>GT-, c.753delG) was found in a patient with dominant RP, but was absent in the patient's affected sister (data not shown). Finally, the missense change Asp326Asn (GAT>AAT, c.976G>A) was present in nine patients with four different diagnoses. Out of these nine patients only one suffered from recessive RP; his affected sibling did not carry the missense change.

Our evidence was insufficient to unambiguously classify some of the DNA changes as being either pathogenic or nonpathogenic. The insertion-deletion variant Ile244 (ATC>G-C, c.730_731delATinsG) was detected in a heterozygote with recessive RP who was the offspring of a consanguineous relationship (the parents were first cousins, both unaffected). No second mutation was found. Since the proband's father was himself an unaffected carrier of this mutation, we interpreted

Table 2. Polymerase chain reaction primers used in this study

Primer name	Nucleotide sequence (5'-3')	Exon
5511	TCTCTAAGTGGAAATGATTGG	1
4342	ACTTCTGCCTTTCTTCACCCATTC	1
5512	TTTAATTTTGAGTCTACTTTG	2
4344	TTTATTTTGCTTTCATTCCAGTG	2
5513	TGAGATATATTCTAACAATGGAC	3
4346	CATTAAGAAAGCTGAGAAAAGTG	3
5514	AATCAAGCAAGTTTAAAAAGTG	4
4348	CATTAGAAGCATTTGATTATAAAG	4
5155	GGGCCTAAATAAACTAAAAGTC	5
4350	CTCCATGACAACAATTTTTCAAGG	5
5516	CAACCCAGATAGATTTTTACC	6
4352	TACTGCAAGCATTTTAGACATTAC	6
4353	TGCCAGATTTTCCTTTTTTTATCG	7
5517	TGCACTTGATCTAAATGGGC	7

For each exon, the first primer listed is the sense primer, and the second primer is the antisense.

Table 3. DNA variants found and their effects

Intron or exon	DNA change	Effect
1	IVS1+20 T-A, c.106+20T-A	Nonpathogenic
1	Ser18Leu (TCG>TTG), c.53C>T	Nonpathogenic
2	Ile41Ile (ATC>ATT), c.123C>T	Nonpathogenic
2	Cys98Tyr (TGT>TAT), c.293G>A	Possibly pathogenic
3	Asn105Ser (AAT>AGT), c.314A>G	Uncertain
3	Ala120Val (GCT>GTT), c.359C>T	Uncertain
4	Leu153Leu (CTG>CTA), c.459G>A	Nonpathogenic
4	Thr174Thr (ACG>ACA), c.522G>A	Nonpathogenic
4	IVS4-42 G>T, c.552>42G-T	Nonpathogenic
4	IVS4-93 insT, c.552>93_552>92insT	Nonpathogenic
5	IVS5+8 A>G, c.720+8A>G	Nonpathogenic
5	His211Arg (CAT-CGT), c.632A>G	Nonpathogenic
5	IVS5-5 T-C, c.721-5T>C	Nonpathogenic
6	Ile244 (ATC>G>C), c.730_731delATinsG	Likely nonpathogenic
6	Met248Val (ATG>GTG), c.742A>G	Uncertain
6	Val251fs (GTG>GT>), c.753delG	Nonpathogenic
6	Pro274Ser (CCC>TCC), c.820C>T	Likely nonpathogenic
7	Asp326Asn (GAT>AAT), c.976G>A	Nonpathogenic
7	Arg302Gln (CGG>CAG), c.905G>A	Uncertain

These DNA changes were identified in 613 patients with various forms of retinal degeneration by direct nucleotide sequencing of all exons and proximal intron regions of the peropsin gene (see text for details).

this DNA change as not a dominant cause of RP, but we could not rule out the possibility that it is a recessive mutation. The missense change Pro274Ser (CCC>TCC, c.820C>T) was detected heterozygously in two monozygotic twins with Leber congenital amaurosis, recessively inherited. We considered Pro274Ser unlikely to be a recessive, pathogenic mutation because no second *RRH* mutation was found in the twins and because the substitution of a proline with a serine residue at codon 274 is probably not critical, since murine peropsin has a serine at this position. The missense change Met248Val (ATG>GTG, c.742A>G) was found in a heterozygote with recessive cone-rod degeneration. Since no second mutation was found and no other affected relative was available for further testing, we classified this change as of uncertain pathogenicity. A similar situation was observed for DNA variants Asn105Ser (AAT>AGT, c.314A>G), Ala120Val (GCT>GTT, c.359C>T), and Arg302Gln (CGG>CAG, c.905G>A), each found heterozygously in a patient with recessive RP, with recessive cone-rod degeneration, and with diffuse atrophy of the RPE, respectively. No other mutations were found in the peropsin sequence of these cases and, because of the absence of informative family members available for analysis, the pathogenic nature of the DNA changes could not be either proved or excluded. The missense change Cys98Tyr (TGT>TAT, c.293G>A) was found in a heterozygote with retinitis punctata albescens. This patient had no family history of retinal degeneration, and no family members were available for analysis. We considered this change as possibly pathogenic, for the reasons described in the next section.

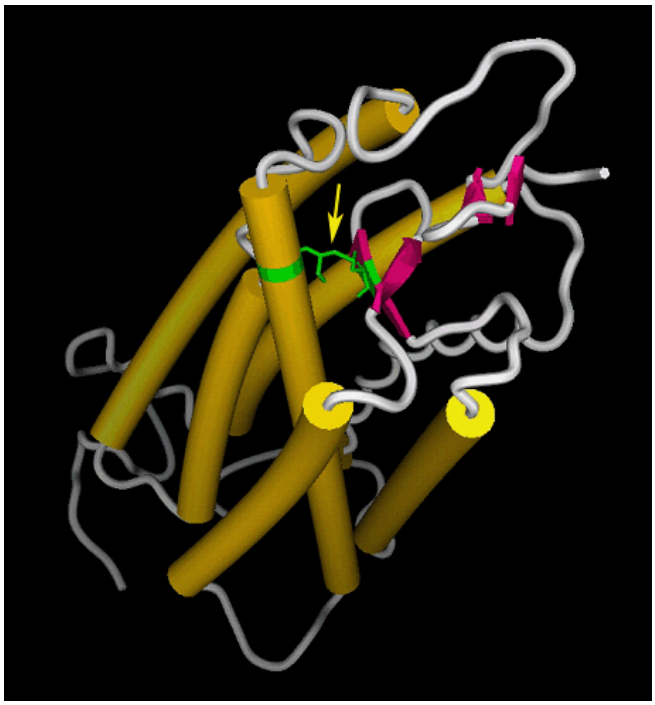

Figure 1. Consequences of the Cys98Tyr change on the putative Cys98-Cys175 disulfide bridge of peropsin. The figure shows the putative 3D structure of peropsin, as modeled on crystallographic data from rhodopsin [16]. According to what is currently known about opsins and their structural features, peropsin's residues Cys98 and Cys175 (green stripes) should be connected by a disulfide bond (yellow arrow). The Cys98Tyr (TGT->TAT) mutant observed in a patient with retinitis punctata albescens would eliminate this bond and could potentially compromise the structure and function of the protein.

Finally, to better assess the nature of all changes that could not be unequivocally considered as nonpathogenic, we PCR-amplified and sequenced *RRH* exons 2, 3, 6, and 7 in 188 chromosomes from 94 control individuals. None of the seven variants were found in these controls. Unfortunately, this result is uninformative since it is consistent with these variants being either rare, nonpathogenic variants or pathogenic mutations.

DISCUSSION

In the present study we analyzed the peropsin gene for mutations in patients with inherited retinal degeneration to search for changes that might cause the surveyed retinopathies. Because of the possible role of peropsin in the retinoid cycle and its specific expression in the RPE, we considered this gene a candidate for inherited photoreceptor diseases. We included in our study patients with typical forms of RP and allied diseases as well as some atypical forms of RP or diseases in which RPE atrophy is a striking characteristic but a genetic basis has not been established. We found no definitely pathogenic mutations in the peropsin gene. These results indicate that peropsin is unlikely to be a major cause of the more common forms of inherited retinal degeneration such as autosomal dominant and autosomal recessive retinitis pigmentosa (Table 1). Specifically, most of the rare variants that we observed either did not segregate with the disease in families with multiple affected relatives or were found in families with no affected relatives available for evaluation.

The most interesting missense change in this context is Cys98Tyr, which was discovered in a patient with retinitis punctata albescens. All known opsins, including peropsin and rhodopsin, have seven transmembrane domains and six hydrophilic loops (three extracellular and three intracellular). In addition, opsins share other structural characteristics, among which is the presence of a disulfide bridge between two cysteine residues (Cys98 and Cys175 in peropsin) located in the first and second extracellular (intradiscal) loops [15,16] (Figure 1). The Cys98Tyr missense would destroy this disulfide bond and potentially impair the structure of the protein. Furthermore, the very same mutation of the corresponding residue in rhodopsin, Cys110Tyr, causes dominant RP [17], indicating the importance of this amino acid residue for normal opsin physiology. Unfortunately, the index patient carrying this change and his relatives were unavailable for further analyses, and thus it is impossible to determine from the data we have gathered whether this missense is a mutation, possibly playing a role in digenic inheritance, or an innocuous rare variant.

ACKNOWLEDGEMENTS

This study was supported by the National Eye Institute (EY00169, EY08683, EY14104) and the Foundation Fighting Blindness, Owings Mills, MD.

REFERENCES

1. McBee JK, Palczewski K, Baehr W, Pepperberg DR. Confronting complexity: the interlink of phototransduction and retinoid metabolism in the vertebrate retina. Prog Retin Eye Res 2001; 20:469-529.
2. Chen P, Hao W, Rife L, Wang XP, Shen D, Chen J, Ogden T, Van Boemel GB, Wu L, Yang M, Fong HK. A photic visual cycle of rhodopsin regeneration is dependent on Rgr. Nat Genet 2001; 28:256-60.
3. Hara T, Hara R. Rhodopsin and retinochrome in the squid retina. Nature 1967; 214:573-5.
4. Hara T, Hara R. Regeneration of squid retinochrome. Nature 1968; 219:450-4.
5. Hao W, Fong HK. Blue and ultraviolet light-absorbing opsin from the retinal pigment epithelium. Biochemistry 1996; 35:6251-6.
6. Koyanagi M, Terakita A, Kubokawa K, Shichida Y. Amphioxus homologs of Go-coupled rhodopsin and peropsin having 11-cis and all-trans-retinals as their chromophores. FEBS Lett 2002; 531:525-8.
7. Hao W, Fong HK. The endogenous chromophore of retinal G protein-coupled receptor opsin from the pigment epithelium. J Biol Chem 1999; 274:6085-90.
8. Sun H, Gilbert DJ, Copeland NG, Jenkins NA, Nathans J. Peropsin, a novel visual pigment-like protein located in the apical microvilli of the retinal pigment epithelium. Proc Natl Acad Sci U S A 1997; 94:9893-8.
9. Tarttelin EE, Bellingham J, Bibb LC, Foster RG, Hankins MW, Gregory-Evans K, Gregory-Evans CY, Wells DJ, Lucas RJ. Expression of opsin genes early in ocular development of humans and mice. Exp Eye Res 2003; 76:393-6.
10. Rattner A, Sun H, Nathans J. Molecular genetics of human retinal disease. Annu Rev Genet 1999; 33:89-131.
11. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. Lancet 2006; 368:1795-809.
12. Morimura H, Saindelle-Ribeaudeau F, Berson EL, Dryja TP. Mutations in RGR, encoding a light-sensitive opsin homologue, in patients with retinitis pigmentosa. Nat Genet 1999; 23:393-4.
13. Staden R, Beal KF, Bonfield JK. The Staden package, 1998. Methods Mol Biol 2000; 132:115-30.
14. Reese MG, Eeckman FH, Kulp D, Haussler D. Improved splice site detection in Genie. J Comput Biol 1997; 4:311-23.
15. Khorana HG. Rhodopsin, photoreceptor of the rod cell. An emerging pattern for structure and function. J Biol Chem 1992; 267:1-4.
16. Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M. Crystal structure of rhodopsin: A G protein-coupled receptor. Science 2000; 289:739-45.
17. Vaithinathan R, Berson EL, Dryja TP. Further screening of the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. Genomics 1994; 21:461-3.
18. Rivolta C, Ayyagari R, Sieving PA, Berson EL, Dryja TP. Evaluation of the ELOVL4 gene in patients with autosomal recessive retinitis pigmentosa and Leber congenital amaurosis. Mol Vis 2003; 9:49-51.

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