RRH, encoding the RPE-expressed opsin-like peropsin, is not mutated in retinitis pigmentosa and allied diseases

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ABSTRACT

Many genes from the retinoid metabolism cause retinitis pigmentosa. Peropsin, an opsin-like protein with unknown function, is specifically expressed in apical retinal pigment epithelium microvilli. Since rhodopsin and RGR, another opsin-like protein, cause retinitis pigmentosa, we screened by D-HPLC the peropsin gene *RRH* in 331 patients (288 retinitis pigmentosa and 82 other retinal dystrophies). We found 13 non pathogenic variants only, among which a c.730_731delATinsG truncates the last 2 transmembrane spanning fragments and the Lys284 required for retinol binding, but does not segregate with the disease phenotype. We conclude that *RRH* is not a frequent gene in retinitis pigmentosa.

INTRODUCTION

Retinitis pigmentosa (RP), accounting for the 2/3 of the inherited retinal dystrophy cases, is characterized by pigment deposits predominant in the peripheral retina [1]. RP leads to blindness after several decades of evolution. To date, 45 known genes/loci have been identified in non syndromic RP, including 15 for autosomal dominant- (14 cloned, 1 mapped), 24 for autosomal recessive- (18 cloned, 6 mapped) and 5 for X-linked- (2 cloned, 3 mapped) inheritance (http://www.sph.uth.tmc.edu/retnet/sum-dis.htm). It has been estimated that the cloned genes account for 50-60 % of dominant RP, 40 % of recessive RP and approximately 80 % of X-linked RP, indicating that many genes remain to be identified [2]. The known gene products localize in rods (sometimes in rods and cones) or in the photoreceptor supporting tissue, *i.e.* the retinal pigment epithelium (RPE).

Many genes encoding proteins from the retinoid metabolism cause retinal dystrophies. They include *RHO*, which encodes rhodopsin and causes RP [3], *ABCA4* responsible for Stargardt disease [4] and RP [5] cases, *RDH12* [6, 7] and *RPE65* [8, 9] which both cause Leber congenital amaurosis (LCA) and childhood-onset severe retinal dystrophy, *LRAT* [10, 11]causing rare cases of RP and LCA, *RLBP1* encoding CRALBP, responsible for retinitis punctata albescens [12], RP [13], Bothnia dystrophy [14] and Newfoundland rod-cone dystrophy [15], *RDH5* causing fundus albipunctatus [16] and the retinal pigment epithelium G protein-coupled receptor (*RGR)* that accounts for rare cases of RP or choroidal sclerosis [17].

Among the mammalian opsin-like protein family, Peropsin, encoded by *RRH*, belongs to a unique phylogenetic clade which also includes RGR [18] and neuropsin [19]. These proteins are expressed in non photoreceptive cells and their genes are composed of 7 exons instead of 5 or 6 for photoreceptor opsins [20]. RGR binds to all-*trans* retinal and is able to photoisomerise it

into 11-*cis* retinal [21]. Photoisomerisation has not been demonstrated in peropsin yet, but it is presumed to possess this activity since the peropsin amphioxus homolog is doing it [22]. One difference with RGR though, is the peropsin expression in RPE apical microvilli while RGR is present in the endoplasmic reticulum [23]. This suggests that peropsin could control light-driven rythmic processes in the retina or exchanges of retinoids.

In view of the involvement of *RHO* and *RGR* in retinal dystrophies, it was important to check for *RRH* mutations in retinal dystrophies. Here we show that none of the patients in a 331-sample panel carried pathogenic mutations in *RRH*, although our screening revealed one presumed deleterious variant for the peropsin function.

MATERIALS AND METHODS

Patients

A panel of 331 unrelated patients was screened, including 249 patients with RP (134 cases with simplex or multiplex RP, 79 cases with autosomal dominant RP, 36 cases with undetermined inheritance) and 82 patients with various types of flecked retinal dystrophies. Additional 50 normal individuals served as normal controls. All patients had standard ophthalmologic examination (refractometry, visual acuity, slit-lamp examination, applanation tonometry, funduscopy). Kinetic visual fields were determined with a Goldman perimeter with targets V_{4e}, III_{4e} and I_{4e}. Fluorescein angiograms were available for some patients. Full-fields ERG was recorded using a ganzfeld apparatus (Metrovision, France) with a bipolar contact lens electrode on maximally dilated pupils. Rod-isolated responses were elicited to 20-ms flashes of dim blue light at 1.25 Hz. Mixed cone and rod responses were elicited to 20-ms flashes of white light at 1.25 Hz with a background luminance at 5cd/m^2.

Mutation screening

PCR reaction

Primers for the 7 coding exons of *RRH* were designed according to the GenBank # NM_006583 (Bellingham et al., 2003) human sequence, to include flanking intronic sequences (Table 1). PCR reactions contained 10 pmoles of forward and reverse primers, 50-70 ng of genomic DNA, 2 mM MgCl2, 200 µM of each dNTP, 0.6 U of *Taq* DNA polymerase (Optimase, Transgenomics or AmpliTaq Gold, Applied Biosystems) in a 30-µl volume with the appropriate buffer. Following the denaturation step at 95°C for 5 min (Optimase) or 10 min (AmpliTaq Gold), the amplification was carried out for 35 cycles at 95°C for 30 sec, the appropriate annealing temperature for 40 sec and 72°C for 1 min 10 sec, ending with a final extension step at 72°C for 5 min (Optimase) or 10 min (AmpliTaq Gold). Amplicons were run on 2% agarose gels in 1X TAE buffer to check for the quality and specificity of the PCR reaction.

D-HPLC

PCR products were denatured at 95°C for 5 min and then gradually re-annealed by slow cooling (-1.5°C/min) to 25°C over a period of 60 min using the Mastercycler gradient thermocycler (Eppendorf). Amplicons were analysed on a 3500 High Throughput Wave system (Transgenomics) using elution and oven temperatures as predicted by WAVEMAKER Software version 4.1 (Transgenomics). An aliquot of 5 to 10 µl of the PCR product was injected into a DNASep column. DNA fragments were then eluted with a linear acetonitrile gradient, obtained by mixing buffer A (0.1 M TEAA, pH 7.0 in water) and buffer B (0.1 M TEAA and 25% acetonitrile, pH 7.0 in water) as determined with the WAVEMAKER Software, over a 2-min period at a constant flow rate of 1.5 ml/min. Two or three elution temperatures were necessary to ensure complete coverage of the fragments. The

chromatograms were compared to those of wild type controls and all samples displaying variant profiles were sequenced.

Sequencing

The purified PCR products (QIAquick PCR purification Kit, QIAGEN and ExoSAP-IT CLEEN UP, Amersham Biosciences) were sequenced in both directions, using the BigDye Terminator Cycle Sequencing Ready Reaction kit V1.1. (Applied Biosystems) on an ABI PRISM 3130 capillary sequencer (Applied Biosystems). Sample sequences were aligned to the wild-type ones and analyzed with the Collection and Sequence Analysis software package (Applied Biosystems). Splicing score analysis was performed at the http://rulai.cshl.edu/new_alt_exon_db2/HTML/score.html.

RESULTS

A panel of 331 unrelated patients was screened by D-HPLC and variant profiles were sequenced. Several sequence changes were found, which are summarized in table 2. Some DNA changes are already described in databases as single nucleotide polymorphisms (SNPs). These are c.1056G>T (dbSNP rs7681770), c.1067G>A (dbSNP rs:7657133), c.1132T>C (dbSNP rs9990841) and c.1191T>C (dbSNP rs6842245).

Eight new sequence changes were found. Two intronic variants (IVS2-14T>G, IVS3+46G>A) are frequently encountered and two others, IVS3+149G>C and IVS6-5T>C, were much less frequent. However, none of the 4 intronic variants change the score value of the corresponding splice site. A fifth variant (c.-159T>C) was found in only one patient in the presumed promoter sequence, 159 nt before the initiation codon. The nucleotide at this

position is not conserved in the mouse *Rrh* promoter and the unaffected brother of the patient also carried the change making it unlikely to be pathogenic.

The sequence variant c.618C>T (GCG>GTG) changes the encoded amino acid (Ala196Val). It was found at the heterozygote state in two unrelated patients with recessive cone rod dystrophy (CRD) but not in 100 control chromosomes. One patient was a simplex case. He did not carry any other *RRH* sequence change and his parents and siblings were not available for the familial analysis. The second patient was born from unaffected consanguineous parents (Figure 1A, individual V3). One of her healthy sister (V5) also had the Ala196Val change; both of them did not carry any other *RRH* sequence change. Three other sisters, including one with CRD (V1) and two healthy ones (V2 and V4), did not show the Ala196Val but carried a c.762A>G (GAT>GGT) at the heterozygote state that also changes the amino acid (Asp244Gly). None had any other *RRH* sequence alteration. We concluded that Ala196Val is a rare peropsin polymorphism which is not responsible for the observed retinal dystrophy.

We had examined a consanguineous family in which 3 out of 6 children had typical RP (Figure 2 A-D and table 3). We found that the index case (V5 in Figure 1B) carried the complex sequence alteration c.730_731delATinsG (Figure 1C) at the heterozygote state resulting in a frameshift at codon 244 and causing a premature STOP at codon 245. The patient did not carry any other *RRH* sequence change and the sequence alteration was present in a healthy sister (V1) and absent in another RP sister (V2). We concluded that this *RRH* sequence alteration was not responsible for the RP phenotype in this family.

DISCUSSION

Non syndromic RP is a highly genetically heterogenous condition with 45 genes/loci registered today (Hamel, 2006). As several genes from the opsin family have already been described in RP or other types of retinal dystrophies, we search for mutations in *RRH* encoding the RPE preferentially expressed peropsin protein. In a panel of 331 unrelated patients, we found no mutations responsible for the disease.

Among the 13 sequence changes that we are reporting, the c.730_731delATinsG presumably severily impairs the peropsin function. This deletion/insertion will cause the truncation of the 94 C-terminal amino acids, that is 27.9 % of the protein. Thus, the alteration deletes the last 2 transmembrane spanning fragments, and, more importantly, abolishes the lysine 284 which is required to bind the retinol. Thus, if the function of peropsin is related to retinal binding, it is likely that this allele causes a peropsin loss of function. A knock out animal model would be necessary to test whether the loss in peropsin expression is detrimental to retinal function and could cause a retinal dystrophy.

Our study cannot exclude that mutations in *RRH* could rarely cause retinal dystrophies, or alternatively could cause a more subtle phenotype or be involved in multifactorial diseases.

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LEGEND TO FIGURE

Figure 1 : (A) and (B) : pedigrees of families segregating retinitis pigmentosa. Squares indicate male, circles indicate female. Blackened symbols are individuals affected with RP. A double horizontal line between a mating pair indicates consanguinity. Genotypes are as follows : "+" indicates wild-type genotype, del in (B) indicates the c.730_731delATinsG mutation. (C) electropherogram of individual V3 in pedigree (B) carrying the c.730_731delATinsG mutation. Arrows show the deletion of the two nucleotides A and T, and the insertion of a G.

Figure 2 : Fundus photographs of patients from family shown in figure 1B in which the heterozygous *RRH* c.730_731delATinsG change was found. **A**, posterior pole and **B**, temporal region, of the left eye of patient V5, showing narrowing of retinal vessels, atrophy of the retina while optic disc remains healthy, and numerous bone spicule-shaped pigment deposits in mid periphery. **C**, posterior pole of patient V3 shows small drusenoid deposits in the temporal side of the macula and perifoveal radial folds indicating an epiretinal membrane. Retinal vessels are slightly narrowed and the optic disc is normal. **D**, posterior of pole of the unaffected brother V6.