Genetic Analysis of the Rhodopsin Gene Identifies a Mosaic Dominant Retinitis Pigmentosa Mutation in a Healthy Individual

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Genetics

Retinitis pigmentosa (RP) is a heterogeneous group of the most common form of inherited retinal degenerations (IRDs), with a prevalence of approximately 1:4500 in the United States and Europe1–4 and 1:2100 in the vicinity of Jerusalem.5 Early clinical symptoms include loss of night and peripheral vision, leading eventually to total vision loss in most patients at later stages of the disease.6 The disorder can be inherited in different patterns including autosomal recessive (arRP), autosomal dominant (adRP), and X-linked (xlRP).7

Rhodopsin (RHO, OMIM *180380) was the first gene in which mutations were reported to cause RP.8 A single mutation (p.Pro23His) was identified in a large number of American patients with adRP and is currently known to be the most common RP mutation in North America.9 More than 150 RHO mutations have been identified so far that are responsible for 16% to 35% of adRP cases and for approximately 10% of all RP cases in Europe and the United States.10–18 In addition, a few RHO mutations are known to cause arRP19 and adCSNB20 (autosomal dominant congenital stationary night blindness).

Genetic analyses of the Israeli and the Palestinian populations in the last decade have revealed a large number of founder mutations as the cause of arRP21–24; however, no mutations associated with adRP have been reported so far in these populations. Owing to the high prevalence of RHO mutations as the cause of adRP in other populations, we selected this gene as the first to be systematically screened for mutations among our adRP cohort. Our analysis revealed previously reported mutations as well as a novel mutation. In addition, we identified the first mosaic individual to be reported with an RP-causing mutation.

Keywords: retinal degeneration, inherited blindness, genetic defects, rhodopsin, mosaicism

METHODS

Thirty-two adRP families participated in the study. Mutation detection was performed by whole exome sequencing (WES) and Sanger sequencing of RHO exons. Fluorescence PCR reactions of serially diluted samples were used to predict the percentage of mosaic cells in blood samples.

RESULTS.

Eight RHO disease-causing mutations were identified in nine families, with only one novel mutation, c.548-638dup91bp, identified in a family where WES failed to detect any causal variant. Segregation analysis revealed that the origin of the mutation is in a mosaic healthy individual carrying the mutation in approximately 13% of blood cells.

CONCLUSIONS.

This is the first report of the mutation spectrum of a known adRP gene in the Israeli and Palestinian populations, leading to the identification of seven previously reported mutations and one novel mutation. Our study shows that RHO mutations are a major cause of adRP in this cohort and are responsible for 28% of adRP families. The novel mutation exhibits a unique phenomenon in which an unaffected individual is mosaic for an adRP-causing mutation.

Informed Consent

Informed consent was obtained from patients and family members who participated in this study.

Conflict of Interest

No potential conflicts of interest were disclosed.

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Clinical evaluations included a detailed family history, a full ophthalmologic examination, electrooculography, full-field electrotoretinography, color vision testing using the Ishihara 38-plate test and Farnsworth D-15 panel, optical coherence tomography (OCT), color and infrared fundus photography, autofluorescence imaging, and fluorescein angiography, performed as previously described.25

Genetic Analysis

Thirty-two families with adRP were enrolled in the study: three Arab-Muslim families of Palestinian origin and 29 Israeli Jewish families. Genomic DNA was extracted from peripheral blood of the participants by using FlexiGene DNA kit (QIAGEN, Hilden, Germany). Nine samples underwent an in-house whole exome sequencing (WES) analysis (see Supplementary Methods). DNA samples of the 32 index cases were screened for mutations in \( RHO \) (NCBI Reference Sequence NM_000539.3) exons (and flanking intronic sequences) with PCR followed by Sanger sequencing. Primers (Supplementary Table S1) were designed by using the PRIMER3 program (http://frodo.wi.mit.edu/; provided in the public domain by the Whitehead Institute for Biomedical Research, Cambridge, MA, USA). For fluorescent PCR of \( RHO \)-exon 3, the forward primer was \( 5^\prime \) labeled with Fam. Twenty-seven PCR cycles were performed in a volume of 30 µL. A genomic DNA sample of a heterozygous patient (MOL1076 III:1) was serially diluted with a sample of a wild-type family member (MOL1076 I:2), to obtain mutation ratios of 50% (no dilution), 25%, 12.5%, 6.25%, 3.13%, and 1.56%. The fluorescence was measured with ABI PRISM 3100 Genetic Analyzer (Life Technologies, Grand Island, NY, USA). Peaks were analyzed with Peak Scanner 2 program.

RESULTS

In the last decade, we recruited 649 families with RP, 32 of whom showed a clear AD inheritance pattern. We performed WES analysis on nine cases (from seven families) and identified the cause of disease in two families: a \( PRPF3 \) mutation in MOL0108 and a previously reported \( RHO \) mutation (p.Arg135Trp) in MOL0843 (Table 1). We subsequently performed Sanger sequencing analysis of the five \( RHO \) exons in all of the remaining 30 index cases with no identified mutation. A combination of WES and Sanger sequencing revealed eight disease-causing mutations in nine families; only one of the mutations is novel (Fig. 1; Table 1). The seven mutations reported previously are missense, and the novel mutation is a tandem duplication of 91 nucleotides. Five of the seven reported missense mutations alter amino acid residues that have been previously shown to be affected by other mutations (Table 1). While seven of the mutations were family-specific, one (c.403C>T, p.Arg135Trp) was observed in two unrelated families from different origins (MOL0843 of North African Jewish origin and MOL0418 of Turkmenistan Jewish origin).

Clinical data of 12 patients with heterozygous \( RHO \) mutations were collected and all had clinical symptoms and signs of RP (Table 2). The age of disease onset ranged from 6 to 43 years and visual acuity was from 0.3 to 1.0. The fundus appearance was compatible with typical RP except for one patient (MOL0908 II:1), who was diagnosed with sector RP. Scotopic and photopic ERG amplitudes were nondetectable in approximately half of the cases.

Interestingly, while we were not able to identify the disease-causing mutation by WES in family MOL1076, the subsequent Sanger sequencing revealed the cause of disease (Table 1). MOL1076 includes four affected family members from two generations (Fig. 1). The index case (III:4) was diagnosed at the age of 6 years with RP accompanied by high myopia, pigmentary changes at the posterior pole and the peripheral retina, severely reduced ERG amplitudes, and normal color vision (Table 2). Samples of three family members (mother and two affected daughters) underwent WES analysis, which revealed 126,114 variants on average; of these, 77,950 were heterozygous. Filtering the WES data for minor allele frequency, pathogenicity scores, and shared genotypes revealed 27 sequence changes that were heterozygous and shared by all affected individuals. These changes were considered non-
pathogenic because they were in the 5′ or 3′ untranslated region and not in the open reading frame of the main transcript, or missense changes affecting amino acids that were not evolutionarily conserved. In parallel, the index case (1076 III:4) was analyzed by Sanger sequencing for RHO mutations. The PCR analysis of exon 3 revealed three PCR products (Fig. 2A, bottom) in the affected individuals; the size of the shorter band (No. 1) was compatible with the expected size of the wild-type (WT) product (349 bp) and the two additional bands (Nos. 2, 3) were approximately 100 bp longer. Sequencing analysis of the PCR products from the affected individuals revealed a heterozygous tandem duplication of 91 bp (c.548-638dup91 bp) (Fig. 2B). The appearance of three PCR bands is likely due to the heating–cooling PCR cycles, generating two homoduplexes (WT-WT and mutant-mutant) and a heteroduplex (WT-mutant) that migrate to three different locations on the agarose gel.

Following the identification of the duplication by Sanger sequencing, we took a closer look at the WES data of RHO exon 3. A base coverage analysis along exon 3 of three patients and three controls did not reveal any significant differences (Supplementary Fig. S1). In addition, no variants in the duplication region were identified in the WES data.

The duplication of 91 nucleotides is predicted to result in a frameshift; such frameshift mutations usually create a premature stop codon. However, the nucleotide sequence of the new mutated frame did not contain a premature termination codon, and the mutation therefore is expected to result in a 360-amino acid (aa) long protein (12 aa longer than the WT protein) with a termination codon in the terminal exon 5 (Fig. 2D).

A bioinformatics analysis using OCTOPUS showed that the last two transmembrane domains (Nos. 6, 7) of the rhodopsin protein are eliminated by the frameshift mutation, and the C-terminal mutated protein sequence is expected to be located intracellularly, forming a globular loop (Supplementary Fig. S2A). This topology modification is also supported by a Phyre2 analysis (Supplementary Fig. S2B). The affected C-terminus region includes the retinal-binding site, sites of interaction with cytoplasmic proteins, and phosphorylation sites. In addition, the mutation is expected to cause a conformational change of the protein, not only in the secondary structure level, as predicted by OCTOPUS and Phyre2, but also in the tertiary structure.
TABLE 2. Ocular Information of Patients With RHO Mutations

| Patient ID (Age, y) | Mutation            | Visual Acuity | Refraction | Cone Flicker | Rod Response |
|---------------------|---------------------|---------------|------------|--------------|--------------|
|                     |                     | Right Eye     | Left Eye   | Right Eye    | Left Eye     |
| MOL0418 II:1 (12)   | c.403C>T, p.Arg135Trp | 0.7           | 0.8        | Plano        | ND           |
| MOL0418 II:2 (8)    | c.403C>T, p.Arg135Trp | 0.5           | 0.8        | Plano        | ND           |
| MOL0553 III:2 (35)  | c.511C>T, p.P171S    | 0.9           | 0.9        | NA           | NA           |
|                     |                     | 0.9           | 0.9        | Trace responses | Trace responses |
| MOL0596 III:7 (43)  | c.647T>G, p.Pro216R  | 1.00          | 1.60       | NA           | NA           |
|                     |                     | 0.9           | 0.6        | Trace responses | Trace responses |
| MOL0692 III:1 (17)  | c.1040C>G, p.Pro347Arg | 1.00          | 1.00       | NA           | NA           |
|                     |                     | 1.00          | 1.00       | Trace responses | Trace responses |
| MOL0843 II:2 (20)   | c.403C>T, p.R135W    | 0.5           | 0.33       | /C0          | /C0          |
|                     |                     | 5.0           | 4.0        | /C0          | /C0          |
| MOL0908 II:1 (37)   | c.800C>T, p.T267L    | 0.86          | 0.86       | 1.0          | 1.0          |
|                     |                     | 0.86          | 0.86       | Trace responses | Trace responses |
| MOL1076 III:4 (6)   | c.548-638dup91bp     | 1.00          | Trace responses | Trace responses | Trace responses |
| MOL1076 III:1 (10)  | c.548-638dup91bp     | 1.00          | Trace responses | Trace responses | Trace responses |

IT, implicit time; NA, not available; ND, not detected; RE, right eye.

DISCUSSION

We report here the first genetic analysis of an adRP gene in general and the rhodopsin gene in particular, in the Israeli and Palestinian populations. Our data showed that RHO mutations are a major cause (and are probably the major cause) of adRP in these populations and are responsible for 28% (9/32 families) of adRP families (including 36 RP patients) in this cohort. Our data are consistent with those obtained from other countries: Italy: 28%, United Kingdom: 30%, United States: 24%, and France: 10.5%. A review analysis has reported that 25% of adRP cases worldwide are caused by RHO mutations.
Of the eight mutations we identified, only one (c.548-638dup91bp) was novel and the remaining are likely to represent mutational hotspots. The fact that most mutations identified here were already reported in other populations is surprising since marked differences in mutation spectrum have been reported in autosomal recessive RP-causing genes among the Israeli/Palestinian populations versus the European/US populations. The similarity in adRP mutation spectrum among different populations might justify a relatively simple mutation analysis protocol for RHO in populations that were not studied earlier, while such an analysis for autosomal recessive genes is unlikely to be efficient.

At least 20 amino acids in the rhodopsin protein sequence are affected by different multiple mutations (http://www.retina-international.org/files/sci-news/rhomut.htm; provided in the public domain by Retina International); it is therefore not surprising that five of the mutations identified here are located in such positions. Only one of the mutations (c.403C>T, p.Arg135Trp) was shared by patients from unrelated families, and since the two families are from different origins (Turkmenistan Jews and North African Jews), this is also likely to be due to a mutational hotspot.

Patient MOL0908 II:1 was found to be heterozygous for the p.Pro267Leu mutation and was diagnosed with a relatively mild form of RP that is compatible with sector RP, by fundus appearance and ERG analysis. Interestingly, the same mutation has been reported elsewhere in four patients with a mild phenotype of retinitis pigmentosa. Another mutation affecting the same amino acid (p.Pro267Arg) is reported to cause RP; however, no clinical data are reported.

The c.548-638dup91bp mutation we identified uncovers a unique and novel phenomenon in the genetics of RP and can potentially aid in our understanding of the required amount of normal rhodopsin protein level that is sufficient to prevent retinal degeneration and hence the clinical features of RP.
Figure 3. (A) Electropherograms of fluorescence-based PCR of WT, heterozygous, and mosaic individuals visualized by the Peak Scanner software. The x-axis shows the size of the fragment and the y-axis shows the fluorescence intensity. The WT product is 292-bp long and the mutated product is 383-bp long. The area underneath the peaks is presented in a box next to the peak. (B) Standard curve of the c.548-638dup91bp allele quantity, derived from serial dilutions of DNA from a heterozygous patient (MOL1076 III:1) and a control (MOL1076 I:2), in which 50%, 25%, 12.5%, 6.25%, 3.13%, and 1.56% of the DNA has a mutation. For calculations, the area under the peaks was used. The mutated allele in the grandfather MOL1076 I:1 exhibits approximately 6.5% of the PCR product, which means that he carries approximately 13% heterozygous mutated blood cells.
who are heterozygous for the duplication were diagnosed with RP at a relatively early age. The mutant transcript does not include a premature stop codon and is likely to produce a mutant protein that will lead to adRP. It was therefore intriguing that none of the grandparents reported any visual problems at 75 years of age. Indeed, a detailed ocular evaluation, including ERG, did not reveal any visual abnormalities in the grandparents. However, a detailed genetic analysis of the grandfather revealed that a small proportion of his blood cells carry the mutation. In addition, two of his five children are affected with RP, one of whom was available for the study and carried the duplication heterozygously. It is therefore reasonable to assume that individual 1076 I:1 carries a de novo pathogenic RC-causing mutation, appearing both in somatic cells and in the germline, making him the first individual, to the best of our knowledge, to be reported as a mosaic for an RP-causing mutation. Identification of mosaics for disease-causing mutations is highly important, as it might shed light on the fraction of WT cells needed to protect a person from developing an inherited disease. It is obvious that one cannot measure the fraction of mutated alleles in the relevant tissue, the retina; however, by using a sensitive PCR assay, we were able to accurately determine the fraction of mutated alleles in blood cells (6.5%), resulting in 13% cells that are heterozygous for the mutation. If indeed this value reflects other organs as well as the retina, such allelic fraction is not sufficient to cause retinal degeneration. In addition to the mosaic mutation we reported here, three cases of de novo RHO mutations have been reported thus far (c.538C>G; p.I214N, 33 and c.158C>G; p.P53R34), Such mutations will therefore not be identified in the traditional WES analysis. Recent analyses of WES data reported by others and by our group have shown that in only 50% to 60% of IRD cases analyzed by WES, a disease-causing mutation can be identified.26,56–59 One should therefore bear in mind that duplications, similar to the one we report here, might be present, requiring a specialized WES analysis that is based on base coverage along each exon in the human genome.

In conclusion, we described here for the first time the spectrum of RHO mutations in the Israeli and Palestinian populations and showed that (1) most mutations are family-specific; (2) RHO is a major adRP-causing gene in the Israeli and Palestinian populations and is responsible for the disease in 28% of families; and (3) RHO mutations may appear in mosaicism in unaffected individuals, followed by autosomal dominant inheritance in successive generations.

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