An Unusual Retinal Phenotype Associated With a Novel Mutation in RHO

Isabelle Audo, MD, PhD; Anne Friedrich, PhD; Saddek Mohand-Saïd, MD, PhD; Marie-Elise Lancelot, BS; Aline Antonio, BS; Veselina Moskova-Doumanova, MS; Olivier Poch, PhD; Shomi Bhattacharya, PhD; José-Alain Sahel, MD; Christina Zeitz, PhD

Objective: To report a new genetic variant in the rhodopsin gene (RHO) associated with an unusual autosomal dominant retinal phenotype.

Methods: Detailed phenotypic characterization was performed on affected family members spanning 4 generations, including family history, best-corrected visual acuity, fundus examination, kinetic and static perimetry, full-field and multifocal electroretinography, fundus autofluorescence, and optical coherence tomography. For genetic testing, coding exons and flanking intronic regions of RHO were amplified with the use of polymerase chain reaction, purified, and sequenced. Cosegregation and control analysis were performed by direct sequencing of exon 3. Subsequent in silico analysis of the mutational consequence on protein function was undertaken.

Results: The onset of symptoms appeared in the fourth decade of life in this family, with moderate night blindness and asymmetrical visual loss. Affected members showed patchy areas of chorioretinal atrophy with decreased electroretinographic response amplitudes for both scotopic and photopic responses but no implicit time shift, consistent with restricted disease. A novel mutation in exon 3 of RHO was identified and represents a c.620T>A transition leading to a p.Met207Lys substitution. It cosegregated with this phenotype and was not identified in a control population.

Conclusions: We report the phenotype-genotype correlation of an unusual autosomal dominant, late-onset restricted chorioretinal degeneration cosegregating with a novel RHO mutation, p.Met207Lys. A p.Met207Arg substitution has previously been reported to cause a distinct, generalized early-onset rod-cone dystrophy.

Clinical Relevance: These data outline the phenotypic variability associated with RHO mutations. Depending on the localization and the amino acid substitution, patients may show congenital stationary night blindness, rod-cone dystrophy, sector retinitis pigmentosa, or localized chorioretinal atrophy.


OD-CONE DYSTROPHIES, ALSO known as retinitis pigmentosa (RP), are a heterogeneous group of inherited disorders primarily affecting rods with secondary cone degeneration. Retinitis pigmentosa is the most common inherited form of severe retinal degeneration, with a frequency of about 1 in 4000 births and more than 1 million affected individuals worldwide. Patients affected with RP initially report night blindness due to rod dysfunction followed by progressive visual field constriction, abnormal color vision, and eventual loss of central vision due to secondary cone degeneration leading to severe visual handicap and blindness. Inheritance of RP is typically autosomal recessive, autosomal dominant, or linked to the X chromosome. Rare cases of mitochondrial or digenic inheritance have been reported. Mutations in the rhodopsin gene (RHO; OMIM + 180380) were the first molecular defects identified in RP and represent the most frequent cause of autosomal dominant RP, accounting for about 25% of cases in the United States. RHO is located on chromosome 3q21-q24 and spans approximately 5.5 kilobases, with a coding sequence of 1044 base pairs distributed across 5 exons. RHO encodes for the rod-specific protein rhodopsin, which belongs to the superfamily of 7 transmembrane G-protein–coupled receptors. Rhodopsin consists of an apoprotein (opsin) that is covalently bound to a small conjugated chromophore (11-cis-retinal), which is derived from vitamin A. Upon light absorption, the chromophore isomerizes into all-trans-retinal, with subsequent rhodopsin conformational changes leading to the activation of the phototransduction cascade. More than 120 mutations in RHO have been identified (http://www.retina-
clear biochemical or cellular defect or uninvestigated defect and mainly lead to CSNB. Other mutations with uninvestigated folding defect but show an increased activation rate of transducin. In the absence of the chromophore and rhodopsin mutations that fold correctly but are not transported to the outer segment. Class II refers to mutations that misfold, are retained in the endoplasmic reticulum, and cannot easily reconstitute with 11-cis-retinal. Class III refers to mutations that affect endocytosis. Class IV mutations do not affect folding per se but might affect rhodopsin stability and posttranslational modification. Similarly, Class V mutations have no obvious folding defect but show an increased activation rate for transducin. In the absence of the chromophore and in the dark, mutations that appear to fold correctly but lead to the constitutive activation of opsin make up Class VI and mainly lead to CSNB. Other mutations with unclear biochemical or cellular defect or uninvestigated defect were not classified.9 These different molecular defects will differentially affect cellular functions and potentially cause distinct phenotypes.

Herein we report the detailed phenotype-genotype correlation of a family with a novel RHO mutation leading to an unusual autosomal dominant, late-onset, restricted chorioretinal degeneration. We also discuss potential physiopathological mechanisms based on mutation analysis results.

**METHODS**

**CLINICAL INVESTIGATION**

Members of a family with a presumed diagnosis of autosomal dominant retinal dystrophy were ascertained. Informed consent was obtained from each family member and healthy control subject after explanation of the study and its potential outcome. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Family members available for the study underwent full ophthalmic examination with assessment of best-corrected visual acuity, kinetic and static perimetry, and color vision using 15 desaturated hues. Full-field and multifocal electroretinography (ERG) was performed using Dawson, Trick, Litzkow (DTL) recording electrodes and incorporated the International Society for Clinical Electrophysiology of Vision standards (Espion e system and ColorDome Ganzfeld stimulator [Diagnosys UK Ltd, Cambridge, England] for full-field ERG and Veris II system [Electro Diagnostic Imaging Inc, San Francisco, California] for multifocal ERG). Clinical assessment was completed with fundus autofluorescence imaging and optical coherence tomography (OCT) (with the use of HRA II and Spectralis OCT, respectively; Heidelberg Engineering, Dossenheim, Germany).

Blood samples were collected from examined family members and from other members who were not able to undergo clinical examination but were willing to participate in the study. Clinical details for the latter were obtained from their personal health records.

Commercial control samples were used to validate the pathogenicity of the sequence variant (human random control panel 1-3; Health Protection Agency Culture Collections, Salisbury, England).

**MUTATION ANALYSIS**

Total genomic DNA was extracted from peripheral blood leukocytes according to manufacturer recommendations (Puregene Kit; Qiagen, Courtaboeuf, France). The 5 coding exons of rhodopsin (RHO RefSeq NM_000539.2) and the flanking intronic regions were amplified with oligonucleotides previously described21 by using 100 ng of genomic DNA, a commercially available polymerase (HOT FIREPol DNA Polymerase; Solis BioDyne, Tartu, Estonia), 3 mM magnesium chloride (exons 1, 2, and 3) or 1.3 mM magnesium chloride (exons 4 and 5), 0.2 mM deoxynucleotide triphosphates (So-lis BioDyne), and Buffer B2 (Solis BioDyne) at different annealing temperatures: exons 1 and 2, 35°C; exon 3, 62°C; exon 4, 60°C; and exon 5, 52°C. Then, 0.8 to 1.0 µL of the polymerase chain reaction product was enzymatically purified with 5 µL of a 1:50 dilution (ExoSAP-IT, USB Corporation, Cleveland, Ohio, purchased from GE Healthcare, Orsay, France) at 37°C for 15 minutes, and the enzyme was deactivated at 80°C for 15 minutes. Next, 0.5 µL of a sequencing kit (BigDye Term v1.1 CycleSeq kit; Applied Biosystems, Courtaboeuf), 1.25 µL of a 5× sequencing buffer (Big-Dye Sequencing buffer; Applied Biosystems), and 0.8 µL of 5 mM or 10 mM oligonucleotide were added to the purified polymerase chain reaction product. The cycling conditions were as follows: 95°C for 20 seconds and 25 cycles at 96°C for 20 seconds, 50°C for 10 seconds, and 60°C for 2 minutes. The sequenced product was purified on a presoaked (in 300 µL of water for 3 hours, centrifuged at 1500 rpm for 1 minute, then added to 150 µL of water, and centrifuged at 950 rpm for 5 minutes) gel filtration medium (Sephadex G-50; GE Healthcare) in a 96-well multiscreen filter plate (Millipore, Molsheim, France) and eluted with an additional 10 µL of water. The purified product was then analyzed on an automated 16-capillary sequencer (ABI 3100 Genetic Analyzer; Applied Biosystems), and the results were interpreted by applying a software (SecScape, Applied Biosystems).

**WEB RESOURCES**

The PolyPhen (Polymorphism Phenotyping, http://tux.embl-heidelberg.de/tmnenksy/) and SIFT (Sorting Intolerant From Tolerant; http://blocks.fhcrc.org/sift/SIFT.html) software were used to predict the pathogenic character of the sequence alteration.

**SEQUENCE ALIGNMENT**

The human rhodopsin sequence (UniProtKB identifier P08100) was used as a probe for similarity searches in the UniProtKB database with the use of the BlastP program.26 In total, 114 meta-

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zoan sequences (excluding fragments) that were annotated or predicted as rhodopsin were highlighted and aligned with a customized version of the PipeAlign program.27

THREE-DIMENSIONAL HOMOLOGY MODELS

The three-dimensional (3-D) models of the human wild-type and the 2 mutated (p.Met207Lys and p.Met207Arg) rhodopsins were generated by homology modeling with MODELLER software.28 The template used to construct these models was the x-ray crystal structure of the bovine RHO in the dark state (PDB 1F88). For each 3-D model construction, 10 homology models were constructed, and the ones with the best normalized DOPE (discrete optimized potential energy) score29 were selected.

These 3-D models were visualized and analyzed by means of the SM2PH-db (structural mutation to pathology phenotypes in human) Web server (http://decrypthon.igbmc.fr/sm2ph), and figures were constructed with PyMOL software (version 0.99; http://pymol.sourceforge.net/).

RESULTS

The family described in this study spans 4 generations (Figure 1). The proband (IV-3), his 2 sisters (IV-1 and IV-2), his mother (III-2), and his maternal grandmother (II-1) were available for clinical investigation, and the examination results are summarized in the Table. Additional clinical details from personal health records were obtained for the proband’s uncles (III-1 and III-4), grandaunt (II-3), and great-grandmother (I-2).

Symptoms included late-onset night vision disturbances after age 40 years and asymmetric decreased vision starting at the same age. Visual acuity ranged from 20/13 for the youngest family member (IV-3) to hand motions in 1 eye for the mother (III-2). Refractive errors were variable, with a majority of moderate hyperopia and astigmatism (Table).

Examination results of the anterior segments, including lenses and vitreous cavities, were unremarkable for all patients. Fundus examination of the proband and his older affected sister showed similar findings, with no optic disc pallor or narrowing of the blood vessels, a relatively normal posterior pole appearance despite a few perifoveolar retinal pigment epithelial (RPE) clumps, and a few small, round lesions of chorioretinal atrophy for the sister. The retinal periphery had a slight salt-and-pepper appearance for the sister. The proband’s uncles (III-1 and III-4), grandaunt (II-3), and great-grandmother (I-2) obtained for clinical investigation, and the examination results are summarized in the Table. Additional clinical details from personal health records were obtained for the proband’s uncles (III-1 and III-4), grandaunt (II-3), and great-grandmother (I-2).

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Table. Summary of Clinical Findings

<table>
<thead>
<tr>
<th>Patient No./ Sex/Age, y</th>
<th>BCVA</th>
<th>Refraction</th>
<th>Visual Symptoms</th>
<th>Fundus Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-1/F/58 RE: 20/200 LE: 20/25</td>
<td>RE: +1.75 (−0.50)20° LE: +2.25 (−0.50)140°</td>
<td>Night blindness since age 40 y</td>
<td>Patchy chorioretinal atrophy with some RPE clumps in posterior pole and midperiphery; no disc pallor and no narrowing of blood vessels; salt-and-pepper aspect in retinal periphery; no bone spicules</td>
<td></td>
</tr>
<tr>
<td>III-2/F/46 RE: 20/25 LE: HM</td>
<td>RE: +1 (−0.50)160° LE: plano</td>
<td>Sudden decreased vision in LE at age 38 y; night blindness since age 40 y</td>
<td>Patchy chorioretinal atrophy with some RPE clumps in posterior pole and midperiphery; no disc pallor and no narrowing of blood vessels; salt-and-pepper aspect in retinal periphery; no bone spicules</td>
<td></td>
</tr>
<tr>
<td>IV-1/F/26 RE and LE: 20/15</td>
<td>None</td>
<td>None</td>
<td>Normal aspect of posterior poles besides some perifoveolar RPE clumps and 1 small area of atrophy; moderate salt-and-pepper appearance of retinal periphery</td>
<td></td>
</tr>
<tr>
<td>IV-2/F/25 RE and LE: 20/15</td>
<td>RE: +0.50 (−0.25)175° LE: +0.25 (−0.50)180°</td>
<td>None</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>IV-3/M/23 RE: 20/13 LE: 20/15</td>
<td>RE: 0 (−1)110° LE: 0 (−1.50)15°</td>
<td>None</td>
<td>Normal aspect of posterior poles besides some perifoveolar RPE clumps; moderate salt-and-pepper appearance of retinal periphery</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BCVA, best-corrected visual acuity (Early Treatment Diabetic Retinopathy Study chart); HM, hand motions; LE, left eye; RE, right eye; RPE, retinal pigment epithelium.
Figure 2. Retinal abnormalities seen in the index patient (IV-3) and his affected sister (IV-1), mother (III-2), and grandmother (II-1). A, Color fundus photographs of IV-3 and IV-1 show a few perifoveal retinal pigment epithelial (RPE) clumps with round atrophic lesions in IV-1 within the posterior pole and some reticular changes in the periphery. III-2 and II-1 show patchy and asymmetrical areas of chorioretinal atrophy within the posterior pole with reticular changes and a few RPE clumps in the periphery but no optic disc pallor and no bone spicules nor narrowing of blood vessels. B, Autofluorescence images in IV-3 and IV-1 show preservation of normal autofluorescence at the fovea, some hyperautofluorescent dots, and some round areas of loss of autofluorescence in the parafoveal region corresponding to RPE clumps and atrophic lesions, respectively, in IV-1 and a salt-and-pepper autofluorescence appearance in the mid-periphery. In III-2 and II-1, atrophic lesions are clearly seen with loss of autofluorescence and a salt-and-pepper appearance outside these areas. C, Optical coherence tomography (OCT) images of IV-3 and IV-1 show some focal rupture of the inner/outer segments' laminar structure. Areas of hyperautofluorescent RPE clumps appear as round hyperreflecting lumps (IV-3, white arrow). Atrophic changes manifest as disappearance of the photoreceptor structure, with cystic modifications in the overlying inner retinal layers (IV-1, white arrows). The OCT images in III-2 show retinal thinning with loss of the outer retinal structures in atrophic areas associated with increased deep backscatter and subretinal pseudocystic lesions (III-2, white arrow). Green arrows represent orientation of the OCT scans. LE indicates left eye; RE, right eye.
confirmed the relative preservation of the foveal area. Perifoveal RPE clumps appeared hyperautofluorescent, whereas round, atrophic lesions in the sister showed a loss of autofluorescence. The midperipheral retina had a salt-and-pepper appearance with a combination of hypoautofluorescence and normal or hyperautofluorescence (Figure 2B, IV-3 and IV-1). Examination results in the younger sister (IV-2) showed a normal fundus and normal autofluorescence (data not shown).

The mother showed no optic disc pallor or narrowing of the blood vessels on her fundus but rather patchy and asymmetrical areas of chorioretinal atrophy involving the posterior pole. The midperipheral and far-peripheral retina showed some reticular changes, with a few RPE clumps but no typical bone spicules (Figure 2A, III-2). Autofluorescence imaging outlined the chorioretinal atrophy, with loss of autofluorescence and relative conservation of normal autofluorescence of the foveal region in the right eye. Autofluorescence outside the foveal region, when present, showed the heterogeneous salt-and-pepper aspect seen in her 2 affected children (Figure 2B, III-2).

The grandmother’s fundus and autofluorescence has changes similar to her daughter’s, with more extensive chorioretinal areas of atrophy at the posterior pole and midretinal periphery (Figure 2A and B, II-1).

Optical coherence tomographic examination of the proband and his affected sister showed some focal ruptures of the outer/inner segments’ laminar structure. Areas of RPE clumps that were seen on funduscopy with hyperautofluorescence appeared as round, hyperreflecting lumps between the RPE and outer segments. Atrophic changes seen on fundus examination manifested as a disappearance of the photoreceptor structures, with cystic modification in the overlying inner retinal layers (Figure 2C, IV-3 and IV-1). Optical coherence tomographic examination of the mother showed retinal thinning, with loss of the outer retinal structures in atrophic areas associated with increased deep backscatter and subretinal pseudocystic lesions that might correspond to the vascular structure. In addition, there was a decrease in capillary density in the anterior choroid (Figure 2C, III-2). The grandmother had similar OCT abnormalities (data not shown).

Color vision test results with 15 desaturated hues were normal for the proband and his affected sister and showed mild dyschromatopsia with a tritanopic defect for his mother and grandmother (Figure 3A, IV-3 and III-2).

Kinetic visual field test results were normal for the proband and his sisters and showed moderate peripheral constriction for the mother and maternal grandmother that was associated with central abnormalities and correlated with atrophic fundus changes (Figure 3B, IV-3 and III-2).

Static central visual field test results for the proband and his affected sister showed relatively preserved retinal sensitivity thresholds within the central degrees and decreased retinal sensitivity corresponding with atrophic changes in the fundus of his mother and grandmother when visual acuity allowed testing.

Full-field ERG responses in the younger affected patients showed moderate reduction of both a- and b-wave amplitudes during scotopic testing. The amplitudes for the a- and b-waves of the scotopic 3.0 ERG response, which was a mixed rod-cone response dominated by rods, were 80% of the normal value. However, there was a normal implicit time for scotopic responses and normal photopic ERG responses (Figure 4, IV-3). Electroretinographic responses in the mother showed further reduction in both scotopic and photopic responses (amplitudes for the scotopic 3.0 ERG and photopic ERG responses were 65% and 90% of normal values, respectively) with no implicit time shift (Figure 4, III-2). The absence of implicit time shift suggests a restricted disease rather than generalized retinal dysfunction.30–32 Findings in the mother would be in keeping with restricted rod dysfunction, with additional restricted cone dysfunction appearing in the older family members.

**MUTATION ANALYSIS**

Based on family history and night blindness symptoms, the family was included in our autosomal dominant rod-cone dystrophy panel for the screening of known genes implicated in this subgroup. We screened the major gene, rhodopsin (RHO), for underlying rod-cone dystrophy by direct sequencing of the coding exons and flanking intronic regions in the proband. By doing so, a novel mutation in exon 3 was identified (Figure 5), representing a c.620T>A transition leading to a p.Met207Lys substitution. Similarly, the other affected family members (IV-1, III-2, II-1, II-3, and I-2) showed the same mutation, whereas the unaffected members (IV-2, III-2, III-4, III-3, and II-2) did not carry this mutation (Figures 1 and 5). The mutation was not predicted as a single-nucleotide polymorphism and did not appear in 274 controls. Interestingly, in 1992, Farrar and coauthors33 identified a c.620T>G exchange leading to a p.Met207Arg substitution at the same codon.

**BIOINFORMATICS ANALYSES**

Both the PolyPhen and SIFT programs annotated the amino acid substitution p.Met207Lys to be a possible pathogenic change. Multiple alignment analysis of 114 metazoan rhodopsin sequences shows strict conservation of the methionine at position 207 (Met207) in vertebrates and mollusk sequences, whereas a tyrosine is found in arthropods (data not shown).

Structural analysis localized Met207 within the fifth transmembrane helix in the 3-D homology model of human RHO (Figure 6A, blue), which is part of the retinal binding pocket.34 Moreover, the Met207 side-chain extremity lies close to the retinal β-ionone ring in the dark state (Figure 6A, pink) and is proposed to come in close contact with retinal in the activated metarhodopsin II state.35,36

Lysine and arginine are commonly considered to be close residues because of their physicochemical properties: both amino acid exchanges p.Met207Lys and p.Met207Arg introduce a positive charge and thus may alter the stability of the retinal binding cavity in the same manner through charge introduction. Thus, charge modification cannot explain the strong phenotypic differences associated with the 2 different amino acid substitutions.
To gain insight into the deleterious molecular effects associated with the mutation p.Met207Lys and the previously identified p.Met207Arg, these substitutions were simulated by homology modeling (Figure 6B), with Lys207 in yellow and Arg207 in red. In fact, the arginine side chain is longer than that of lysine. Our homology model predicts a reduction of the distances between the 2 mutations and retinal. However, the distance between Lys207 and retinal is still 0.202 nm, compared with 0.365 nm in the normal rhodopsin with Met207, whereas Arg207, with a distance of 0.095 nm to retinal, is much closer. (To convert nanometers to angstroms, multiply by 10.) These findings suggest that steric constraints would be less drastic for lysine than for arginine.

**COMMENT**

We describe a family with an unusual chorioretinal atrophy phenotype associated with a novel mutation in RHO. The younger affected family members showed a relatively preserved posterior pole with RPE clumping that was hyperautofluorescent and irregular pseudogranular auto-

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Figure 3. Psychophysical tests. A, Color vision with 15 desaturated hues is normal in the younger patient (IV-3) and shows a mild tritanopic defect in the older patient (III-2) (the left eye [LE] of III-2 could not be tested owing to poor visual acuity). RE indicates right eye. B, Goldmann visual field results are normal in the younger patient (IV-3) and show moderate peripheral constriction with central abnormalities correlated with atrophic fundus changes in the older patient (III-2).
fluorescence outside the fovea. These abnormalities could correspond to focal disturbances in photoreceptor outer-segment turnover or to phagocytosis by the RPE. Small patchy areas of atrophy, when seen, are associated with cystic changes of the underlying retina that may correspond to remodeling of the inner retina secondary to photoreceptor degeneration. In the older affected family members, areas of chorioretinal atrophy enlarged and coalesced at the posterior pole and midperiphery, with no inferior/superior predilection. Electroretinographic abnormalities with decreased amplitudes and no implicit time shift were consistent with restricted rod dysfunction, and additional restricted cone dysfunction appeared in the older affected individuals.30-32 Long-term follow-up will complete the precise characterization in this family.

The mutation represents a nucleotide exchange, c.620T>A, leading to a p.Met207Lys substitution. According to the phenotypic classifications proposed by Cideciyan and coworkers,17 this mutation, which shows regional differences, belongs to class B1. Regional predilection for retinal degeneration has been described in autosomal dominant families with “sector” RP carrying various RHO mutations (p.Thr17Met,21,17 p.Asn15Ser,38 p.Pro23His,39-41 p.Thr58Arg,42,43 p.Gly106Arg,43,44 and p.Gly182Ser37). In these cases, retinal lesions predominate in the inferior retina, with typical RPE changes, bone spicule migration, and a possible role of light explaining this location.45 This clearly differs from the family we describe herein, who showed chorioretinal atrophy with little pigment migration at the posterior pole and midperiphery. However, the influence of light exposure cannot be ruled out and may explain this location to some extent.

![Figure 4. Full-field electroretinograms (ERGs) incorporating International Society for Clinical Electrophysiology of Vision recommendations. Electroretinography in the younger patient (IV-3) shows decreased amplitudes and no implicit time shift (denoted by vertical dotted lines), which is consistent with moderate restricted rod dysfunction. An additional restricted cone dysfunction appears in the ERG of the older patient (III-2). LE indicates left eye; RE, right eye.](image-url)
Bass and Noble described an autosomal dominant family with a p.Thr17Met amino acid substitution in RHO who showed chorioretinal atrophy, which would resemble the lesions noted in the family we describe. However, in the family described by Bass and Noble, the lesions predominate in the inferior retina. This differs from our family, who showed preferential posterior pole and midperipheral involvement. Therefore, to our knowledge, the phenotype reported herein has never been described in association with RHO mutation.

The mechanism by which dysfunction of a rod-specific gene leads to restricted chorioretinal atrophic lesions resembling choroideremia, albeit with a different location and course of disease, is unclear. It has been suggested that rod photoreceptor degeneration is the first event in the course of choroideremia, whereas choroidal atrophy occurs only secondary to rod photoreceptor degeneration. The gene product associated with choroideremia, REP-1, is expressed in both photoreceptors and choroid and thus could support this theory. However, RHO expression is restricted to rod photoreceptors and thus does not explain the secondary choroidal atrophy observed in our family.

To our knowledge, the p.Met207Lys mutation has never been described before. However, in a previous study, Farrar and colleagues reported a p.Met207Arg substitution in a family with severe classic RP. A similar observation of distinct phenotypes associated with different amino acid substitutions at the same position in RHO has also been previously described: although the substitution of p.Gly90Asp leads to night blindness, p.Gly90Val has been associated with classic autosomal dominant RP. The phenotypic variability seen between families with the p.Met207Arg and the p.Met207Lys mutations could be explained by differences in genetic background or by...
environmental factors, even though both of the reported families are of European decent and have comparable lifestyles.

Bioinformatics analysis gives further insight into the functional consequences of amino acid substitutions. Such analysis reveals the conservation of the methionine at position 207 across species, suggesting a specific role in the vertebrate/mollusk rhodopsin function. This is further supported by the position of Met207 within the fifth transmembrane domain of rhodopsin, which is part of the retinal binding pocket.34 As a result of molecular dynamics and nuclear magnetic resonance studies,35,36 it has been proposed that Met207 comes in close contact with the β-ionone of the retinal in the activated Meta II state and is thus important for further activation of the phototransduction cascade. Induction-of-charge modification is similar for both lysine and arginine substitution and cannot explain phenotypic differences linked to the respective mutations. Differences could be a consequence of differences in steric constraints, which is supported by the homology domain models shown in Figure 6. Aguila and coworkers53 recently studied the functional consequences of different amino acid exchanges, including p.Met207Ala, p.Met207Gly, p.Met207Arg, and p.Met207Cys. Purified normal and mutated rhodopsin transiently expressed in mammalian cells was regenerated with 11-cis-retinal. All of the mutations could be regenerated with native 11-cis-retinal except Met207Cys, probably because of protein mis-folding. The Met207Arg mutation showed dark-altered absorption spectra, a less-compact structure in the dark, reduced affinity for the retinal, and very short-lived active conformation, which accounts for its defective function in transducin activation. Met207Ala and Met207Gly mutations regenerated with 11-cis-retinal were able to activate transducin but with somewhat lower levels of activation than that resulting from normal rhodopsin. The authors argued that the amino acids alanine and glycine are less disruptive compared with the bulky arginine, which introduces a positive charge into the membrane domain resulting in a higher energy cost for the protein. The predicted functional consequence of the p.Met207Lys mutation identified herein might lie between the effect of the p.Met207Ala/p.Met207Gly and p.Met207Arg mutations. We hereby propose that both Met207Lys and Met207Arg mutations are still able to regenerate with 11-cis-retinal, but only Met207Lys is able to appropriately activate transducin, although at a lower rate owing to steric constraints compared with Met207Ala, Met207Gly, and the normal protein. Thus, phenotypic consequences would be directly linked to the size of the mutated residue side chain. According to Mendes and coworkers,10 both the novel mutation p.Met207Lys described herein and the previously described mutation p.Met207Arg might belong to the class IV mutations, which do not affect folding per se but might affect rhodopsin stability by steric constraints. Further functional studies are under way to document these hypotheses.

Our data emphasize the phenotypic variability associated with rhodopsin mutations. They also suggest that, despite RHO being the first and probably the most studied gene implicated in retinitis pigmentosa, a better understanding of its associated disease mechanisms and phenotype/genotype correlations is still needed, with potential new mutations associated with novel, unusual phenotypes yet to be discovered. Our findings also underline the value of systematic mutation-screening strategies of known genes that include families with an unusual phenotype.

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Author Affiliations: Institut National de la Santé et de la Recherche Médicale (INSERM), U968, (Drs Audo, Mohand-Said, Bhattacharya, Sahel, and Zeitz and Ms Lancaster, Antonio, and Moskova-Doumanova), Institut de la Vision, Université Pierre et Marie Curie 6, UMR_S 968 (Drs Audo, Mohand-Said, Bhattacharya, Sahel, and Zeitz and Ms Lancelot, Antonio, and Moskova-Doumanova), CNRS, UMR 7210 (Drs Audo, Mohand-Said, Bhattacharya, Sahel, and Zeitz and Ms Lancelot, Antonio, and Moskova-Doumanova), and Centre de Référence Maladies rares “dystrophies rétiennnes d’origine génétique,” Centre Hospitalier National d’Ophtalmologie des Quinze-Vingts, INSERM-DHOS CIC 503 (Drs Audo, Mohand-Said, and Sahel), Paris, France; Department of Molecular Genetics, Institute of Ophthalmology, London, England (Drs Audo and Bhattacharya); and Laboratoire de Bioinformatique et de Génomique Intégratives, Institut de Génétique et de Biologie Moléculaire et Cellulaire, UMR7104, Illkirch, France (Drs Friedrich and Poch).

Correspondence: Isabelle Audo, MD, PhD, Department of Genetics, Institut de la Vision, 17 Rue Moreau, Paris 75012, France (isabelle.au@inserm.fr).

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