Different Amino Acid Substitutions at the Same Position in Rhodopsin Lead to Distinct Phenotypes

John Neidhardt,1 Daniel Barthelmes,2 Firouzeh Farahmand,1 Johannes C. Fleischbauer,2 and Wolfgang Berger1

PURPOSE. Identification of a novel rhodopsin mutation in a family with retinitis pigmentosa and comparison of the clinical phenotype to a known mutation at the same amino acid position.

METHODS. Screening for mutations in rhodopsin was performed in 78 patients with retinitis pigmentosa. All exons and flanking intronic regions were amplified by PCR, sequenced, and compared to the reference sequence derived from the National Center for Biotechnology Information (NCBI, Bethesda, MD) database. Patients were characterized clinically according to the results of best corrected visual acuity testing (BCVA), slit lamp examination (SLE), funduscopy, Goldmann perimeter (GP), dark adaptometry (DA), and electroretinography (ERG). Structural analyses of the rhodopsin protein were performed with the Swiss-Pdb Viewer program available on-line (http://www.expasy.org/spdbv/ provided in the public domain by Swiss Institute of Bioinformatics, Geneva, Switzerland).

RESULTS. A novel rhodopsin mutation (Gly90Val) was identified in a Swiss family of three generations. The pedigree indicated autosomal dominant inheritance. No additional mutation was found in this family in other autosomal dominant genes. The BCVA of affected family members ranged from 20/25 to 20/20. Fundus examination showed fine pigment motting in patients of the third generation and well-defined bone spicules in patients of the second generation. GP showed concentric constriction. DA demonstrated monophasic cone adaptation only. ERG revealed severely reduced rod and cone signals. The clinical picture is compatible with retinitis pigmentosa. A previously reported amino acid substitution at the same position in rhodopsin leads to a phenotype resembling night blindness in mutation carriers, whereas patients reported in the current study showed the classic retinitis pigmentosa phenotype. The effect of different amino acid substitutions on the three-dimensional structure of rhodopsin was analyzed by homology modeling. Distinct distortions of position 90 (shifts in amino acids 112 and 113) and additional hydrogen bonds were found.

CONCLUSIONS. Different amino acid substitutions at position 90 of rhodopsin can lead to night blindness or retinitis pigmentosa. The data suggest that the property of the substituted amino acid distinguishes between the phenotypes. (Invest Ophthalmol Vis Sci. 2006;47:1630–1635) DOI:10.1167/iovs.05-1317

Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of retinal dystrophies characterized by progressive photoreceptor cell death, which initially affects the peripheral retina. Its prevalence is currently estimated at 1 in 3500 individuals. Typical symptoms of RP include night blindness (NB), progressive constriction of the visual field, and diminished or absent electroretinogram. Eventually, it can lead to complete blindness.

In contrast to the progressive RP, NB is a clinical symptom of various, mostly stationary retinal diseases. These stationary diseases can be classified into two groups: without and with fundus changes. The first group contains the congenital stationary night blindness and its subtypes, whereas Oguchi’s disease, Kandori’s disease, and fundus albipunctatus constitute the second group.1,2 The phenotypes within the second group show unique changes of the fundus.

RP and the stationary retinal diseases associated with NB phenotypically share the impairment of night vision, whereas the reduction of day vision, due to apoptosis of photoreceptors, is only observed in patients with RP.

Rhodopsin (RHO) mutations account for 8% to 10% of all RP cases. Mutations in RHO can cause not only the RP phenotype but also NB without affected day vision, even in later stages of the disease. Of over 100 RHO mutations described so far, three lead to autosomal dominant NB.3–5

Most RHO mutations are inherited in an autosomal dominant pattern, but autosomal recessive traits have also been described.

RHO belongs to the seven transmembrane receptor family and comprises approximately 80% of the total outer disc proteins in rod photoreceptors of the retina. Light absorption is mediated by the chromophore 11-cis-retinal embedded within the molecular environment of RHO and is transmitted into the phototransduction cascade under dim light conditions. The chromophore is covalently linked to the amino acid Lys296 and stabilized by the counterion of Glu113. Light absorption of the chromophore initiates a conformational change of the core protein, which ultimately leads to activation of both the G-protein transducin and the phototransduction cascade.6

In this report, we describe a novel RHO mutation (Gly90Val) in a three-generation family with RP. A different substitution of the same amino acid residue leads to a phenotype best described as NB in a previously reported family.4

METHODS

Mutational Screening

Genomic DNA from 78 Swiss patients with RP was extracted from blood samples (Chemagic Magnetic Separation Module I; Chemagen AG, Baesweiler, Germany). Five PCR fragments, spanning the entire coding region and splice sites of RHO (RefSeq number NM000539.2), were generated from 100 ng genomic DNA of RP patients, by using the

From the 1University of Zurich, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, Switzerland; and the 2Department of Ophthalmology, University Hospital Zurich, Switzerland.

Supported by the Velux Foundation and the Paul Schiller Foundation, Zurich, Switzerland.

Submitted for publication October 6, 2005; revised November 18, 2005; accepted February 13, 2006.

Disclosure: J. Neidhardt, None; D. Barthelmes, None; F. Farahmand, None; J.C. Fleischbauer, None; W. Berger, None.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked ‘advertisement’ in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: John Neidhardt, University of Zurich, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, Schoenenstrasse 16, 8603 Schwerzenbach, Switzerland; neidhardt@medgen.unizh.ch.

Investigative Ophthalmology & Visual Science, April 2006, Vol. 47, No. 4
Copyright © Association for Research in Vision and Ophthalmology
following primer combinations: Ex1-forward (fwd) 5’AGCTCATGGCCCT-TGCGCACGAT, Ex1-reverse (rev) 5’GAGGGCTTTGGGATAACATTT; Ex2-fwd 5’GAGTGCCCTCCTCTTACGCA, Ex2-rev 5’TCTCTAGTGGAGGAG; Ex3-fwd 5’CTGTTCCAAGTCCCTCAACA, Ex3-rev 5’CTG-GACCTCTAGGCTGTGA; Ex4-fwd 5’CAGCTAGTCTGGGGCTC, Ex4-rev 5’TCTGGGAAGTAGCTTGACC; and Ex5-fwd 5’GCCAGTG-CAAGCACACTGT, Ex5-rev 5’GACCTGGTCATCTGTGACAG. The PCR
was performed (HotstarTaq Polymerase; Qiagen AG, Hombrechtikon, Switzerland) as recommended by the manufacturers. No MgCl2 or Q-solution was added. Cycling conditions of the PCR were denatur-
ation 95°C for 1 minute, annealing 55°C for 1 minute, elongation 72°C for 1 minute, and 35 cycles. All PCR fragments were used for direct sequencing (model 3100; Applied Biosystems, Inc. [ABI], Rotkreuz, Switzerland) as recommended by the manufacturers. No MgCl2 or Q-solution was added. Cycling conditions of the PCR were denatur-
ation 95°C for 1 minute, annealing 55°C for 1 minute, elongation 72°C for 1 minute, and 35 cycles. All PCR fragments were used for direct sequencing (model 3100; Applied Biosystems, Inc. [ABI], Rotkreuz, Switzerland). In addition, PCR and sequencing was performed for 340 control alleles.

Clinical Evaluation of Patients
This study was performed in accordance with the Declaration of Helsinki. Informed consent was obtained from patients and control individuals after explanation of the nature and possible consequences of the study.

All patients underwent slit lamp examination (SLE) with funduscopy, Ganzfeld-electroretinography (ERG), dark-adaptometry (DA), Goldman perimetry (GP), fundus-photography, and best corrected visual acuity (BCVA) testing. Ganzfeld-ERG was performed according to the ISCEV standard protocol (UTAS 3000 ERG system; LKC Technologies Inc., Gaithersburg, MD). After a phase of dark-adaptation, single dim-white and bright-white flashes were used for stimulation. After a phase of light adaptation bright-white flashes were used for recording of single responses as well as 30-Hz flicker responses. A Goldmann-Weekers adaptometer (Haag-Streit, Könitz, Switzerland) was used for dark adaptometry measurements.

Homology Modeling
The three-dimensional structure of RHO in two mutated forms was generated by homology modeling using the x-ray crystal structure of RHO (Protein Data Bank identifier 1F88.pdb), thus simulating the structural consequences of mutations Gly90Val and Gly90Asp. For evaluation of the results, software provided by the Swiss Model (http://swissmodel.expasy.org/) for homology modeling and the Deep-View analysis tool were applied (http://www.expasy.org/spdbv/ both provided in the public domain by the Swiss Institute of Bioinformatics, Geneva, Switzerland). [9,10]

RESULTS

Mutation Screen
A novel mutation was identified by direct sequencing of the coding region and flanking intronic sequences of RHO. The mutation shows a heterozygous change at the nucleotide position 269 from G to T (c.269G>T), which affects codon 90 (GGC>GTC) and thus mutates the amino acid glycine to valine (p.Gly90Val). The mutation concordantly segregates with the disease and was found in two brothers, their mother, and their uncle in a Swiss family (Fig. 1). The father and aunt of the two brothers did not carry the mutation. The family pedigree suggests an autosomal dominant inheritance, which is in accordance with most mutations in RHO. No additional sequence alteration was found in this family in RHO or other genes causing RP (peripherin/RDS, NRL, CXR, RP2; data not shown). The mutation was not identified in 340 ethnically matching control alleles.

Clinical Findings
Diagnosis of retinitis pigmentosa in affected family members was established based on ophthalmic findings, such as extin-
guished scotopic and severely impaired photopic Ganzfeld-ERG, progressive concentric visual field constriction, and progressive loss of visual acuity. All affected patients were of the typical autosomal dominant RP phenotype.

Visual field constriction and a decrease in BCVA in patient II:2 (Fig. 2) manifested when she was in her late 30s. At first examination in 1995, BCVA was OD 20/25 and OS 20/30. In follow-up examinations, BCVA decreased to OD 20/35 and OS 20/65. Cystoid macular edema and cataract had developed. Fundus examinations showed retinal atrophy with bone spicules in the periphery (Fig. 2). Vessels are rarefied, especially in the periphery. Accumulation and progression of atrophy as well as pigment clumping could clearly be seen when comparing photographs from 1996 and 2005 (Fig. 2). Dark adaptometry showed a monophasic curve, indicating a sensitivity loss of the rod system of almost 3 log units. Anterior segment examinations and intraocular pressure (IOP) were normal.

Patient II:4 (Fig. 1) shows a very similar clinical appearance concerning VA and visual field (Table 1). From 1992 to 2005, progressive visual field constriction, increased pigment clumping, and progressive deterioration of Ganzfeld-ERG findings was present (Table 1). This is especially shown by almost

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933597/ on 06/24/2017)
absent dark-adapted and an obvious decline of light-adapted ERG recordings. Fundus examinations reveal a narrowing and deprivation of vessels. Like his sister, patient II:4 also showed monophasic dark adaptation with a sensitivity loss of approximately 2.6 log units after 35 minutes. Anterior segment morphology and IOP were normal.

In contrast, family member II:3 was not affected and showed a normal, healthy phenotype. Ganzfeld-ERG results, visual field measurements, IOP, and BCVA were without pathologic findings. Funduscopia showed normal anterior segment morphology, no pigment clumping, and no other abnormalities.
Both sons of patient II:2 were already showing pathologic findings in dark adaptometry and ERG at ages 11 and 13, whereas VA was normal and visual fields were well preserved. Fundus examinations showed atrophic areas as well as narrowed vessels. Bone spicules were not detectable at the present time. The father of III:1 and III:2 was not affected (Fig. 1) and showed no abnormal findings.

**Homology Modeling**

Previously, Sieving et al. reported a family with a Gly90Asp mutation. The mutation characterized in this study also leads to an exchange of glycine at position 90, but results in a different amino acid substitution (Gly90Val). The two mutations lead to distinct phenotypes. Sieving et al. found that affected members of the family had early-onset NB but not classic RP. In contrast, our patients with the Gly90Val substitution show a phenotype compatible with a classic RP. In contrast, patients with a different amino acid substitution at the same position in RHO have been described with a phenotype that is different from classic RP and resembles NB. The present study describes a novel mutation in RHO that leads to additional hydrogen bonds incorporated into the structure between the aspartic acid side chain and Leu112 and Ala117 in the third transmembrane domain. The orientation of the backbone at position 90 shows a distortion of 0.31 Å and 0.28 Å in the Gly90Asp and Gly90Val isoforms, respectively. Second, the backbone oxygens of amino acids Leu112 and Glu113 in the nonmutated closely matches in all but two regions of the molecule. First, the orientation of the backbone is shifted from their normal position by 0.55 Å and 0.37 Å, respectively. The Gly90Asp mutation leads to additional hydrogen bonds in the third transmembrane domain.

The homology model shows amino acid 90 located within the second transmembrane domain of RHO. Its side chain is oriented toward the third transmembrane domain. In RP and NB, either a nonpolar valine or a basic aspartate is introduced at position 90, respectively. The different characteristics of these side chains have the potential to influence not only the orientation of the amino acid 90 backbone, but also that of opposing amino acids. The overlay of the two mutated structures with the nonmutated closely matches in all but two regions of the molecule. First, the orientation of the backbone is shifted from their normal position by 0.55 Å and 0.37 Å, respectively. The Gly90Asp mutation leads to additional hydrogen bonds incorporated into the structure between the aspartic acid side chain and Leu112, Glu113, and Ala117 (Fig. 3).

**DISCUSSION**

The present study describes a novel mutation in RHO that leads to a phenotype compatible with a classic RP. In contrast, patients with a different amino acid substitution at the same position in RHO have been described with a phenotype that is different from classic RP and resembles NB.

In RP, the severity of the disease seems to correlate with the localization of the RHO mutations. Mutations in the cytoplasmic side of the molecule lead to a more severe phenotype, whereas mutations within the transmembrane domains lead to a milder phenotype. The orientation of the amino acid 90 backbone, but also that of opposing amino acids. The overlay of the two mutated structures with the nonmutated closely matches in all but two regions of the molecule. First, the orientation of the backbone is shifted from their normal position by 0.55 Å and 0.37 Å, respectively. The Gly90Asp mutation leads to additional hydrogen bonds incorporated into the structure between the aspartic acid side chain and Leu112, Glu113, and Ala117 (Fig. 3).

**FIGURE 3.** Three-dimensional (3-D) structural localization of amino acids mutated at position 90 in RHO. (A) Side view of the 3-D model showing the seven transmembrane domains of RHO. Each transmembrane helix is numbered and marked by a specific color. The shorter red helix (horizontally oriented) runs parallel to the cytoplasmic surface of the membrane. (B) Overlay of three different homology models using the Gly90Val, Gly90Asp, or wild-type RHO sequences. The amino acid side chains introduced by the mutations Gly90Val and Gly90Asp are shown in pink and yellow, respectively. Note that the overlay of the three structures matches in all positions except residues 90, 112, and 113. Green dotted lines: additional hydrogen bonds with amino acid Leu112 and Ala117 that are only present in Gly90Asp mutated RHO. Arrow: additional hydrogen bonds that influence the chromophore counterion Glu113.

**Table 1.** Overview of Clinical Findings Showing All Family Members

<table>
<thead>
<tr>
<th></th>
<th>II-1</th>
<th>II-2</th>
<th>II-3</th>
<th>II-4</th>
<th>III-1</th>
<th>III-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>48</td>
<td>46</td>
<td>44</td>
<td>42</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Intraocular pressure</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Dark adapted ERG responses</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Light adapted ERG responses</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Visual field</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Anterior segment morphology</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Pigment clumping</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Narrow vessels</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Macular edema</td>
<td>N</td>
<td>+</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Column headings are patient designations. N, normal or unaffected; -, diminished; --, impaired; ---, severely impaired; +, weak phenotype; ++, intermediate phenotype; ++++, strong phenotype.
mic part of RHO show a more severe phenotype than mutations affecting the discal space region of the protein. In terms of severity of the disease progression, mutations in transmembrane domains resemble intermediate forms. However, intrafamilial variability often complicates conclusions from such observations.

To the best of our knowledge, this is the first report that shows distinct phenotypes correlated with different amino acid substitutions at the same position in RHO.

The mutational spectrum of RHO shows as many as 19 examples where the same position is substituted by different amino acids. These include positions Pro235, Gly51, Gly106, Cys110, Gly114, Arg125, Ala164, Cys167, Pro171, Tyr178, Ser186, Gly188, Asp190, His211, Met216, Pro267, Lys296, Val345, and Pro347. The total number of different amino acids at these 19 positions amounts to more than 50. Of note, all of these substitutions lead to the RP phenotype.

The family described herein showed a typical phenotype of an autosomal dominant form of RP, with marked fundus changes developing in later stages of life. Distinction between NB and RP in the 11- and 13-year-old family members was possible with ERG recordings. Diagnosis of RP was established using visual fields, ERG recordings, and fundus morphology.

NB is characterized by an abolished dark adaptation and severely impaired night vision. There are two major types: the complete and the incomplete form—mainly differentiated by their characteristic ERG findings. Three RHO mutations have been associated with a phenotype that resembles NB without marked reduction in day vision. These mutations are Gly90Asp, Thr94Ile, and Ala292Glu. The molecular mechanism leading to NB has been suggested to be similar in all cases. The chromophore-binding pocket is mainly build by hydrophobic amino acids, but the polar residues Glu113 and Lys296 are of utmost importance. The chromophore is covalently bound by a Schiff linkage to Lys296 resulting in a positive charge of the Schiff base nitrogen. This positive charge is stabilized by an electrostatic interaction with the Glu113 carboxylate side chain that serves as a counterion. Mutagenesis of RHO showed in vitro that Lys296 and Glu113 are crucial positions in maintaining the inactive conformation of RHO by the salt bridge between the chromophore and its counterion Glu113. The mutations Gly90Asp and Ala292Glu introduce carboxylate side chains in close proximity to Glu113 and might serve as an alternative coutherion in mutated RHO molecules, which could disrupt the naturally occurring salt bridge. Without this salt bridge, RHO undergoes constitutive activation as shown for Gly90Asp, Thr94Ile, and Ala292Glu. The constitutively activated RHO is capable of initiating the phototransduction cascade, even after dissociation from the chromophore. Consequently, rod photoreceptors are activated without light, which gives rise to desensitization and less photoresponse, resembling the phenotype of NB. This mechanism was confirmed in transgenic mice expressing wild-type and Gly90Asp mutated Rho in rods on an Rho knockout background. Furthermore, mice expressing only Gly90Asp Rho had minimal photoreceptor degeneration at 1 year of age, which supports the observation from Gly90Asp carriers.

Our homology model suggests that amino acid 113 is affected by the mutation Gly90Val and thus provides the basis for an explanation of the functional relevance of this mutation in RHO. The crystal structure showed that the retinylidene group of the chromophore is oriented almost parallel to the transmembrane helix 3, which involves amino acids 113, 114, 117, 118, and 120. The orientation of the chromophore is likely to be disturbed by the amino acid side chains of valine in the Gly90Val mutation, due to an increase in required space of the valine side chain in comparison to glycine. Crystallographic studies also suggested that RHO undergoes a conformational change from the inactive to the active state including a movement of transmembrane domain 3 and/or 4. This movement may initiate the phototransduction cascade. A common feature of the transmembrane domains in RHO, which is conserved among G-protein-coupled receptors, is a bended shape of the helix. On the molecular level, the bends often occur at proline or glycine-glycine amino acids and are thought to be necessary for the activation process or stabilization of the protein structure. The positions Gly89 and Gly90 build a pair of amino acids involved in bending the helical structure in transmembrane domain 2, which leads to a strong change in the helix orientation by 30° at Gly89. It is likely that the functional properties of the Gly89-Gly90 pair are lost in the Gly90Val mutated RHO. Thus, an altered bending of the helix may be part of the pathologic processes that leads to RP instead of NB.

In addition to Gly90Val, a Gly90Asp mutation introduces hydrogen bonds into the structure mediated by the polar side chain. These additional hydrogen bonds may stabilize the activated form of RHO, which is not possible with a valine at position 90. In vitro mutagenesis studies of Thr94 showed that eight different amino acid substitutions lead to constitutive activation of RHO. Although the degree of activation varies over the different amino acid substitutions, no discernable trend was observed when considering the amino acid properties. This demonstrates the difficulty in predicting the functional consequences of amino acid substitutions. Moreover, additional genetic, epigenetic, or environmental factors may modify the phenotype in the Swiss family described herein. It is not completely clear why the different properties of the amino acids valine and aspartic acid give rise to different phenotypes in patients, but a major difference in aspartic acid with impact on structural stability and conformation is the ability to form hydrogen bonds with opposite amino acids. Our data suggest that the adverse structural effects of Gly90Val are based on the incapacity to stabilize RHO by hydrogen bonds like Gly90Asp and thus lead to classic RP.

Acknowledgments

The authors thank Gunther Niemeyer for expert contributions concerning the ERG findings and clinical evaluation as well as discussion of the results, and Esther Glaus for technical support during DNA extraction and sequence evaluation.

References