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Permalink
https://escholarship.org/uc/item/2wd9c2vg

Journal
OPHTHALMOLOGY, 102(2)

ISSN
0161-6420

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Publication Date
1995-02-01

Peer reviewed
A Peripherin/Retinal Degeneration Slow Mutation (Pro-210-Arg) Associated with Macular and Peripheral Retinal Degeneration

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Background: Mutations in the peripherin/retinal degeneration slow (RDS) gene have been identified in patients with retinitis pigmentosa and pattern macular dystrophy. The authors initially examined a large family affected with both peripheral and macular degeneration, inherited as an autosomal dominant trait. Screening for peripherin/RDS mutations identified a previously unreported nucleotide alteration in all of the affected individuals. Two additional families later were found to have this same mutation.

Methods: DNA samples from the members of three unrelated families were screened for peripherin/RDS mutations by denaturing gradient gel electrophoresis of the polymerase chain reaction-amplified peripherin/RDS coding sequences. The sequence change that was detected was further characterized by DNA sequencing. Family members were examined and evaluated with psychophysical and electrophysiologic methods.

Results: A proline to arginine mutation in codon 210 of peripherin/RDS was found in all clinically affected individuals. Macular changes included extensive geographic atrophy, pigment epithelial changes, and/or drusen. The proline to arginine mutation was not found among 100 healthy individuals, making it unlikely to be a nondisease-causing polymorphism.

Conclusions: The authors identified a novel peripherin/RDS gene mutation associated with autosomal dominant retinal degeneration in patients from three different families. The largest family showed a broad variability in the expressivity of the mutation. The overlap of clinical features with those of age-related maculopathy highlights the need to consider photoreceptor-specific genes as potential factors in the etiology of the latter condition. Ophthalmology 1995;102:246-255


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The retinal degeneration slow (RDS) gene was first identified in the *rds* mouse, which manifests a semidominant retinal degeneration. The RDS gene product is peripherin, which is an integral membrane protein present in the rim region of the outer segments of both rod and cone photoreceptors.

The recent identification of mutations in the photoreceptor-specific gene rhodopsin stimulated investigators to screen the peripherin/RDS gene for mutations in families with retinitis pigmentosa. Many disease-causing mutations were found. The expansion of this candidate gene screening to patients with other retinal and pigment epithelial dystrophies showed a spectrum of phenotypes to be associated with different peripherin/RDS mutations, including butterfly-shaped pigment epithelial dystrophy and panretinal degeneration. Although the function of peripherin/RDS is unknown, it may stabilize outer segment discs through interactions with other membrane-associated proteins. Because these interactions may involve other cone- or rod-specific proteins, there is the potential for peripherin/RDS mutations to have differential effects on the rod and cone photoreceptor cell populations.

In almost all previous reports of peripherin/RDS mutations, affected individuals within a family have shared common phenotypes with varying degrees of severity. Recently, a family was reported in which a single peripherin/RDS mutation was associated with retinitis pigmentosa, pattern dystrophy, or fundus flavimaculatus in different affected family members. In this report, a new peripherin/RDS mutation is found to be associated with a broad spectrum of clinical phenotypes within the same family.
Patients and Methods

The proband of the largest family (family 1) was a 41-year-old white woman who had visual blurring for 1 year and who initially had received a diagnosis of fundus flavimaculatus. Upon further questioning, it was apparent that a number of other family members had had central visual loss, and a family study was undertaken. The majority of the family members initially were screened at a large family reunion. The complete pedigree of this family is shown in Figure 1. Medical histories, informed consents, and blood samples either were taken at the time of the screening or in person’s homes. Whenever possible, affected individuals had more extensive evaluations, including color testing, visual fields, fundus photography, and angiography. Electroretinography and electrooculography were performed on a subset of these individuals. Blood samples also were obtained from members of two other families affected with an autosomal dominant macular dystrophy. Family 2 originally was described by Gass et al., defining the entity known as peculiar foveomacular dystrophy. Family 3 consists of a woman, who was originally believed to have Stargardt disease, and her son.

DNA Studies

Genomic DNA was purified from peripheral blood lymphocytes by standard methods using the Applied Biosystems 340 DNA extractor (Applied Biosystems, Inc, Foster City, CA). The primer sequences used for GC-clamped denaturing gel electrophoresis (DGGE) analysis of the peripherin/RDS gene previously were reported by Nichols et al., whereas those used for SSCP analysis previously were reported Wells et al.

Polymerase chain reaction (PCR) reactions were carried out in a 25-μl total volume containing 400 ng genomic DNA as a template and the following: 2.7 μl 10X buffer (100 mM TRIS-HCl pH 8.8, 500 mM KCl, 15 mM MgCl₂, 0.1% gelatin, 1.25 mM each of dNTPs), 0.4 μl of each 20 μM primer, 0.4 μl of 5 U/μl Taq polymerase (Perkin-Elmer/Cetus, Norwalk, CT), and 0.1 μl of α³²P-dCTP. Samples were overlaid with mineral oil and incubated in a DNA thermocycler (Perkin-Elmer/Cetus) for 30 cycles under the following conditions: 94°C for 30 seconds, 61°C for 30 seconds, and 72°C for 30 seconds. Ten μl formamide loading buffer was added to the PCR product. After denaturation at 80°C for 3 minutes, 3 μl of each sample was loaded on a nondenaturing 6% polyacrylamide gel. Electrophoresis was carried out for 16 hours at room temperature. The gel was transferred on to 3-mm filter paper (Whatman, Inc, Clifton, NJ) and dried using a heated gel dryer in vacuo. Autoradiographs were made by exposing Kodak X-OMAT AR film to the dried gel for 24 to 48 hours. Denaturing gradient gel electrophoresis and DNA sequencing were performed as described by Nichols et al.

Results

Denaturing Gel Electrophoresis and SSCP Analyses

GC-clamped DGGE screening of the complete peripherin/RDS coding sequence in two affected individuals from each of the three families identified the existence of a mutation in exon 2 in all of them. Direct DNA sequencing of the exon 2 PCR products of one individual in each family showed the sequence change to be a C to G transversion mutation in the second nucleotide of codon 210, resulting in the substitution of arginine for proline (Pro-210-Arg) at this position (Fig 2). SSCP analysis then was
used to screen all available family members of family 1, and the mutation was identified in 17 individuals, including all 14 clinically affected patients (Fig 3). This mutation was not detected in 100 control individuals (200 chromosomes—data not shown).

**Clinical Phenotypes**

Fifty-one members of family 1 were examined. The 12 clinically affected family members ranged in age from 16 to 75 years. Table I lists some of the clinical features and visual function test results in these affected individuals.

Figure 4 illustrates the macular findings in the affected individuals. Retinal findings included diffuse, fine, hard drusen in the macula; a butterfly pattern; extensive retinal atrophy; diffuse pigment epithelial disturbances; and severe atrophic macular degeneration. Fluorescein angiography detected subtle abnormalities in the fovea of the youngest molecularly affected individual (V-10) (Fig 4) and in the individual with extensive fine drusen. In other individuals, the fluorescein angiograms showed widespread pigment epithelial alterations or parafoveal disturbances, without staining of the vitelliform lesions. Similar to the family reported by Nichols et al, there were no cases of choroidal neovascularization. Five individuals with normal results of fundus examinations, including one 39-year-old individual, were found to harbor the peripherin/RDS mutation.

Visual function varied considerably among the members of family 1 with the mutation. Visual acuities ranged from 20/20 to less than 20/200. Central visual loss seemed to correlate with the degree of central retinal atrophy. As shown in Figure 5, perimetric findings also varied widely. Severe peripheral field loss was documented in several members of the pedigree, whereas other affected individuals showed general threshold elevation and/or central/paracentral scotomas. Eleven patients in family 1 underwent electrophotography. Only one had normal responses. The other ten individuals showed rod and cone system abnormalities, some having more cone than rod dysfunction. The electroretinograms were normal in 4 of 11 persons tested. The Arden ratios were between 1.5 and 2.0 for five persons and below 1.5 for two.

The fundus features of many of the affected members of family 1 are identical to those of family 2 who were initially described by Gass as having peculiar foveomacular dystrophy. Affected individuals in family 2 ranged in age from 30 to 70 years and had well-preserved visual acuities (range, 20/25–20/50). The onset of symptoms ranged from 24 to 44 years of age, and the visual disturbances were slowly progressive. The proband of family 3 initially was seen at 44 years of age with a 1-year history of metamorphopsia. Her initial visual acuities were 20/50 and 20/60 in the right and left eyes, respectively. At the time of her most recent evaluation at 50 years of age, her visual acuity had declined to 20/200 and 20/100 in the right and left eyes, respectively. Results of examination of her retinas showed yellowish subfoveal lesions with multiple radial projections and yellowish flecks in the retinal periphery. The woman’s son was asymptomatic when he was first evaluated at 11 years of age because of his mother’s retinal findings. His visual acuities were 20/30+3 in the right eye and 20/25 in the left. Similar to individual V-10 in family 1 (Fig 4), he had small yellowish subfoveal lesions in each eye. Five years later, there was increased prominence of the subfoveal lesions and some early peripheral flecks. His visual acuity remained 20/25 in both eyes.

**Discussion**

**Clinical Heterogeneity of a Single Peripherin/Retinal Degeneration Slow Mutation**

Within a single family, most peripherin/RDS mutations have a fairly homogeneous phenotype, with only age-dependent variations of severity. However, Weleber et al recently reported a family in which a deletion of codon 153 was associated with three distinct phenotypes. The Pro-210-Arg mutation in this report has a similar intrafamilial variability. A number of individuals in family 1 initially were seen by ophthalmologists who were unaware of the nature of the retinal disease in other family members. The diagnoses made in this single-patient setting included age-related macular degeneration, retinitis pigmentosa, Stargardt disease, adult vitelliform degeneration, dominant drusen, and pattern dystrophy. The youngest symptomatic individual in family 1 was 16 years of age at the time of evaluation (patient V-10, Fig 4). In contrast, individual IV-24, carried the Pro-210-Arg mutation but had no visual symptoms or electrophysiologic or fundus abnormalities at 39 years of age. Several affected individuals in family 1 with fundus abnormalities had normal visual acuities and few or no visual symptoms even in the sixth decade of life (Table 1). Only two of the affected individuals in family 1, III-08 and IV-20, were sufficiently impaired that they could not legally drive.

The two individuals from family 3 illustrate that the Pro-210-Arg mutation can be associated with fairly severe visual loss as well as early onset of retinal pathology. The best visual acuity of the proband’s best eye was 20/100 at 50 years of age, and her 11-year-old son had reduced visual acuity of 20/25 at 11 years of age.

The complete lack of penetrance of the mutant gene in one 39-year-old patient and its mild expression in several others should not raise doubts about the involvement of the peripherin/RDS gene in the retinal disease of these three families. Every clinically affected family member whose DNA was available for testing was found to harbor the mutation (18/18 patients). The odds of this association occurring by chance are less than 1 in 10,000. The other factors that argue strongly for the pathogenicity of the Pro-210-Arg mutation are (1) its discovery in three unrelated but phenotypically similar families; (2) its absence in control individuals; and (3) the severe nature of the amino acid substitution.
<table>
<thead>
<tr>
<th>Pedigree No.</th>
<th>Sex/Age (yrs)</th>
<th>Best-corrected Visual Acuity</th>
<th>Fundus Findings</th>
<th>Fluorescein Angiogram</th>
<th>Visual Field*</th>
<th>Color</th>
<th>ERG†</th>
<th>EOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>III-08</td>
<td>M/75</td>
<td>CF, 1 ft CF, 1 ft</td>
<td>Macular geographic atrophy, no significant drusen</td>
<td>Geographic atrophy in maculae and extensive punctate defects in RPE throughout posterior pole</td>
<td>Central scotomas and peripheral field constriction-Goldmann</td>
<td>Unable</td>
<td>R-Ăś</td>
<td>266%</td>
</tr>
<tr>
<td>III-10</td>
<td>F/72</td>
<td>20/30 20/30</td>
<td>Diffuse RPE changes with areas of atrophy, few drusen, and no flecks; surface wrinkling retinopathy in macula, OD; indistinguishable from atrophic age-related macular degeneration</td>
<td>Extensive focal patches of RPE atrophy becoming almost coalescent in some areas</td>
<td>Bilateral temporal paracentral scotomas and diffuse threshold elevations-Humphrey</td>
<td>Abnormal, B/Y</td>
<td>R-Ăś</td>
<td>166%</td>
</tr>
<tr>
<td>III-12</td>
<td>M/68</td>
<td>20/32 20/25</td>
<td>Mixture of flecks and atrophic patches of RPE in the posterior pole, OU; retinal arteriolar attenuation, OU</td>
<td>Extensive patches of RPE atrophy becoming almost coalescent in some area, OU</td>
<td>Widespread loss of paracentral and peripheral vision, OU-Humphrey</td>
<td>Abnormal, B/Y</td>
<td>R-Ăś</td>
<td>136%</td>
</tr>
<tr>
<td>IV-15</td>
<td>M/52</td>
<td>20/25 20/20-1</td>
<td>Small parafoveal areas of RPE atrophy, no significant drusen or flecks</td>
<td>Parafocal transmission defects corresponding to RPE atrophy and additional 250-μm spots of atrophy in the midperiphery, OU</td>
<td>Borderline diffuse threshold elevations-Humphrey</td>
<td>Abnormal, D-15</td>
<td>R-Ăś</td>
<td>150%</td>
</tr>
<tr>
<td>IV-19</td>
<td>F/43</td>
<td>20/40 20/32</td>
<td>Irregularly shaped vitelliform lesions in the macula, and scattered areas of RPE atrophy in the midperiphery</td>
<td>Diffuse 250-500-μm patches of transmission defects corresponding to RPE atrophy throughout the posterior pole</td>
<td>Patchy elevations of thresholds within 30° fields, OU-Humphrey</td>
<td>Abnormal, D-15</td>
<td>R-Ăś</td>
<td>125%</td>
</tr>
</tbody>
</table>

*Visual Field:
- Central scotomas and peripheral field constriction-Goldmann
- Bilateral temporal paracentral scotomas and diffuse threshold elevations-Humphrey
- Widespread loss of paracentral and peripheral vision, OU-Humphrey
- Borderline diffuse threshold elevations-Humphrey

†ERG:
- R-Ăś
- R-Ăś
- R-Ăś
- R-Ăś
- R-Ăś

‡EOG:
- 266%
- 350%
- 166%
- 157%
- 136%
- 120%
- 150%
- 166%
- 125%
- 125%
<table>
<thead>
<tr>
<th>Case</th>
<th>Age/Gender</th>
<th>VA</th>
<th>VA</th>
<th>Clinical Findings</th>
<th>Color Vision</th>
<th>Thresholds</th>
<th>ERG Amplitudes</th>
<th>Goldmann</th>
<th>Humphrey</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV-20</td>
<td>F/43</td>
<td>20/20</td>
<td>20/25</td>
<td>Numerous drusen-like flecks in the midperipheral retina and macula; parafoveal, irregular vitelliform lesions; mild arteriolar attenuation</td>
<td>Spots of hyperfluorescence-midperipheral and parafoveal; RPE atrophy not exactly corresponding to yellow flecks or parafoveal vitelliform lesions</td>
<td>General threshold elevations with increased blind spots and midperipheral relative scotomas-Humphrey</td>
<td>Normal, FM-100</td>
<td>R-↓↓, C-↓↓</td>
<td></td>
</tr>
<tr>
<td>IV-22</td>
<td>F/34</td>
<td>20/50</td>
<td>20/32</td>
<td>Parafoveal, irregularly shaped atrophic lesions and diffuse RPE changes</td>
<td>Transmission defects corresponding to the area of RPE atrophy</td>
<td>Dense constriction of peripheral vision to the central 20°-Humphrey</td>
<td>Normal, D-15</td>
<td>R-↓, C-↓↓</td>
<td></td>
</tr>
<tr>
<td>IV-23</td>
<td>M/41</td>
<td>20/20</td>
<td>20/20</td>
<td>Irregular, vitelliform lesions in the foveas with yellow flecks in the posterior poles of both eyes</td>
<td>Hyperfluorescent spots corresponding to some but not all of the flecks, no staining of foveal lesion</td>
<td>Elevation of peripheral thresholds-Humphrey</td>
<td>Abnormal, FM-100</td>
<td>R-↓, C-↓↓</td>
<td></td>
</tr>
<tr>
<td>IV-24</td>
<td>M/39</td>
<td>20/16</td>
<td>20/16</td>
<td>Normal</td>
<td>Diffuse mottling of the RPE without focal defects</td>
<td>Within normal limits, slight elevation of thresholds peripheral to the central 20°-Humphrey</td>
<td>Normal HRR, D-15</td>
<td>R-±, C-↓</td>
<td></td>
</tr>
<tr>
<td>IV-27</td>
<td>M/36</td>
<td>20/16</td>
<td>20/16</td>
<td>Extensive fine drusen in the midperiphery, OS &gt; OD</td>
<td>Scattered small punctate hyperfluorescent spots primarily anterior to maculae, slight irregularity of parafoveal RPE</td>
<td>Normal-Humphrey</td>
<td>Normal HRR, D-15</td>
<td>R-↓, C-↓</td>
<td></td>
</tr>
<tr>
<td>V-04</td>
<td>F/24</td>
<td>20/16-2</td>
<td>20/16-1</td>
<td>Granular RPE in periphery, but normal foveal reflexes and no focal abnormalities</td>
<td>Normal</td>
<td>Scattered points within central 30°with mild threshold elevations-Humphrey</td>
<td>Normal HRR, D-15, FM-100</td>
<td>R-normal, C-normal</td>
<td></td>
</tr>
<tr>
<td>V-10</td>
<td>F/16</td>
<td>20/15</td>
<td>20/20</td>
<td>Normal foveal reflexes, OU, but underlying RPE appeared to be irregularly pigmented, OS &gt; OD</td>
<td>Subtle RPE changes beneath the foveal avascular zones, OU</td>
<td>Normal-Humphrey</td>
<td>Normal HRR, D-15</td>
<td>No data</td>
<td></td>
</tr>
</tbody>
</table>

OD = right eye; OS = left eye; ERG = electroretinogram; EOG = electro-oculogram; CF = counting fingers; RPE = retinal pigment epithelium; R = rod amplitudes; C = cone amplitudes; B/Y = blue-yellow axis defect; D15 = D15 desaturated test; FM-100 = Farnsworth-Munsell 100 hue test; HRR = Hardy-Rand-Rittler color plates.

*Goldmann = standard kinetic perimetry; Humphrey = 30-2 static perimetry program.

↓↓↓ = markedly reduced (but detectable); ↓↓ = moderately reduced; ±↓ = borderline normal; ↓ = mildly reduced.
Figure 4. Fundus photographs and fluorescein angiograms of selected affected individuals. Pedigree numbers correspond to those in Figure 1 and Table 1. Top left, II-05 right eye (OD). Top right, III-12 left eye (OS). Second row left, III-12 (fluorescein angiogram) OS. Second row right, IV-22 OD. Third row left, IV-22 (fluorescein angiogram) OD. Third row right, IV-23 OD. Fourth row left, IV-19 OD. Fourth row right, IV-19 (fluorescein angiogram) OD. Bottom left, V-10 2x macula OS. Bottom right, III-08 OS. (Fig 4 continues.)
Relation of Photoreceptor Genes to Age-related Macular Degeneration

Our findings provide additional evidence that a photoreceptor-specific gene can cause a primarily macular disease. Other proteins involved in the phototransduction pathway and photoreceptor structure may interact with peripherin, and polymorphisms within such proteins may explain the range of disease phenotypes seen within a single family. The histopathologic studies conducted by Gass almost 20 years ago on a person with this Pro-210-Arg mutation showed that the alterations in the pigment epithelium were indistinguishable from the drusen associated with dominantly inherited drusen and those of age-related macular degeneration. Several of us have reported a family with an X-linked cone-rod degeneration that maps to the region of RP2 and RP3. The affected members of this family also show varying degrees of macular degeneration. It is possible that mutations of one of the genes responsible for X-linked retinitis pigmentosa also may
contribute to the development of progressive macular disease. Macular degeneration also has been observed in individuals with deletions in the red cone pigment gene on the X-chromosome. An autosomal dominant, Stargardt's-like macular disease recently has been linked to chromosome 13 and chromosome 6.

The role of photoreceptor-specific genes in the development of age-related macular degeneration is unknown. The demonstration of macular degeneration associated with multiple peripherin/RDS mutations expands the potential role of the photoreceptors in the pathogenesis of this disorder. Age-related macular degeneration is a clinically heterogeneous set of disorders that frequently demonstrates autosomal dominant patterns of inheritance. Although many investigators have emphasized pigment epithelial dysfunction and basement membrane abnormalities as potential causes of age-related macular degeneration, it is clear that genes expressed anywhere within the entire retina–pigment epithelium–choriocapillaris complex are potential candidates for involvement in the pathogenesis of age–related macular degeneration.

References