A Peculiar Autosomal Dominant Macular Dystrophy Caused by an Asparagine Deletion at Codon 169 in the Peripherin/RDS Gene

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Objective: To describe the clinical and genetic findings in a family with a peculiar autosomal dominant macular dystrophy with peripheral deposits.

Methods: All family members underwent an ophthalmic examination, and their genomic DNA was screened for mutations in the human retinal degeneration slow (peripherin/RDS) and rhodopsin genes. In selected cases, fluorescein angiography and electrophysiologic testing were performed.

Results: The age at onset of the disease was between the third and fourth decades of life, starting with mild visual acuity loss and periods of metamorphopsia. Clinical signs included subretinal yellowish macular deposits evolving into geographic atrophy and retinal hypopigmentation and hyperpigmentation. Electroretinography demonstrated rod dysfunction, and electro-oculograms were mildly to severely disturbed. All affected members were found to carry a 3-base pair deletion affecting codon 169 of the peripherin/RDS gene. This mutation resulted in an asparagine (Asn) deletion in the peripherin/RDS protein and was not found in 155 control individuals.

Conclusion: A deletion of Asn169 in the peripherin/RDS protein causes a peculiar form of autosomal dominant macular dystrophy in a large family from the Netherlands.

Clinical Relevance: Characterizing the phenotype and genotype in this family may, in the long term, result in a better understanding of the precise mechanism underlying this retinal degeneration.

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Blood samples were obtained from 19 family members, and their genomic DNA was isolated as described elsewhere. For mutation analysis of the peripherin/RDS gene, 25 ng of genomic DNA was amplified by means of the polymerase chain reaction under the following conditions: initial denaturation for 5 minutes at 95°C, denaturation for 30 seconds at 94°C, annealing for 30 seconds at 55°C, extension for 1 minute at 72°C, and a final extension for 5 minutes at 72°C. For peripherin/RDS exon 1, part a (complementary DNA [cDNA] nucleotides 209–1453), we used the forward primer 5'-GTGGGAAGCAACCCGGAC-3' and the reverse primer 5'-AGATCTTCCCAGCAGGCG-3'. For exon 1, part b (cDNA nucleotides 431–673), we used the forward primer 5'-TGATAGGGATGGGGGTGC-3' and the reverse primer 5'-AGATCTTCCCAGCAGGCG-3'.

RESULTS

CLINICAL FINDINGS

The pattern of inheritance in the family was consistent with autosomal dominant inheritance. Dominant X-linked inheritance could be excluded because there were 6 father-to-son transmissions of the genetic defect (Figure 1). We examined all known clinically affected family members except individuals II:1 and II:2 (Table 1). Complaints of metamorphopsia started in the fourth decade of life for all affected persons, but macular changes were already seen in the third decade of life in individual IV:1. Visual acuity varied from 20/100 to 20/16. Seven subjects had periods of metamorphopsia and/or paracentral scotomas. Six subjects complained of night blindness, in 2 individuals since the ages of 19 and 20 years (subjects III:8 and III:10, respectively). Two other individuals without any fundus changes (IV:4 and IV:10) had very mild complaints of night blindness but no other visual symptoms. We classified the retinal findings into 3 stages, varying from small subretinal macular deposits in stage I (individuals III:1, III:2, and III:4) and larger butterfly-shaped macular lesions or lesions with a scrambled-eggs appearance accompanied by 0.1-disc diameter large midperipheral nonelevated deposits in stage II (individuals III:4, III:6, III:7, III:8, and III:10) to large atrophic scarring in stage III (individuals III:1 and III:2) (Figure 2). Fluorescein angiography showed a small area of central hypofluorescence surrounded by a hyperfluorescent rim in stage I. In stage II this developed into a more pattern dystrophy–like hypofluorescent or hyperfluorescent central lesion. The peripheral deposits seemed hyperfluorescent and did not always correspond to the deposits visible by ophthalmoscopy. In stage III the central choroidal vessels became visible within the lesion because of chorioretinal atrophy, and the central atrophic area was surrounded by a mottled hyperfluorescence (Figure 2). In individuals III:1, III:5, III:9, IV:4, IV:5, IV:6, IV:7, IV:8, and IV:10, fundus examination demonstrated no abnormalities. In cases III:1, III:2, and III:4, ERG testing demonstrated pathologic b-wave amplitudes for dark-adapted rods (Table 2). The b-wave amplitudes for cones and the maximal dark-adapted com-

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bined responses were diminished in subject III:2 (Table 2). Individuals III:10 and IV:2 showed normal scotopic and photopic ERG values (data not shown). Results of EOG testing were normal in 3 cases (IV:2, IV:5, and IV:6). The Arden ratio was between 1.5 and 2.0 in 2 subjects (IV:4 and IV:9) and less than 1.5 in 6 subjects (III:1, III:2, III:4, III:8, IV:1, and IV:3). Individuals III:6, III:7, III:10, and IV:10 were not tested.

MOLECULAR GENETIC FINDINGS

Direct sequencing of the polymerase chain reaction products from cases III:4 and III:10 showed a 3-bp deletion in exon 1 of the peripherin/RDS gene affecting either codon 168 or 169, both coding for asparagine (Figure 3). According to the nomenclature for the description of human sequence variations, the most 3’ copy is arbitrarily assigned to have been changed in case of deletions in amino acid stretches, so we state that the deletion involves codon 169.12 This novel deletion was found in all affected individuals and in 4 asymptomatic individuals of this family (Figure 1), but not in 155 control individuals (data not shown). Individual IV:3 was not tested. In addition, the sequence variant 558C→T (Val106Val) was found, representing a previously reported synonymous mutation.13 The rhodopsin gene showed no disease-causing mutations.

This family demonstrated a retinal dystrophy characterized by an age at onset in the third to fourth decade of life, with a typical history of periods of metamorphopsia and paracentral scotomas, mild visual acuity loss, sub-retinal macular deposits, which slowly evolved into lesions resembling geographic atrophy in age-related macular degeneration, and posterior pole deposits. On ophthalmoscopy, these deposits did not always correspond to the angiographic appearance. Possibly, lesions showed blocked hypofluorescence because of accumulation of lipofuscin, with adjacent small areas of atrophy causing hyperfluorescence. The EOG values were normal or subnormal in the beginning (stage I) but gradually became abnormal in later stages (stages II and III). The scotopic ERG was abnormal in stages II and III. Currently, 18 loci (10 cloned genes) are associated with nonsyndromic autosomal dominant dystrophies of the retina and choroid with primary involvement of the macular area (available at: http://www.sph.uth.tmc.edu/Retnet/disease.htm). The phenotype of the family presented herein differs from all of them. The first stage of our dystrophy resembles AVMD.14 In AVMD the age at onset is in the middle age group, the progression of visual loss is slow, patients complain of metamorphopsia, the EOG is often reduced, and fundus features consist of bilateral symmetric and slightly raised small yellow sub-retinal lesions.14 Families with AVMD may display an autosomal dominant pattern of inheritance.15,16 All of these features are compatible with the dystrophy in the family described herein, but incompatible features are also seen. In our family the EOG became nonrecordable in later stages, while in AVMD the EOG findings stay normal or subnormal.15 Abnormal scotopic ERGs as observed in several individuals of our family are not found in patients with AVMD. Adult-onset vitelliform macular dystrophy is known to be associated with mutations in the periph-

### Table 1. Clinical Characteristics of the Affected Family Members and 4 Clinically Unaffected Family Members With the 3-bp Deletion

<table>
<thead>
<tr>
<th>Pedigree No.</th>
<th>Clinically Affected</th>
<th>Age at Onset, y</th>
<th>Age at Examination, y</th>
<th>VA OD</th>
<th>VA OS</th>
<th>Symptoms</th>
<th>Fundus Stage*</th>
<th>ERG</th>
<th>Scotopic</th>
<th>Photopic</th>
<th>EOG</th>
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<tr>
<td>III:1</td>
<td>Yes</td>
<td>38</td>
<td>68</td>
<td>20/25</td>
<td>20/25</td>
<td>Metamorphopsia, night blindness</td>
<td>III</td>
<td>AN</td>
<td>N</td>
<td>OU: 1.0</td>
<td></td>
</tr>
<tr>
<td>III:2</td>
<td>Yes</td>
<td>30-40</td>
<td>65</td>
<td>20/100</td>
<td>20/50</td>
<td>Metamorphopsia, paracentral</td>
<td>III</td>
<td>AN</td>
<td>AN</td>
<td>OU: 1.0</td>
<td></td>
</tr>
<tr>
<td>III:4</td>
<td>Yes</td>
<td>36</td>
<td>64</td>
<td>20/30</td>
<td>20/20</td>
<td>Metamorphopsia, night blindness, paracentral scotoma</td>
<td>II</td>
<td>AN</td>
<td>N</td>
<td>OU: 1.4</td>
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<tr>
<td>III:6</td>
<td>Yes</td>
<td>35</td>
<td>49</td>
<td>20/20</td>
<td>20/16</td>
<td>Metamorphopsia, night blindness</td>
<td>II</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>III:7</td>
<td>Yes</td>
<td>30-40</td>
<td>48</td>
<td>20/20</td>
<td>20/20</td>
<td>Metamorphopsia</td>
<td>II</td>
<td>...</td>
<td>...</td>
<td>...</td>
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<tr>
<td>III:8</td>
<td>Yes</td>
<td>20</td>
<td>44</td>
<td>20/40</td>
<td>20/40</td>
<td>Night blindness</td>
<td>II</td>
<td>...</td>
<td>...</td>
<td>OU: 1.0</td>
<td></td>
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<tr>
<td>III:10</td>
<td>Yes</td>
<td>19</td>
<td>60</td>
<td>20/20</td>
<td>20/20</td>
<td>Night blindness, metamorphopsia from 39 y</td>
<td>II</td>
<td>N</td>
<td>N</td>
<td>...</td>
<td></td>
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<td>IV:1</td>
<td>Yes</td>
<td>–</td>
<td>24</td>
<td>20/16</td>
<td>20/16</td>
<td>No complaints</td>
<td>I</td>
<td>...</td>
<td>...</td>
<td>OD: 1.4</td>
<td></td>
</tr>
<tr>
<td>IV:2</td>
<td>Yes</td>
<td>35-40</td>
<td>40</td>
<td>20/20</td>
<td>20/20</td>
<td>Metamorphopsia, night blindness</td>
<td>I</td>
<td>N</td>
<td>N</td>
<td>OD: 4.0</td>
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<tr>
<td>IV:3</td>
<td>Yes</td>
<td>?</td>
<td>38</td>
<td>20/20</td>
<td>20/20</td>
<td>Unknown</td>
<td>I</td>
<td>...</td>
<td>...</td>
<td>OU: 1.3</td>
<td></td>
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<tr>
<td>IV:4</td>
<td>No</td>
<td>30-35</td>
<td>35</td>
<td>20/20</td>
<td>20/20</td>
<td>Mild night blindness</td>
<td>N</td>
<td>...</td>
<td>...</td>
<td>OU: 1.7</td>
<td></td>
</tr>
<tr>
<td>IV:5</td>
<td>No</td>
<td>–</td>
<td>20</td>
<td>20/16</td>
<td>20/16</td>
<td>No complaints</td>
<td>N</td>
<td>...</td>
<td>...</td>
<td>OD: 2.0</td>
<td></td>
</tr>
<tr>
<td>IV:6</td>
<td>No</td>
<td>–</td>
<td>16</td>
<td>20/20</td>
<td>20/20</td>
<td>No complaints</td>
<td>N</td>
<td>...</td>
<td>...</td>
<td>OD: 2.2</td>
<td></td>
</tr>
<tr>
<td>IV:9</td>
<td>Yes</td>
<td>?</td>
<td>39</td>
<td>20/20</td>
<td>20/20</td>
<td>Unknown</td>
<td>I</td>
<td>...</td>
<td>...</td>
<td>OD: 1.7</td>
<td></td>
</tr>
<tr>
<td>IV:10</td>
<td>No</td>
<td>30</td>
<td>30</td>
<td>20/20</td>
<td>20/20</td>
<td>Mild night blindness</td>
<td>N</td>
<td>...</td>
<td>...</td>
<td>OD: 1.5</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AN, abnormal; bp, base pair; ellipses, not performed; EOG, electro-oculogram (Arden ratio); ERG, electroretinogram; N, normal; VA, visual acuity; –, no symptoms; ?, unknown.

*See text for explanation of stages.
erin/RDS gene and in the vitelliform macular dystrophy (VMD2) gene.17,18

Similarities to Best disease are the progression of the foveal lesions to lesions with a scrambled-egg appearance and the diminished EOG values.19,20 Nevertheless, the characteristic configuration of the early lesions in Best disease, often already seen in children between the ages of 5 and 15 years, including the intact egg yolk and pseudohypopyon cyst and the “fluid-level” appearance of the fluorescein staining within the lesion, were not seen in any of our family members. Furthermore, the ERG in Best disease is normal,20 while in some of our family members the ERG was disturbed. Best disease is associated with mutations in the VMD2 gene.17,18

Another entity from which this family’s findings need to be differentiated is dominant slowly progressive macular dystrophy.21 In these patients, the age at onset is in the fifth decade of life, but the visual acuity remains good until the seventh decade. Several patients have had visual acuity fluctuations and metamorphopsia. Obvious retinal changes include perifoveal pigment epithelial atrophy, posterior pole flecks, and lesions resembling an

Figure 2. A, Fundus photograph of patient IV:2 at age 37 years (stage I). B, Fluorescein angiogram of patient IV:2 at age 37 years (stage I). C, Fundus photograph of patient III:4 at age 52 years (stage II). D, Fluorescein angiogram of patient III:4 at age 52 years (stage II). E, Fundus photograph of patient III:2 at age 65 years (stage III). F, Fluorescein angiogram of patient III:2 at age 65 years (stage III).
atrophic form of age-related macular degeneration. The ERG is normal or has slightly reduced photopic as well as scotopic responses. Nevertheless, the EOG is normal in these patients, and this entity has recently been found to be associated with mutations in the elongation of the very long-chain fatty acids 4 (ELOVL4) gene.

For other dystrophies with subretinal deposits, distinguishing features involving the clinical history, electrophysiologic findings, and form and distribution of deposits are easily found. These dystrophies include autosomal dominant Stargardt disease, caused by mutations in the ELOVL4 gene and an undefined gene at 4p, macular drusen, caused by mutations in the epidermal growth factor–containing fibrillin-like extracellular matrix 1 (EFEMP1) gene; and butterfly-shaped dystrophy, caused by mutations in the peripherin/RDS gene. Half of the mutations found in the peripherin/RDS gene are identified in families with diseases of the central retina, but in a few of these families deposits in the peripheral retina are found. Within 2 of these families, clinically disparate phenotypes are observed. These vary from RP, pattern dystrophy, and fundus flavimaculatus in a family with a 3-bp deletion of codon 153 or 154 of the peripherin/RDS gene to Stargardt disease, dominant drusen, adult vitelliform dystrophy, and RP in a family with a P210R mutation of the peripherin/RDS gene. In 3 families with point mutations at codon 172 of the peripherin/RDS gene and macular dystrophy, normal rod electrograms were found, while our family demonstrated rod dysfunction.

It is likely that the novel peripherin/RDS deletion of codon 169, resulting in a deletion of asparagine, is associated with the clinical appearance in this family. Four individuals, IV:4, IV:5, IV:6, and IV:10, varying in age from 16 to 35 years, with normal results of fundus examinations, have been found to harbor the 3-bp deletion in the peripherin/RDS gene. Two of these (subjects IV:4 and IV:10) had mild complaints of night blindness. Most likely these individuals will develop more symp-
tom's in the future, although we cannot rule out nonpenetrance in some cases.

Peripherin/RDS, an integral membrane glycoprotein, is exclusively expressed in the outer segments of both rod and cone photoreceptor cells and is thought to play a role in morphogenesis and maintenance of the disc structure of the photoreceptor outer segments. The majority of mutations are located in the large second intradiscal loop. Some of the autosomal dominant RP-associated mutations affect protein folding and subunit assembly, which might lead to the inability of the peripherin/RDS mutant protein to form intermolecular disulfide bonds that are required for oligomerization. Oligomerization and dimerization of the peripherin/RDS protein are necessary for the maintenance of flattened photoreceptor disc morphologic characteristics. This is critical for the visual process, since disc flattening maximizes the area available for photoreception and allows efficient renewal of the rod outer segments. The in-frame deletion found in the present family is located in the large second intradiscal loop and might affect the disc flattening through the mechanism we described, precipitating the retinopathy in this family. It has been described that peripherin/RDS molecules carrying the P216L and C165Y mutations are unable to flatten membrane vesicles, implicating such mutations as the primary cause of RP. On the contrary, the R172W peripherin/RDS mutation, associated with macular dystrophy and also located in the second intradiscal loop, has no effect on the expression, dimerization, glycosylation, and subunit assembly of the peripherin/RDS molecule. This could mean that R172W peripherin/RDS–ROM-1 complexes can form in vivo and that a relatively subtle change in their structure undermines their function in cone, but not rod, photoreceptors. These findings are consistent with the fact that only a subset of the retinal photoreceptors (cones) appears to be affected. Further analysis of the asparagine deletion in the peripherin/RDS gene should allow elucidation of the structural defect in the protein.

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