Clinical Findings in a Multigeneration Family With Autosomal Dominant Central Areolar Choroidal Dystrophy Associated With an Arg195Leu Mutation in the Peripherin/RDS Gene

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Objective: To characterize clinical findings associated with a mutation in codon 195 (Arg195Leu) of the peripherin/RDS gene in a large multigeneration family of European decent.

Methods: Sixteen members from 2 generations underwent ophthalmologic examination, including best-corrected visual acuity, examination of the anterior segments, and inspection of the ocular fundus after pharmacologic mydriasis. All affected family members underwent Farnsworth Panel-D15 color testing. Five selected family members with early stages of the disease underwent multifocal electroretinography. Full-field electroretinography was performed in 2 family members with more advanced fundus changes. Finally, former patients’ records and fundus images were analyzed to determine the course of the disease in affected individuals.

Results: Nine family members in 2 generations were diagnosed as having autosomal dominant central areolar choroidal dystrophy. The family demonstrated an age-dependent increase of central granular fundus abnormalities with progressive development of geographic atrophy. Interindividual phenotypic variability was apparent and ranged from predominantly drusenlike depositions to single perifoveal pigment clumps. Age of onset of visual disturbances varied between 27 and 48 years. All individuals who manifested signs of disease were found to carry an Arg195Leu mutation in the peripherin/RDS gene.

Conclusions: Age of onset, progression of the disease, and characteristic fundus abnormalities share similarities to previous reports on families with central areolar choroidal dystrophy associated with peripherin/RDS gene mutations in codons 172, 142, and 195, respectively. However, striking variability in individual phenotypic findings and age of onset in our family suggests that additional factors that modify the defined peripherin/RDS gene mutation Arg195Leu likely influence the severity of the disease.

Clinical Relevance: Caution should be advised in predicting the clinical course and severity of the disease based solely on a specific mutation in the peripherin/RDS gene.

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A NUMBER OF MUTATIONS IN the peripherin/RDS gene that affect codons 142, 172, and 195 (Arg172Trp, Arg172Gln, Arg142Trp, and Arg195Leu) are known to cause progressive central areolar choroidal dystrophy (CACD). Early stages of the disease are characterized by a fine, at times barely discernible, mottling of the parafoveal pigment epithelium. Later, progressive macular atrophy leads to a significant decline in the central visual acuity (VA) in most cases.

Several reports on clinical findings focus on differences in age of onset of visual complaints with respect to distinct mutations in the peripherin/RDS gene. So far, a single family with CACD from Japan has been shown to carry an Arg195Leu mutation; 3 affected members of this family were described to develop visual symptoms between the fourth to fifth decades of life. Herein, we report the clinical findings of a large family from Bavaria, Germany, with 9 affected members in 2 generations cosegregating an Arg195Leu mutation in the peripherin/RDS gene.

METHODS

Sixteen members from 2 generations underwent ophthalmologic examination, including best-corrected VA, examination of the anterior segments, and inspection of the ocular fundus after pharmacologic mydriasis. Digital fundus (Funduskamera FF 450 plus; Zeiss, Jena, Germany) and confocal autofluorescence im-
ages (HRA 1; Heidelberg Engineering, Heidelberg, Germany) were obtained in all individuals. Autofluorescence images were recorded according to a standard protocol described previously. All affected family members underwent Farnsworth Panel-D15 color testing. Five selected family members with early stages of the disease underwent multifocal electroretinography (mfERG). This method identifies localized dysfunction of the retina (eg, maculopathies) where the full-field electroretinogram is only mildly affected. The mfERG recording procedures adhered to the corresponding International Society for Clinical Electrophysiology of Vision guidelines. The mfERGs were recorded binocularly with dilated pupils and DTL (Dawson-Trick-Litzkow) electrodes using a VERIS system (version 4.16; Electro-Diagnostic Imaging, Inc, San Mateo, Calif). Full-field electroretinography was performed in 2 family members with more advanced fundus changes. The method was performed in concordance with the International Society for Clinical Electrophysiology of Vision standard protocol. Finally, former patients’ records and fundus images were analyzed to determine the course of the disease in affected individuals.

Mutational analysis of the 3 coding exons of the peripherin/RDS gene, including at least 20 base pairs of intervening sequences representing the splice consensus motifs, was performed with the following primer combinations: exon 1: rds1c.f (5’-CCG GAC TAC ACT TGG CAA G-3’)/rds1b.r (5’-ATA GCT ATC TCC AGC TGT CTG-3’); exon 2: rds2a.f (5’-AGG CCC ATC TTC AGG TCT TCT GAG TG-3’)/rds2b.r (5’-TTG GAT GGT GGT GGG GAG-3’); and exon 3: ex3F (5’-CCA GGG ATT CTC CCA GAT T-3’)/ex3R (5’-AGG TTG GAT GAG GGG GAG AT-3’). Polymerase chain reaction fragments were directly sequenced with the Big-Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Weiterstadt, Germany) and analyzed on an ABI 310 automated sequencer (Applied Biosystems, Weiterstadt, Germany).

RESULTS

The pattern of disease inheritance in 4 generations was consistent with an autosomal dominant trait of CACD in this family (Figure 1). After obtaining informed consent, 9 siblings (III-1 through III-3, III-5, III-6, and III-8 through III-11) (aged 43–61 years) were examined clinically. Five of them revealed signs of the disease. In addition, 7 children (IV-1 through IV-7) aged 14 to 36 years of 2 affected siblings (III-5 and III-6) underwent close ophthalmologic examination. Affected family members reported progressive decrease in central VA, with the onset ranging from age 27 to 48 years. Three individuals (III-5, IV-3, and IV-6) aged 14 to 45 years were not aware of their disease until examination. The age–VA relationship is plotted in Figure 2. None of the affected individuals was aware of problems with night vision or side vision.

Four clinical stages were defined according to the classification established by Piguet et al (Table). Consistent with stage 1, two affected family members with perifoveal pigment mottling and tiny foveal, hard drusenlike deposits; preserved central VA (1.0–1.25); and normal color perception were identified (individuals IV-3 and III-5, aged 14 and 45 years). Perifoveal pigment clumps (Figure 3) revealed high autofluorescence properties indicative of abnormal accumulation of retinal pigment epithelium (RPE) lipofuscin. Adjacent spots of low autofluorescence are suggestive of incipient RPE atrophy (arrows in Figure 4). The images depicted central oval-shaped areas of diffusely increased autofluorescence up to 11.0° eccentricity. This area was not assessable by conventional fundus imaging. The mfERG (Figure 5) showed latencies out of normal limits in rings 1 through 3, indicating photoreceptor dysfunction up to approximately 9.6° eccentricity. Reduction of amplitudes, indicating photoreceptor degeneration, was found in rings 2 and 3.

Two affected family members (IV-6 and IV-7), aged 32 and 30 years, respectively, were classified as having stage 2 disease defined by scattered pigment clumping of the central posterior pole without the presence of focal patches of geographic atrophy (GA). The VA was slightly reduced to 0.8 OU.

Farnsworth Panel-D15 color testing identified specific color disturbances that affected the blue axis. Funduscopy revealed diffuse depigmentation of the central posterior pole, including tiny clumps of pigment and yellow, hard drusenlike deposits. Family member IV-7 had been examined at 25 years of age with VAs of 1.0 OU. During the past 5 years a change...
from a coarse-grained pigmentation of the central fundus to a more dusty aspect was observed. Autofluorescence images exhibited a speckled central oval-shaped area (Figure 6A and B) reaching eccentricities up to 14° (IV-6). Individual tiny spots of high autofluorescence intensities corresponded to larger pigment clumps in the color image. The mfERG disclosed delayed latencies and reduced amplitudes in all rings of both family members.

Their elder sister (IV-4, aged 34 years, stage 3 disease) experienced her first visual symptoms by age 27 years. Six years later she was found to have a severe decrease in central acuity to 0.05 OU. Results from a saturated Farnsworth Panel-D15 color test showed unspec-

<table>
<thead>
<tr>
<th>Stage*/No.</th>
<th>Age, Mean ± SD, y</th>
<th>VA†</th>
<th>Fundus</th>
<th>mFERG</th>
<th>AF</th>
<th>Farneᠩş워 Panel-D15 Color Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>29 ± 22</td>
<td>1.0-1.25 (20/20-20/16)</td>
<td>Perifoveal pigment clumps, single hard drusenlike deposits</td>
<td>Latencies: out of limits (rings 1-3); amplitudes: reduction (rings 2-3)</td>
<td>Focal spots of high AF (pigment clumps) and diffuse high AF up 11° eccentricity</td>
<td>Normal</td>
</tr>
<tr>
<td>2/2</td>
<td>31 ± 1</td>
<td>0.8 (20/25)</td>
<td>RPE mottling and hard drusenlike deposits up to 14° eccentricity</td>
<td>Latencies: out of limits (rings 1-5); amplitudes: reduction (rings 1-5)</td>
<td>Speckled AF up to 14° eccentricity</td>
<td>Tritanopic deficiency</td>
</tr>
<tr>
<td>3/1</td>
<td>34</td>
<td>0.05 (20/400)</td>
<td>Focal GA, speckled hyperpigmentation, hard drusenlike deposits</td>
<td>Latencies and amplitudes out of limits (mFERG); normal scotopic, subnormal photopic ERG</td>
<td>Speckled AF up to 14° eccentricity, solitary spot of GA</td>
<td>Unspecific dyschromatopsia</td>
</tr>
<tr>
<td>4/4</td>
<td>56 ± 3.9</td>
<td>CF-0.1 (CF-20/200)</td>
<td>Advanced GA, diffuse hyperpigmentation, hard drusenlike plaques</td>
<td>Scotopic ERG within the lower normal limits, photopic ERG markedly reduced</td>
<td>Large areas of GA, surrounded by speckled AF, extending beyond vessel arcades and nasal side optic disc</td>
<td>Unspecific dyschromatopsia</td>
</tr>
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Abbreviations: AF, autofluorescence; CF, counting fingers; ERG, electroretinogram; GA, geographic atrophy; mFERG, multifocal electroretinogram; RPE, retinal pigment epithelium; VA, visual acuity.

*Staging according to Piguet et al.4
†Range of visual acuity (Snellen equivalent) in both eyes.
cific dyschromatopsia with no preference of axis in both eyes. Autofluorescence images depicted characteristic speckling within the central oval area similar to the pattern observed in her younger siblings. In addition, the left eye revealed an isolated patch of sharply demarcated chorioretinal atrophy (Figure 6C). The mFERG revealed latencies outside normal limits in all rings whereas amplitudes were extinguished in rings 1 through 4 and markedly reduced in ring 5. Full-field electroretinography displayed a normal scotopic response whereas photopic responses were near the lower limit of normal.

The elder 4 siblings of generation 3 (III-1 through III-3 and III-6, aged 52-61 years) presented with markedly reduced central acuity (range, counting fingers to 0.1) and advanced GA consistent with stage 4. The eldest sibling (III-6, aged 61 years) was first seen at the age of 37 years when he experienced a significant loss in VA to 0.05 OD and 0.1 OS. He had not been aware of visual symptoms before that time. His eldest sister (III-1) underwent examination at the age of 43 years with a bilateral VA of 0.4. She reported gradual visual deterioration during the past 15 years, down to 0.05 in the right and counting fingers in her left eye at the age of 57 years. The 2 youngest sisters (aged 52 and 54 years) realized severe decline in VA (defined as \( \leq 0.1 \)) by the age of 48 years (III-2) and 32 years (III-3),

Figure 4. Autofluorescence images of individual IV-3, aged 14 years (A), and individual III-5, aged 45 years (B), depict central oval areas of diffusely increased autofluorescence up to 11.0° eccentricity. This area is not assessable by conventional fundus imaging. Speckled autofluorescence structures form a ringlike configuration in correspondence to funduscopic pigment clumps. Note the tiny dark spots of incipient retinal pigment epithelial atrophy next to high autofluorescence clumping (white arrows).
traces as derived from our database of 50 healthy individuals. The mfERG latency of each ring average were evaluated by fitting template mfERG the retina. preserved amplitude, indicating a beginning dysfunction in the foveal part of rings 1 through 3. In the central ring, the latency delay is accompanied by a indicated by the gray background (dark blue ±1 SD, light blue ±2 SDs) in of individual III-5 (right eye) exhibits latencies above normal limits as contained hexagonal stimulus fields of identical eccentricity. Amplitude and electroretinogram (mfERG) traces were averaged across 5 rings that for individuals III-1 and III-2, who disclosed more advanced alterations in their left eyes. Peripheral retina was inconspicuous in all affected family members. respectively. Farnsworth Panel-D15 color test findings showed unspecific dyschromatopsia with no preference of axis in all family members with stage 4 disease. Fundus examination depicted multiple areas of sharply demarcated RPE atrophy surrounded by typical tiny scattered or confluent spots of hyperpigmentation and small, hard drusenlike depositions. Family members III-2 and III-3 displayed an overall yellowish fundus, including myriads of tiny, hard drusenlike deposits, partly forming confluent plaques. Older family members showed fundus changes to extend beyond the vessel arcades and the nasal side of the optic disc. The pattern of the macular lesion, shape, and size of the GA depicted striking symmetry between both eyes except for individuals III-1 and III-2, who disclosed more advanced alterations in their left eyes. Peripheral retina was inconspicuous in all affected family members. Former fundus images of individual III-6, aged 37 years, and of individual III-1, aged 43 years, confirmed progression of the disease from a pattern of central RPE motting to advanced GA. Autofluorescence images disclosed the characteristic speckled pattern extending beyond the vessel arcades and the optic nerve head by the sixth decade of life. Sharply demarcated dark areas of RPE atrophy preferentially involved the papillomacular area in individual III-3 (Figure 6D-G). Full-field electoretinography revealed markedly reduced photopic responses, whereas scotopic responses reached the border of normal limits (individual III-6, aged 61 years). Peripheral visual fields were unremarkable.

Sequence analysis of the 3 coding exons in the peripherin/RDS gene revealed a heterozygous point mutation (nucleotide 584 G>T) in codon 195, resulting in an arginine to leucine change at this position in all 9 affected family members analyzed. Conversely, 7 unaffected relatives in generations III and IV did not harbor the Arg195Leu mutation. Thus, the molecular findings show strict cosegregation of the Arg195Leu mutation with the clinical phenotype of CACD in the family.

The clinical features associated with the Arg195Leu mutation in the peripherin/RDS gene in our family can be categorized as a progressive macular dystrophy with relative preservation of peripheral retinal function. There is a marked similarity to patients carrying the codon 172 (Arg172Trp) and codon 142 (Arg142Trp) mutation with regard to the characteristic RPE motting within a central oval macular configuration and the progression toward GA with age. The appearance of drusenlike deposits as observed in individuals III-2 and III-3 in this family has also been noted in some members of the Dutch families with the Arg142Trp mutation and in patients with the Arg172Trp mutation originating from the Zermatt area of Switzerland.

Consistency in the age of onset and progression of the disease has been reported in families segregating the Arg172Trp mutation in the peripherin/RDS gene. For example, 11 individuals of 11 ancestrally related British families were found to exhibit first visual symptoms by the age of 30 years, and a severe decrease in central acuity (=20/200) was reported by the late 40s and early 50s in these families. Similarly, 24 affected members of the Swiss family with the same mutation had initial visual symptoms in their 30s (stage 2; VA, 0.8-1.0; mean±SD age, 33±3 years) and decreases in VA below 0.1 by their 40s to 50s. Comparably, severe visual impairment (VA ≤20/200) in 7 members of a Swedish family segregating the Arg172Trp mutation was noted in their fourth to fifth decades of life. Affected members of the Swedish family were found to develop a degeneration of more peripheral parts of the retina with advancing age, suggesting additional involvement of rods late in the disease. Age of onset of first visual symptoms was also reported in 30 affected family members of 7 Dutch families that harbored the Arg142Trp

Figure 5. For amplitude and latency analysis, the multifocal electoretinogram (mfERG) traces were averaged across 5 rings that contained hexagonal stimulus fields of identical eccentricity. Amplitude and latency of each ring average were evaluated by fitting template mfERG traces as derived from our database of 50 healthy individuals. The mfERG of individual III-5 (right eye) exhibits latencies above normal limits as indicated by the gray background (dark blue ±1 SD, light blue ±2 SDs) in rings 1 through 3. In the central ring, the latency delay is accompanied by a preserved amplitude, indicating a beginning dysfunction in the foveal part of the retina.

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Figure 6. Age-dependent variability in pattern of fundus autofluorescence: left eyes of 7 individuals affected by central areolar choroidal dystrophy stages 2 through 4. Individuals IV-7 (A), IV-6 (B) (stage 2 disease), and IV-4 (C) (stage 3 disease) show an abnormal speckled pattern of autofluorescence that reveals enlargement with age and encloses a single patch of geographic atrophy in individual IV-4 (C). There was a range of autofluorescence variability within individuals disclosing central areolar choroidal dystrophy stage 4: III-3 (D), III-2 (E), III-1 (F), and III-6 (G). The characteristic speckled pattern extends beyond the vessel arcades and the optic disc with more advanced age. Sharply demarcated dark areas indicate loss of lipofuscin-loaded retinal pigment epithelial cells. Note that the size of geographic atrophy is not necessarily linked to age.
mutation. In contrast to individuals affected by the Arg172Trp mutation, onset of visual symptoms in the Dutch families occurred later in life; affected family members experienced visual deterioration in their mid 40s, and disabling decrease of vision was noted by their 60s. Finally, 3 Japanese family members segregating the Arg195Leu mutation were reported to manifest first visual disturbances between the fourth and fifth decades of life. The time of severe visual loss was not assessable in this relatively small family. The authors suggested that the clinical course of CACD due to the Arg195Leu mutation may be less severe than the one associated with Arg172Trp.

In contrast to the previous reports, our family revealed a striking variability both in the age of onset of visual impairment and in the age at which there was severe visual loss. Onset of first visual symptoms occurred between the ages of 27 and 48 years; one affected member did not complain about visual problems at the age of 45 years. Five family members experienced a severe decrease in central VA to 0.1 or less between the ages of 33 and 52 years. In contrast to the Swedish family, function of rods remained preserved up to the age of 61 years.

The peripherin/RDS gene appears to play an essential role in the assembly, orientation, and structural stability of outer segment discs and to account for an increased turnover of instable membranous segments. The encoded glycoprotein has been localized to the rim region of the disc membranes of both rods and cones. The functional basis of why some mutations of this gene account for predominantly rod and others for mainly cone dysfunction has been widely discussed. Interactions with other gene products, as previously shown, for example, with ROM1 and peripherin/RDS mutations in retinitis pigmentosa, have been proposed as a possible explanation. However, no such associations have so far been reported to occur in peripherin/RDS-related CACD. Recently, a transgenic mouse model with cone-dominated macular dystrophy carrying the Arg172Trp mutation in the peripherin/RDS gene has been reported. The authors speculated that the preferential damaging effect of this mutation on cones may be accounted for by differences in the cone outer segment structure, which may render cones more susceptible to conformational changes than rods. On the other hand, this hypothesis does not explain the relatively late involvement of foveal cones in the course of the disorder in contrast to the early alteration of perifoveal cone photoreceptors. Outer segments of perifoveal cones may be more susceptible to alterations in stabilizing peripherin/RDS molecules than foveal cones. Blue cones may play a role, since they are mostly absent from the foveal center, are most numerous at 0.3° to 0.5° from the foveal center, and are markedly different from red and green cones in their anatomical structure and cellular function. The ring of high blue cone density corresponds to the perifoveal region, where the disease is initially observed in our family. Two of 4 young family members had blue cone deficit. The involvement of blue cones, however, is not supported by the facts that they are relatively sparse (compared with red and green cones) and that the disease spreads into neighboring areas that affect other types of photoreceptors. Finally, we cannot rule out the presence of thus far unknown protective factors for foveal photoreceptors or segment stability factors at the site of the fovea, which could account for the preservation of central acuity up to the age of 45 years, such as in family member III.

In summary, we observed a striking variability in age of onset and macular phenotype in our relatively large family with an Arg195Leu mutation in the peripherin/RDS gene. Also, a single mutation in this gene has been reportedly noted to cause various phenotypic features, including retinitis pigmentosa, fundus flavimaculatus, and pattern dystrophy in affected members of a single family. Thus, a wide phenotypic range associated with an identical mutation in the peripherin/RDS gene is not uncommon. Further investigations may identify whether additional genetic factors may exert an influence on individual phenotype expression and/or whether environmental influences are responsible for the wide range of pathologic changes.

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REFERENCES


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