Clinical Features of Codon 172 RDS Macular Dystrophy

Similar Phenotype in 12 Families

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Objective: To report the phenotype associated with the codon 172 RDS (gene for retinal degeneration slow) mutation in 11 separate families with an arginine-to-tryptophan substitution with common ancestry, and 1 family with an arginine-to-glutamine transition.

Patients: Screening for RDS gene mutations was performed in 400 subjects with autosomal dominant retinal degeneration. Twelve families were identified with a mutation in codon 172. Haplotype analysis was performed. Full ophthalmic evaluation was performed, including electrophysiologic and psychophysical investigation and imaging of autofluorescence using confocal laser scanning ophthalmoscopy.

Results: Haplotype analysis demonstrated that the 11 families were ancestrally related. All 12 families showed a common phenotype of macular dysfunction, with the deficit increasing with age. Abnormally high autofluorescence predated loss of visual acuity or visual field changes. Pattern electroretinographic (PERG) findings were affected early in disease. There was high intrafamilial and interfamilial consistency of phenotype.

Conclusion: These families demonstrate a striking conformity of symptoms and signs.

Clinical Relevance: In the codon 172 RDS mutation, unlike disease resulting from other RDS mutations, prediction of approximate age of onset and progression of visual deficit is possible. This should assist diagnosis and counseling.
SUBJECTS AND METHODS

SUBJECTS

This research was performed in accord with the Declaration of Helsinki and was approved by the Hospital Ethics Committee of Moorfields Eye Hospital, London, England. Four hundred subjects with dominantly inherited retinal degenerations (170 with macular dystrophies and 230 with RP or cone-rod dystrophy phenotypes) underwent screening for mutations in the RDS gene. From this heterogeneous group, 12 families were identified with a codon 172 RDS mutation. For comparison, 100 healthy subjects underwent screening for codon 172 mutations; none were identified. All 12 families with a codon 172 RDS mutation and more than 95% of the 400 autosomal dominant retinal degeneration and comparison groups were white Europeans. To represent different generations from each of the 12 families, at least 1 member (and preferably 2 members) were contacted and enrolled in the study. Informed consent for clinical and molecular genetic assessment was obtained from all subjects involved in the study.

Nineteen subjects with a codon 172 RDS mutation were recruited (Table). Pedigrees of 11 of the 12 families are shown in Figure 1 (the pedigree of family L has been published previously), and the Table shows the clinical characteristics of these families. Clinical information regarding family L also has been published previously, as have details on families A, D, and G. MUTATION SCREENING, HETERO DupLEX ELECTROPHORESIS, AND DIRECT GENOMIC SEQUENCING

Peripheral blood samples (10 mL) were obtained from each subject, and genomic DNA was extracted using a genomic DNA blood extraction kit (Nucleon 2; Scotlab Ltd, Strathclyde, Scotland). The DNA samples were screened for mutations in the RDS gene by amplifying the complete coding region of RDS with polymerase chain reaction, using primers and conditions as previously published. We also screened for retinal outer segment membrane protein 1 (ROM1). The amplified exons were analyzed using electrophoresis on mutation detection enhancement heteroduplex gels (FMC BioProducts, Rockland, Me) run at 180 V overnight on a discontinuous gel electrophoresis system (Hoeffer 600S apparatus; Pharmacia, Cambridge, England). Products of polymerase chain reaction amplification of the gene then were sequenced using a commercially available kit (ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Sequencing Kit; Perkin Elmer Corp, Norwalk, Conn) and analyzed on an automated sequencer (ABI 373; Perkin Elmer Corp). Haplotypic analysis was performed as described elsewhere.

CLINICAL AND FUNCTIONAL INVESTIGATIONS

Phenotypic characterization included a full ophthalmic history and detailed fundus examination. In addition, fundus photography, confocal laser scanning ophthalmoscopy (cLSO), and psychophysical and electrophysiologic evaluations were performed. In 4 subjects, review of the findings of more than 5 years of detailed electrophysiologic and psychophysical evaluation was possible.

Electrophysiologic Assessment

Electrophysiologic assessment was undertaken on 17 patients. Electroretinography (ERG) was performed using standard techniques. Although developed before the intro-

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**Table: Summary of Clinical Data**

<table>
<thead>
<tr>
<th>Family Members</th>
<th>Visual Acuity</th>
<th>Original Diagnosis</th>
<th>30-Hz Cone Flicker Response</th>
<th>Rod ERG</th>
<th>Standard Flash</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Age at Onset of Symptoms, y)</td>
<td>EOG</td>
<td>PERG</td>
<td>Impl, Amp</td>
<td>N</td>
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<tr>
<td>A-II-2</td>
<td>50</td>
<td>20/80</td>
<td>20/80</td>
<td>RP (30)</td>
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<tr>
<td>A-IV-1</td>
<td>20</td>
<td>30/30</td>
<td>20/30</td>
<td>Asymptomatic</td>
<td>N</td>
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<tr>
<td>B-IV-1</td>
<td>47</td>
<td>20/120</td>
<td>20/120</td>
<td>RP (25)</td>
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<td>C-III-4</td>
<td>46</td>
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<td>20/20</td>
<td>20/20</td>
<td>GACD (27)</td>
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</tr>
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</table>

*Family letters are given in Figure 1 pedigrees; roman numerals refer to generation, and arabic numerals, to position in pedigree. EOG indicates electro-oculogram; PERG, pattern electroretinogram (ERG); RP, retinitis pigmentosa; NP, not performed; N, normal; Amp, amplitude; down arrow, decreased; CF, counting fingers; CACD, central areolar choroidal dystrophy; Impl, implicit time; and up arrow, increased.
duction of guidelines for standard electrophysiology by the International Society for Clinical Electrophysiology of Vision, these comprehensive protocols incorporated recordings similar to those that subsequently were recommended (rod ERG, bright white flash mixed rod-cone ERG, and 30-Hz cone-derived flicker ERG). Pattern ERG (PERG) was performed according to standard guidelines.21

Psychophysical Tests
Static threshold perimetry in the dark- and light-adapted states was performed using a modified Humphrey field analyzer (Allergan Humphrey, Hertfordshire, England).

Photopic Humphrey Visual Fields. These were performed using the standard protocol.22,23

Dark-adapted Visual Fields. The pupil was dilated with 2.5% phenylephrine hydrochloride and 1% cyclopentolate hydrochloride, and the patient underwent dark adaptation for 45 minutes. The Humphrey field analyzer was modified for use in dark-adapted conditions.22,23 An infrared source illuminated the bowl, and an infrared monitor (Phillips, Eindhoven, the Netherlands) was used to detect eye movements. Fields were recorded using central 30-2 and peripheral 30/60-2 programs and macula full-threshold tests. The target size corresponded to Goldmann size V for peripheral testing and to Goldmann size III for macular programs. Each program was performed with a red (predominant wavelength, 650 nm) and blue (predominant wavelength, 450 nm) filter in the stimulus beam.

Dark Adaptometry. Results of the dark-adapted blue central 30-2 field testing were reviewed to determine the most informative locations of dark-adapted visual sensitivity. Two test locations were chosen at 3°, 5°, and 9°, 10° eccentricity, although in a few cases sensitivity losses were too extreme for useful measurements at the 3° location. The Humphrey field analyzer was used for dark adaptometry, and it was controlled by a custom program on a computer (PS/2 model 50; IBM Corp, Armonk, NY) as described elsewhere.24,25 Fully dark-adapted rod thresholds were measured before exposure to the adapting light at the 2 coordinates with the blue filter in the stimulus beam.

Fine Matrix Mapping. A modified Humphrey field analyzer was used to present flashes of blue stimuli under scotopic conditions with 4 red light-emitting diodes in a small diamond configuration used for fixation. One hundred positions on a square 10 × 10 matrix over a 9° × 9° test field were presented with a Humphrey size III target at 1° intervals. Subsequent processing of data produced 3-dimensional representation of rod thresholds, with highest elevations from baseline representing greatest loss of retinal sensitivity.26

cLSO Images. Images of the central macular region were obtained using a prototype confocal laser scanning ophthalmoscope (SM 30-4024; donated by Zeiss, Oberkochen, Germany). An argon laser (488 nm, 250 µW) was used for illumination. Reflective imaging was undertaken using the Zeiss cLSO with a 20° field and an argon blue laser with depth plane adjusted to maximize the visibility of the fundus features. A wide band pass filter with a cutoff at 521 nm inserted in front of the detector was used to detect autofluorescence and was recorded using published techniques.27

Tations were found in this study. In addition, 100 control subjects underwent screening for codon 172 mutations; no mutations were identified. The typical phenotype was seen in all those family members with the mutation, and no abnormality was identified in those without the mutation.

DESCRIPTION OF PATIENTS

Twelve individuals with a codon 172 mutation were identified, and despite the 2 different substitutions, the individuals had familial disease of autosomal dominant inheritance that showed marked clinical homogeneity with intrafamilial and interfamilial consistency. Pedigrees of 11 of the 12 families are shown in Figure 1. Detailed clinical and functional data on 19 subjects (≥1 representative member from each family) is given in the Table. Clinical information on family L is included.16 The ages of the 19 affected members examined ranged from 10 to 69 years.

PHENOTYPE RANGE

The patients had been referred with a wide variety of diagnoses, including cone dystrophy, central areolar choroidal dystrophy (CADD), Sorsby fundus dystrophy, and RP (Table). The usual reason for seeking medical atten-
physiologic findings are also indistinguishable from those of the patients with the Arg172Trp mutation.

Two subjects (D-III-1 and H-III-1) showed a very different phenotype.

Electrophysiologic Findings

Eighteen subjects underwent electrodiagnostic investigation. Subject J-II-3 was unable to complete the tests and only underwent PERG. The findings in all patients, other than subjects D-II-1 and H-III-1, were similar; electro-oculogram (EOG) light rise was normal; rod ERG showed no abnormalities; and photopic and flicker ERG showed reduced cone amplitudes in 4 of the 18 subjects undergoing testing (including the 2 atypical subjects D-III-1 and H-III-1, but normal implicit times were seen in all subjects apart from these 2). The PERGs usually were extinguished, but in 3 young asymptomatic patients
(C-IV-3, E-IV-2, and H-IV-1), they were merely reduced (Figure 6).

The findings in subjects D-III-1 and H-III-1 markedly differed from those in the other patients. The EOG light rise was reduced in H-III-1 and abolished in D-III-1, and the rod response was markedly subnormal in both. In subject D-III-1, the bright white flash response showed a "negative" waveform with relative preservation of the (subnormal) a wave and more pronounced loss of the b wave (Figure 6); 30-Hz flicker ERG showed subnormal findings but normal implicit time; and PERGs were unrecordable. The findings suggest generalized rod and cone dysfunction. The absent PERGs confirm severe macular dysfunction.

Psychophysical Findings

Data were available on 17 of the 19 subjects, but only 7 subjects underwent dark-adaptation testing. Rod and cone sensitivities were severely abnormal, in many cases amounting to more than 3 logarithm units, but usually restricted to the central macula. In addition, the areas of decreased sensitivity were seen to enlarge over time and with increasing age; late in the disease, field loss was noted in some individuals to exceed 30°.

In the 3 young asymptomatic individuals, early losses of central sensitivity in photopic and scotopic conditions were evident, but there was no marked widespread elevation of threshold. Subject I-IV-1, aged 35 years, showed distinct central cone loss of more than 30 dB localized to the central 10° for photopic conditions (Figure 4, C). This is also seen in the rod function, measured under scotopic conditions, as

Figure 2. Age vs visual acuity in the right eye in retinal degeneration slow (RDS) codon 172 mutation (n = 19). CF indicates counting fingers.

Figure 3. A, Atrophic area in central macula of subject E-III-2, aged 47 years. B, Autofluorescence imaging showing the confluent loss of autofluorescence compatible with atrophy. C, Increased autofluorescence at macula in his daughter, subject E-IV-2, aged 10 years. D, Fluorescein angiogram of his aunt, aged 80 years, who did not undergo screening in our study, demonstrating extensive macular and peripapillary atrophy.
illustrated in subject G-IV-5, in whom sensitivities ranged from normal to losses of approximately 15 dB, again predominantly affecting the central macula (Figure 7, A). Two years later, these sensitivity losses had become more marked and extensive, ranging up to 20 dB (Figure 7, B). Similar results were found for the scotopic red stimulus in the extent of the losses and the progression of the loss during the 2-year period (Figure 7, C and D).

Rod sensitivities in the macular region, measured by fine matrix mapping, were mildly abnormal for the 18-year-old subject C-IV-3 (Figure 8, A), with pro-
nounced and more widespread loss of macular rod function in his mother, subject C-III-4 (aged 46 years), in the eye with 20/30 vision (Figure 8, B).

Recovery of dark adaptation showed severe abnormalities in cone function, which were more pronounced in the central macular region. Figure 9 shows an example of severe cone dysfunction coexisting with nearly normal rod function for final dark-adapted sensitivity at the more peripheral location (9°, 9°) but delayed recovery of rod function in the more central location (3°, 3°). Subjects D-III-1 and H-III-1 showed more pronounced abnormalities of rod dark adaptation than the other subjects.

Autofluorescence Findings

Autofluorescence was noted to be highly elevated in those younger than 30 years. The youngest subject (aged 10 years) had abnormally high levels at the fovea (Figure 3, C) long before any other phenotypic expression of disease appeared. Similarly, in subject C-IV-3, aged 18 years, high levels of autofluorescence at the macula preceded abnormality seen using ophthalmoscopy, and he had irregular autofluorescence at the fovea (Figure 5, A-D). A comparison of these images with those of his mother’s fundus autofluorescence shows that she, while 28 years older, has areas of marked irregularity of autofluores-
cence, indicating areas of photoreceptor outer segment loss on a background of increased autofluorescence. The atrophy is seen clearly on the reflectance imaging, and the areas of speckled autofluorescence representing incipient atrophy are seen more clearly in her left eye. These areas are far more extensive in the mother than in the son (Figure 5, E-H). The development of increasing levels of autofluorescence, followed by decreasing levels as atrophy supervenes, is well illustrated by the members of this family. The typical finding of the autofluorescence appearance at the macula becoming increasingly speckled with the onset of atrophy, and subsequently well defined and then confluent, is consistent in this family. High levels of autofluorescence persisted beyond the area of atrophy (Figures 3, 4, and 5). The autofluorescence images are shown for comparison with fundus photographs and fluorescein angiograms (Figures 3, 4, and 5).

**COMMENT**

Although these 12 families were referred with a variety of diagnoses, including cone dystrophy, macular dystrophy, CACD, Sorsby fundus dystrophy, RP, and cone-rod dystrophy, it became clear that a distinctive common phenotype with typical age-associated features was
present in 17 of the 19 members examined. This is a fully penetrant autosomal dominant macular dystrophy. Although symptoms and ophthalmoscopic changes become clinically apparent in the third decade of life, increased autofluorescence and electrophysiologic and psychophysical abnormalities are detectable in the second decade. This is initially a central macular dystrophy with extension occurring up to the temporal, superior, and inferior arcades by 50 years of age, with peripapillary extension with increasing age, although this may occur earlier in some individuals. Autofluorescence, fine matrix mapping, and PERGs all show abnormalities, even when patients are asymptomatic with a normal fundus appearance. Photopic and scotopic fields mirrored central losses, indicating anatomical loss of cones and rods corresponding to the areas of decreased autofluorescence noted on cLSO. The areas of decreased sensitivity enlarged over time.

The RDS codon 172 mutation has been previously described in several families of English, Japanese, Swiss, and Spanish origin. The Spanish and Japanese families show different electrophysiologic findings, which may result from different testing conditions. The phenotype in the Swiss family supports our conclusions. Reduced

rescence would be consistent with an excessive accumulation of lipofuscin in the retinal pigment epithelium. It has been suggested that the mutation may give rise to physical instability of the outer segment and increased membrane turnover. The consequent increase of retinal pigment epithelial metabolic activity would account for accumulation of intracellular material. This clinical feature appears to be common to many disorders associated with mutations in the RDS gene. At later stages of disease, these areas of previously high autofluorescence developed into areas of abnormally reduced autofluorescence consistent with cell death.

In these 12 families, a remarkably consistent macular phenotype was identified in all patients, except 2 in whom global rod and cone involvement were identified. Other consistent phenotypes have been described in relation to the RDS gene, including CACD in association with a mutation in codon 142. Previous publications have attempted to explain the phenotypic variability in RDS mutations. It is difficult to explain why the codon 172 mutation leads to a consistent macular phenotype, when a point mutation elsewhere in the same loop (153/154) leads to a much more severe and variable phenotype.

Although RDS is expressed in rods and cones, it is possible that an amino acid substitution at the codon 172 position is not critical for binding to ROM1, unlike a substitution at the 153/154 position, in which variable and severe degeneration occur. Experimental evidence supports this suggestion. Therefore, although phenotypic consistency in this group may be explained by the simple nature of a transition mutation and its position, an alternative explanation for the 153/154 mutation phenotype may be an undetected digenic change. This possibility has been described previously in RP with ROM1 and RDS mutations. The digenic theory may also account for the more severe and early disease seen in 2 individuals in our study, although no mutation was found in ROM1. As new genes are identified and screened in these individuals, sequence changes in other genes may be shown to modulate the phenotype.

The codon 172 RDS mutation usually is associated with a distinctive phenotype, and this is clearly helpful in diagnosis and counseling.

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