An Early-Onset Autosomal Dominant Macular Dystrophy (MCDR3) Resembling North Carolina Macular Dystrophy Maps to Chromosome 5

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PURPOSE. To characterize the phenotype of an autosomal dominant macular dystrophy and identify the chromosomal locus.

METHODS. Thirteen members of a four-generation, nonconsanguineous British family were examined clinically and also underwent automated perimetry, fundus fluorescein angiography, and fundus autofluorescence imaging. After informed consent was obtained, blood samples were taken for DNA extraction, and genetic linkage analysis was performed.

RESULTS. The retinal changes have an early age of onset and are confined to the macular region. The macular abnormalities vary from mild retinal pigment epithelium (RPE) pigmented change to atrophy. Drusen-like deposits are present to various degrees and are characteristic of the phenotype. Subretinal neovascular membrane (SRNVM) is an established complication. Genetic linkage analysis established linkage to chromosome 5, region p13.1-p13.33 with a maximum LOD score of 3.61 at a recombination fraction of 0.00 for marker D5S630. The locus for this autosomal dominant macular dystrophy lies between flanking markers D5S1981 and D5S2031.

CONCLUSIONS. A novel locus has been identified for early-onset autosomal dominant macular dystrophy on chromosome 5. (Invest Ophthalmol Vis Sci. 2003;44:2178–2183) DOI: 10.1167/iovs.02-1094

The central receptor or macular dystrophies comprise a heterogeneous group of disorders in which there is variable visual loss associated with bilateral symmetrical macular abnormalities. Autosomal dominant, autosomal recessive, X-linked recessive, and mitochondrial inheritance have all been reported, and there is considerable heterogeneity even within these subtypes.1,2 A number of different genes that cause macular dystrophy have been identified, including ABCA4,3 ELOVL4,4 perilipin/RDS,5,7 VMD2,8 TIMP3,9 XLRS1,10 and EFEMP1.11 and the study of gene expression and function of the encoded proteins has improved our understanding of disease pathogenesis.

Although in most macular dystrophies the abnormal fundoscopic appearance is confined to the macular region, there is usually electrophysiologic, psychophysical, or histologic evidence of widespread photoreceptor and retinal pigment epithelial dysfunction.1,2 This is consistent with the fact that most genes identified as causing macular dystrophy are expressed throughout the retina, rather than solely in the macular region.2 There are, however, a few disorders in which the disease appears to be confined to the macular region. For example, in North Carolina macular dystrophy, results of psychophysical and electrophysiologic tests demonstrate that normal peripheral retinal function is retained.12

In the present study we report the clinical and electrophysiologic findings in a family with a dominantly inherited macular dystrophy, resembling North Carolina macular dystrophy (MCDR1). We have excluded the MCDR1 locus on chromosome 6 and have demonstrated linkage in this family to a novel locus on chromosome 5.

PATIENTS AND METHODS

Thirteen members of a four-generation, nonconsanguineous British family with an autosomal dominant macular dystrophy were assessed (Fig. 1). We were also able to review the clinical notes of three additional affected family members who were not available for examination. After informed consent was obtained, a medical history was taken and a full ophthalmic examination performed. Blood samples were taken for DNA extraction and linkage analysis was performed. The protocol of the study adhered to the provisions of the Declaration of Helsinki.

Clinical Assessment

Color vision testing was performed using Hardy, Rand, Rittler (HRR) plates (American Optical Company, New York, NY). Affected subjects also underwent Humphrey automated perimetry (Zeiss-Humphrey Systems; Dublin, CA), color fundus photography, and fundus autofluorescence (AF) imaging, using a confocal scanning laser ophthalmoscope (cSLO; Heidelberg Retina Angiograph; Heidelberg Engineering, Heidelberg, Germany). Electrodiagnostic assessment included an electro-oculogram (EOG) and a flash electroretinogram (ERG) according to the protocols recommended by the International Society for Clinical Electrophysiology of Vision.13,14 Two subjects underwent fundus fluorescein angiography (FFA).

Individuals were diagnosed as affected on the basis of the presence of macular abnormality and, in most cases, associated decreased visual acuity.
Linkage Analysis Method

Genotyping. Genotyping was achieved by using markers from a commercial mapping set (ABI MD-10 and HD-5 Linkage Mapping Sets, ver. 2.0; Applied Biosystems, Foster City, CA). These sets allow approximately 10- and 5-cM resolution of the human genome, respectively, and consist of fluorescently labeled PCR primer pairs for 800 highly polymorphic dinucleotide-repeat microsatellite markers chosen from the Genethon human linkage map (http://www.genethon.fr; provided in the public domain by the French Association against Myopathies, Evry, France).15–17

PCR reactions were performed for each marker individually in a 5-µL reaction volume, containing 25 ng DNA, 15 mM Tris-HCl (pH 8.0), 50 mM KCl, 2.5 mM MgCl₂, 250 µM each dNTP, 1.25 pmol each primer, and 0.25 U Taq polymerase (AmpliTaq Gold; Applied Biosystems). Reactions were performed on a thermocycler (model 9600; Perkin Elmer, Wellesley, MA) with a standard thermocycling profile for all markers. This consisted of an initial denaturation of 12 minutes immediately followed by 10 cycles of 95°C for 15 seconds, 55°C for 15 seconds, and 72°C for 30 seconds, and then by 20 cycles of 89°C for 15 seconds, 55°C for 15 seconds, and 72°C for 30 seconds, with a single final extension step of 72°C for 10 minutes.

PCR products for selected sets of markers were pooled, diluted, and denatured in formamide and size-fractionated with an automated gene analyzer (model 9600; Perkin Elmer, Wellesley, MA) with a standard thermocycling profile for all markers. This consisted of an initial denaturation of 12 minutes immediately followed by 10 cycles of 95°C for 15 seconds, 55°C for 15 seconds, and 72°C for 30 seconds, and then by 20 cycles of 89°C for 15 seconds, 55°C for 15 seconds, and 72°C for 30 seconds, with a single final extension step of 72°C for 10 minutes.

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RESULTS

The disorder is present in a four-generation British family, as shown in Figure 1. Patients III:10, III:11, III:15, IV:2, IV:3, and IV:4, were assessed and were found to be asymptomatic, with normal findings in a clinical examination and were designated as unaffected. The affected individuals showed a range of macular appearances varying from multiple drusen-like deposits to focal atrophy and pigmentation (Table 1, Fig. 2). EOG and flash ERG was normal in all affected individuals.

Markers known to demonstrate linkage to cone–rod dystrophies (CORD) and to MCDRI were examined in the first instance. No significant linkage was found at the following chromosome regions: CORD6 on 17p,20 CORD7 on 6q,21 and MCDRI on 11q.22

Linkage Analysis. Subjects were classified as affected, unaffected or of unknown status according to their clinical status. Linkage analysis was performed with standard lod score methods. Two-point lod scores were calculated using the MLINK program of the LINKAGE (version 5.1) package (http://www.hgmp.mrc.ac.uk; provided in the public domain by the Human Genome Mapping Project Resources Center, Cambridge, UK).23 A fully penetrant dominant model with a disease allele frequency of 0.0001 was assumed. Marker allele frequencies were assumed to occur at equal frequencies, because population allele frequencies were not available.

FIGURE 1. Four-generation pedigree of a family with autosomal dominant macular dystrophy. Individuals are numbered according to generation (indicated) and position in each generation, numbering from left to right. The alleles present for each of the 10 microsatellite markers on 5p are shown. The minimal disease region for each affected individual is boxed. Disease haplotype is defined by recombination events in individuals III:9 and IV:2. Unaffected individual IV:2 has two recombination events in the chromosome inherited from his affected mother which, along with the haplotype information for individual III:9 establishes D5S1981 and D5S2031 as the markers flanking the disease region.
### Table 1. Clinical Findings

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>OD</th>
<th>OS</th>
<th>Visual Acuity</th>
<th>Fundus</th>
<th>AF Imaging</th>
<th>EOG</th>
<th>ERG</th>
<th>Visual Fields</th>
<th>Color Vision</th>
</tr>
</thead>
<tbody>
<tr>
<td>I:2*</td>
<td>F</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td>——</td>
<td>——</td>
<td>——</td>
<td>——</td>
<td>Bilateral SRNVM</td>
<td>Bilateral protan, deutan and tritan defects</td>
</tr>
<tr>
<td>II:1*</td>
<td>F</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
<td>——</td>
<td>N N</td>
<td>Bilateral central scotomata</td>
<td>Bilateral protan, deutan and tritan defects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II:4</td>
<td>F</td>
<td>50</td>
<td>6/18</td>
<td>6/60</td>
<td>Bilateral macular scarring associated with subretinal fibrosis and surrounding fine drusen-like deposits. FFA was consistent with previous bilateral SRNVM</td>
<td>Increased AF at both maculae, corresponding to the drusen-like deposits</td>
<td>——</td>
<td>——</td>
<td>——</td>
<td>——</td>
<td></td>
</tr>
<tr>
<td>II:8</td>
<td>F</td>
<td>60</td>
<td>6/9</td>
<td>6/9</td>
<td>Bilateral fine macular drusen-like deposits with areas of RPE atrophy and pigmentation</td>
<td>——</td>
<td>N N N N</td>
<td>Bilateral protan, deutan and tritan defects</td>
<td>Bilateral protan, deutan and tritan defects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III:2*</td>
<td>F</td>
<td>37</td>
<td>6/60</td>
<td>6/12</td>
<td>Right: SRNVM Left: Macular drusen-like deposits with areas of RPE atrophy and pigmentation (Fig. 2C)</td>
<td>——</td>
<td>——</td>
<td>——</td>
<td>——</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III:4</td>
<td>F</td>
<td>36</td>
<td>6/5</td>
<td>6/5</td>
<td>Bilateral fine macular drusen-like deposits with areas of RPE atrophy and pigmentation</td>
<td>——</td>
<td>N N N N</td>
<td>Bilateral protan and deutan defects</td>
<td>Bilateral protan and deutan defects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III:9</td>
<td>M</td>
<td>16</td>
<td>6/60</td>
<td>6/60</td>
<td>Bilateral extensive macular atrophy and pigmentation</td>
<td>——</td>
<td>N N</td>
<td>Bilateral central scotomata</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III:12</td>
<td>F</td>
<td>27</td>
<td>6/6</td>
<td>6/6</td>
<td>Bilateral fine macular drusen-like deposits with areas of RPE atrophy and pigmentation</td>
<td>——</td>
<td>N N N N</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III:14</td>
<td>M</td>
<td>37</td>
<td>6/6</td>
<td>6/6</td>
<td>Bilateral fine macular drusen-like deposits with associated RPE atrophy and pigmentation (Fig. 2D)</td>
<td>Increased AF at both maculae, corresponding to the drusen-like deposits (Fig. 2E)</td>
<td>N N N N</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV:5</td>
<td>M</td>
<td>11</td>
<td>6/18</td>
<td>6/12</td>
<td>Bilateral macular RPE atrophy and pigment clumping, with surrounding drusen-like deposits (Fig. 2A)</td>
<td>Bilateral decreased AF centrally with a surrounding ring of relative increased AF (Fig. 2B)</td>
<td>——</td>
<td>N N ——</td>
<td>Bilateral protan, deutan and tritan defects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV:6</td>
<td>F</td>
<td>9</td>
<td>6/4</td>
<td>6/4</td>
<td>Bilateral macular atrophy and pigmentation</td>
<td>——</td>
<td>N N N N</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N. Normal.

* Patients I:2, II:1, and III:2 were not available for study, but examination of clinical records, retinal photography, and fluorescein angiography was performed. II:4 had SRNVM before the age of 17 years in her right eye. II:1 had bilateral disciform maculopathy before the age of 20 years.

### Table 2. Lod Scores between Autosomal Dominant Macular Dystrophy and Microsatellite Markers on 5p

<table>
<thead>
<tr>
<th>Marker</th>
<th>0.00</th>
<th>0.05</th>
<th>0.10</th>
<th>0.20</th>
<th>0.30</th>
<th>0.40</th>
<th>Distance from Telomere (Mb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D5S1981</td>
<td>1.69</td>
<td>1.58</td>
<td>1.44</td>
<td>1.19</td>
<td>0.73</td>
<td>0.32</td>
<td>1.3</td>
</tr>
<tr>
<td>D5S417</td>
<td>1.93</td>
<td>1.79</td>
<td>1.63</td>
<td>1.30</td>
<td>0.95</td>
<td>0.50</td>
<td>3.2</td>
</tr>
<tr>
<td>D5S2088</td>
<td>3.55</td>
<td>3.24</td>
<td>2.93</td>
<td>2.25</td>
<td>1.51</td>
<td>0.72</td>
<td>4.4</td>
</tr>
<tr>
<td>D5S406</td>
<td>3.52</td>
<td>3.23</td>
<td>2.92</td>
<td>2.27</td>
<td>1.54</td>
<td>0.75</td>
<td>5.1</td>
</tr>
<tr>
<td>D5S416</td>
<td>3.44</td>
<td>3.14</td>
<td>2.82</td>
<td>2.15</td>
<td>1.41</td>
<td>0.65</td>
<td>16.7</td>
</tr>
<tr>
<td>D5S2031/H9258</td>
<td>——</td>
<td>1.05</td>
<td>1.34</td>
<td>1.52</td>
<td>0.98</td>
<td>0.49</td>
<td>20.8</td>
</tr>
<tr>
<td>D5S419/H9258</td>
<td>——</td>
<td>——</td>
<td>1.01</td>
<td>0.34</td>
<td>0.39</td>
<td>0.20</td>
<td>26.3</td>
</tr>
<tr>
<td>D5S426</td>
<td>——</td>
<td>——</td>
<td>0.86</td>
<td>0.18</td>
<td>0.25</td>
<td>0.29</td>
<td>34.7</td>
</tr>
</tbody>
</table>
CORD8 on 1q, GCAP on 6p, STGD1 on 1p, STGD3 on 6q, STGD4 on 4p, and MCDR1 on 6q. In total approximately 50% of the genome was screened involving genotyping of 195 markers before significant linkage was established to chromosome 5p13.1-15.33, with a maximum lod score of 3.61 at a recombination fraction of 0.00, for marker D5S630 (Table 2, Fig. 1) in a family in which the maximum two-point lod score would be 4.21. Critical recombination events observed in individuals III:9 and IV:2 define the locus for this autosomal dominant macular dystrophy as between flanking markers D5S1981 and D5S2031. This represents a genetic distance of 35 cM and a physical distance of 19.5 Mb. The distance between the flanking markers D5S1981 and D5S2031 and their nearest nonrecombinant markers (D5S417 and D5S416) is 1.9

**Figure 2.** (A) Patient IV:5: fundus photograph showing bilateral macular RPE atrophy and pigment clumping, with surrounding drusen-like deposits and (B) fundus AF imaging showing bilateral decreased AF centrally with a surrounding ring of relative increased AF. (C) Patient III:2: fundus photograph showing acute SRNVM at the right macula and RPE atrophy with fine drusen-like deposits at the left macula. (D) Patient III:14: fundus photograph showing bilateral typical fine macular drusen-like deposits with associated RPE atrophy and pigmentation and (E) fundus AF imaging showing increased AF at both maculae, which corresponds to the drusen-like deposits. In addition, slight decreased AF is seen centrally in both eyes.
and 4.1 Mb, respectively. The chromosome inherited from the affected parent of individual IV:2 has two recombination events separated by 35 cM and enables exclusion of the region telomeric to D5S1981 from the disease interval.

In view of the phenotypic similarity of this disorder to the North Carolina macular dystrophy (MCDR1) that maps to 6q16, we have examined this region in our family in greater detail. Multipoint linkage analysis has previously indicated that the MCDR1 gene is in the interval between D6S249 and D6S1671. The lod scores for these markers in our family were both −5 at θ = 0.0. In addition, haplotype analyses of these and additional markers adjacent to the MCDR1 region confirm that the disease in our family does not map to this region of the genome.

**DISCUSSION**

The autosomal dominant macular dystrophy in this family has an unusual phenotype. It is characterized by an early age of onset and is generally associated with relatively good vision, despite significant macular abnormalities evident on ophthalmoscopy. Mild color vision abnormalities are variably present in affected individuals. The macular appearance varies from multiple drusen-like deposits to focal atrophy and pigmentation. With the exception of one young individual (IV:5) who showed an increase in retinal pigmentation and drusen-like deposits over a 5-year period, there was no evidence of change in macular appearance over time. Furthermore, although the retinal phenotype varied widely within the family, the severity of the changes was unrelated to age. Two individuals had angiographic evidence of SRNVM, and in an additional two, the macular appearances were consistent with this diagnosis. In accordance with the convention established by the nomenclature used for North Carolina macular dystrophy phenotype (MCDR1), we have termed this disorder MCDR3 (MC, macular; D, dystrophy; R, retinal).

Visual field loss in family members was demonstrated only over the central macular lesions. The normal EOG and ERG in all affected individuals suggests that the dystrophy is localized to the macula and that there is no widespread involvement of retinal photoreceptors.

 Autofluorescence (AF) imaging is a relatively new technique to visualize the RPE, taking advantage of its intrinsic fluorescence derived from lipofuscin. Affected subjects showed decreased AF corresponding to areas of atrophy seen ophthalmoscopically (Fig. 2). In addition, concentric perifoveal areas of increased AF were evident and were found to correspond to the AF images in the RPE within the atrophic macular lesion. All three grades of lesion were seen in our pedigree. The electrophysiological changes detected in our family are also consistent with those reported in MCDR1. The only significant differences in the two phenotypes is that, in our family, color vision testing was abnormal in the majority of affected individuals, and there was evidence of disease progression, albeit in a single case.

Linkage studies have mapped MCDR1 to a locus on chromosome 6, region q16. To date, MCDR1 has been described in various countries and no evidence of genetic heterogeneity has been reported. In the family reported herein we have excluded linkage to the MCDR1 locus and have obtained significant linkage to the short arm of chromosome 5. This region contains three members of the cadherin gene family, cadherin-6, -10, and -12, which are all highly expressed in the brain. Retinal expression has yet to be examined. These represent potential candidates, especially in light of the recent identification of mutations in cadherin-23 in patients with Usher syndrome type 1D, a condition that includes retinitis pigmentosa. However, the region is large, and many more potential candidate genes within the disease interval remain to be characterized. Identification of the genes responsible for these disorders will help to improve our understanding of the mechanisms underlying macular development and may shed light on the pathogenesis of drusen and SRNVM.

**Acknowledgments**

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**References**


