Age-related Macular Degeneration

Clinical Features in a Large Family and Linkage to Chromosome 1q

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Objectives: To identify the chromosomal location of a disease-causing gene and to describe the clinical characteristics of a large family with age-related macular degeneration (ARMD).

Methods: An ARMD pedigree was identified, and the disease state of family members was documented by stereoscopic fundus photography and was classified using a modified version of the Wisconsin Age-Related Maculopathy Grading System. A genome-wide screen at approximately 6-centimorgan spacing using a DNA-pooling strategy combined with shared-segment analysis was used to identify likely chromosomal regions. The entire family was then screened at each likely locus, and 1 positive locus was refined by screening with markers at an average density of 0.5 centimorgan and subjected to parametric linkage analysis.

Results: In the 10 affected family members, ARMD was manifest by the presence of large, soft, confluent drusen accompanied by varying degrees of retinal pigment epithelial degeneration and/or geographic atrophy. Age-related macular degeneration segregated as an autosomal-dominant trait, with the disease locus mapping to chromosome 1q25-q31 between markers D1S466 and D1S413, with a multipoint lod score of 3.00.

Conclusion: Age-related macular degeneration localized to chromosome 1q25-q31 (gene symbol, ARMD1) as a dominant trait in a large family with a predominantly dry phenotype.

Clinical Relevance: Identification of ARMD genes will facilitate early diagnosis and aid in understanding the molecular pathophysiological mechanisms of ARMD. This knowledge will contribute to the development of preventive and improved treatment strategies.


Age-related macular degeneration (ARMD) is the leading cause of blindness in the United States and other western countries. Approximately 7% of people 75 years and older have progressed to the late stage of this disease. Although certain risk factors have been identified, no interventions have been proven effective in preventing ARMD. Only laser photocoagulation has been established as an effective treatment, but its use is confined to a small proportion of patients with the exudative or dry form of ARMD. There is no treatment for the more common atrophic or dry form of ARMD.

Although the pathogenesis of ARMD remains unknown, there is growing evidence that genetic influences play an important role. Support for this view includes an increased family incidence of ARMD in affected individuals, a strong concordance of ARMD in monozygotic twins, and the results of segregation analysis suggesting the possibility of a single gene effect in a large proportion of patients. Recently, mutations in the Stargardt disease gene (ABCR) at chromosome 1p21 were reported at a higher frequency in patients with ARMD compared with a control population.

In this report, we describe a large family in which ARMD segregates as an autosomal-dominant trait that maps to chromosome 1q.

RESULTS

CLINICAL FINDINGS

Of the participating 21 family members at risk for ARMD (Figure 1), 10 were affected (Table 1). Blood samples were collected from an affected individual (patient 1009) who subsequently died before undergoing fundus photography. Her eyes, obtained postmortem, demonstrated pigment epithelium, photoreceptor, and choriocapillaris atrophy in the macula (Figure 2, A). These findings are consistent with the clinical diagnosis of geographic atrophy associated with a history of poor vision for several years before death.
PATIENTS AND METHODS

CLINICAL INFORMATION

Twenty-one individuals from a family with ARMD agreed to participate in the study, which was approved by the Institutional Review Board of Oregon Health Sciences University, Portland. For all but 1 individual, stereoscopic fundus photographs of the macula of both eyes were obtained. The remaining individual died before we obtained photographs, but the eyes were retrieved for histopathologic analysis. Clinical information, including visual acuity, was obtained for all affected individuals. In most instances, this information was obtained from the individual's local ophthalmologist or optometrist, and fundus photographs were taken at a local retina facility.

CLASSIFICATION OF ARMD

Stereoscopic fundus photographs of the macula were classified using a modified version of the Wisconsin Age-Related Maculopathy Grading System. Each eye was classified independently by 3 ophthalmologists (M.L.K., A.E., and Robert C. Watzke, MD) without knowledge of the patient's genotype. Disagreements were adjudicated among the 3 graders. The patient's classification was based on the eye with the more advanced ARMD.

The following classification system was used: Group 1, definite ARMD is characterized by exudative ARMD (choroidal neovascularization, pigment epithelial detachment, or disciform macular scar) or geographic atrophy (area, >175 µm). Group 2, probable ARMD is characterized by the presence of large drusen (>125 µm) within 3000 µm of the fovea, with total cumulative drusen area exceeding 393 744 µm² (approximating the area within a 700-µm-diameter circle). Nongeographic pigment atrophy and focal hyperpigmentation may or may not be present, and the features present in group 1 are absent. Group 3, probably no ARMD is characterized by large drusen present (>125 µm), but the cumulative area is less in extent than in group 2. Group 3 is characterized by the absence of exudative ARMD, geographic or nongeographic pigment atrophy, and focal hyperpigmentation. Group 4, no ARMD is characterized by no large drusen (>125 µm), geographic or nongeographic pigment atrophy, focal hyperpigmentation, or exudative maculopathy. Group 5, uncertain is characterized by absence of features seen in groups 1 and 2 and presence of any of the following: extensive small (<63 µm) or intermediate (63-125 µm) drusen, nongeographic pigment atrophy, or focal hyperpigmentation, or factors preventing reliable classification, such as media opacities, concomitant retinal disease, or pigment epithelial disturbance, with or without large drusen (>125 µm).

The definition of group 2 was based on findings (Ronald Klein, MD, e-mail communication, November 18, 1997) demonstrating a 19-fold increase in the probability of developing late ARMD (group 1) for eyes with large drusen (>125 µm) and a minimum cumulative drusen area of 393 744 µm² (equivalent to an approximately 700-µm-diameter circle) located within 3000 µm of the fovea. Eyes classified as group 5, uncertain, are those in which accompanying conditions preclude accurate classification of ARMD or eyes that contain possible risk factors for the later development of late ARMD, including extensive small and intermediate drusen (<125 µm) or pigment epithelial abnormalities.

For purposes of linkage analysis, groups 1 and 2 were classified as affected and groups 3 and 4 were classified as unaffected. Those categorized as group 5 were classified as unknowns in the linkage analysis.

GENOTYPING

Genomic DNA was extracted from the blood of family members using a kit from Epicentre Technologies (Madison, Wis) according to their directions and was quantitated spectrophotometrically. Three pools of DNA were created. The first pool consisted of equimolar amounts of DNA from 8 affected family members (patients 1009, 2101, 1011, 1015, 1007, 2705, 1017, and 2005). The second pool was similarly derived from 4 unaffected family members (patients 2009, 2502, 2011, and 2103). These pools represented all affected and unaffected individuals who had been ascertainment as the pooling was initiated. A third pool consisted of equimolar amounts of DNA from 8 unrelated individuals. Three affected cousins from the family with ARMD (patients 2101, 2705, and 2005) were also individually genotyped. Six hundred fifteen polymorphic microsatellite repeat markers at an average density of 6 centimorgans (cM) were used to genotype DNA from the 3 selected individuals and the 3 DNA pools. Five hundred seventy-two of these markers yielded clearly identifiable banding patterns. After the initial pooled genome-wide screen, markers from areas most suggestive of shared chromosomal segments were used to genotype all potentially informative family members. One region showing positive linkage was then mapped at an average marker density of 0.5 cM. Conditions for polymerase chain reaction, gel electrophoresis, blotting, and staining have been described previously.

LINKAGE ANALYSIS

Two-point linkage between the disease locus and each microsatellite marker locus was tested by the parametric lod score method using a computer program (MLINK). Frequencies of the disease allele and of the normal alleles were assumed to be 0.001 and 0.999, respectively. Based on the pedigree in which there were 3 generations of affected individuals and male-to-male transmission, an autosomal-dominant mode of inheritance was assumed. Family members were placed in 1 of 5 age-related liability classes. Age-dependent penetrances for these classes were set to 0.001 (<30 years), 0.01 (30-54 years), 0.09 (55-64 years), 0.42 (65-74 years), and 0.95 (≥75 years). These values were determined from a set of 20 similar families with ARMD that we identified and are comparable with the prevalence observations reported in 3 studies based on approximately 15 000 individuals. Allele frequencies reported by the Centre d’Etudes du Polymorphisme Humain (CEPH), Paris, France (http://www.ceph.fr/cephdb/), were used except for markers D1S191, D1S202, D1S461, D1S492, and D1S512, which were measured in a set of 92 unrelated individuals. Markers for multipoint linkage analysis, whose order was statistically supported, were identified using genotypes from CEPH pedigrees and the computer programs CRI-MAP and MultiMap. Multipoint linkage analysis was conducted using the VITESSE algorithm.

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For the remaining 9 affected members, the diagnosis was established by stereoscopic fundus photography. Four of these patients had geographic atrophy (Figure 2, B-E) and were classified as group 1, definite ARMD. One individual (patient 2005) had extensive large drusen and pigment changes and a photographically documented history of previous serous pigment detachment in both eyes and was thus also classified as group 1 (Figure 2, G). The remaining 4 individuals had extensive large, soft, confluent drusen in both eyes and were classified as group 2, probable ARMD (Figure 2, F and H-J). One of these individuals, a 70-year-old man (patient 2101), was thought by his local ophthalmologist to have had a pigment detachment in 1 eye. However, recent photographs (Figure 2, J) and results of fluorescein angiography were not conclusive for the presence of pigment detachment. Ten individuals were classified as unaffected (Figure 3, A and B); 7 of these patients were categorized as group 4, no ARMD (patients 2502, 2009, 3506, 2103, 2401, 2405, and 2001), and 3 were classified as group 3, probably no ARMD (patients 2114, 2403, and 2501). One individual (patient 2105) was classified as group 5, uncertain, because of the presence of pigmentary changes in the posterior pole of both eyes but without meeting the criteria for definite or probable ARMD.

The initial classification by the 3 independent graders was in agreement for 15 of the 20 individuals classified by fundus photography. For the remaining 5 individuals, initial classification by 1 of the graders differed
from that of the other 2 with regard to groups 3, 4, or 5. These differences were adjudicated to yield a final classification. For no individual family member was there any grader disagreement between affected (groups 1 and 2) and unaffected (groups 3 and 4) status.

Visual acuity for the 10 affected patients ranged from 20/20 to counting fingers. Age at diagnosis of ARMD ranged from 54 to 77 years (average, 65 years). For the 8 affected patients with visual symptoms, onset began between age 52 and 75 years (average, 67 years).

GENOTYPING

A high-density, genome-wide screen was performed on 6 DNA samples, 3 pools (unrelated, unaffected, and affected), and 3 affected individuals. The 3 individuals were cousins and were the 3 members of the family with ARMD who were separated by the greatest number of meioses. Of 572 microsatellite markers analyzed, 194 (33.9%) demonstrated a shared allele among the 3 cousins who were genotyped. In addition, each marker was scored for the preponderance of the shared allele in the affected pool and for its scarcity in the unaffected pools.

The shared segments were reduced to 46 possible regions containing 2 or more adjacent markers. These regions were ranked and placed in 1 of 7 categories based on the scores of the DNA pools for their respective markers. Members of the pedigree who were used for pooling plus an additional 5 family members were then individually genotyped for linkage analysis, with markers in all 9 of the regions placed in the top category. Markers in 8 of the 9 regions yielded significantly negative lod scores (data not shown). Only 1 region contained markers (D1S238 and D1S2757) that produced lod scores greater than 1.00. This was also the only region in the genome-wide screen in which an allele exhibited at least 50% of the total band intensity in the affected pool and was absent from the unaffected family pool in flanking markers (Figure 4).

LINKAGE ANALYSIS

Twenty-one individuals from the expanded pedigree were then genotyped at high density with 46 microsatellite markers from the Genethon map that encompassed the region containing markers D1S238 and D1S2757. The most informative markers, D1S202 and D1S461, yielded maximum pairwise lod scores of 2.98 and 2.96, respectively, at q = 0.00 with the ARMD locus in this pedigree (Table 2). In total, 21 contiguous microsatellite markers between D1S466 and D1S2840 yielded positive lod scores (data not shown).
Mulitpoint linkage analysis was performed between the disease gene and markers in this region on chromosome 1 using the program VITESSE.\(^3^0\) A subset of the markers between D1S466 and D1S413, whose local relative order was statistically well supported, was chosen for the analysis. Overlapping 5-point analyses were used to span the map of 9 markers. The maximum lod score was 2.98 (Figure 5), which is the same lod score obtained with D1S202 in the 2-point analysis. Multipoint analysis with a phenocopy rate of 0.01 without age dependence, with a disease gene frequency of 0.038, or with both assumptions together yielded lod scores of 2.00, 2.35, and 1.70, respectively. A separate analysis using the age-dependent penetrance calculated from this single family rather than from 20 similar families yielded a multipoint lod score of 3.20. Based on recombinations in 2 individuals (patients 1007 and 1009), the interval of shared haplotypes was approximately 9 cM, defined by markers D1S240 and D1S412 (Figure 1).

Using 3 markers from the Stargardt disease gene locus (ABCR), we evaluated the possibility that this gene could be linked to ARMD in this family. All 3 markers across the locus yielded lod scores of less than −2.00, thus excluding this locus as a major gene effect in this family (Table 3).

**Figure 5.** Multipoint parametric lod scores obtained with the subset of 9 markers by overlapping 5-point analyses using VITESSE.\(^3^0\) Plot shows lod scores plotted against relative chromosomal map position.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Relative Map Position, cM</th>
<th>Marker</th>
<th>Relative Map Position, cM</th>
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</thead>
<tbody>
<tr>
<td>D1S466</td>
<td>0.0</td>
<td>D1S240</td>
<td>0.7</td>
</tr>
<tr>
<td>D1S191</td>
<td>1.3</td>
<td>D1S202</td>
<td>2.2</td>
</tr>
<tr>
<td>D1S238</td>
<td>3.0</td>
<td>D1S461</td>
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</tr>
<tr>
<td>D1S2625</td>
<td>6.6</td>
<td>D1S412</td>
<td>9.6</td>
</tr>
<tr>
<td>D1S413</td>
<td>12.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Two-Point lod Scores Between ARMD and Markers at the ABCR Locus\(^*\)**

<table>
<thead>
<tr>
<th>Marker</th>
<th>lod Score‡</th>
</tr>
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<tbody>
<tr>
<td>D1S424</td>
<td>−3.30</td>
</tr>
<tr>
<td>D1S2804</td>
<td>−2.50</td>
</tr>
<tr>
<td>D1S256</td>
<td>−3.20</td>
</tr>
</tbody>
</table>

\(^*\)ARMD indicates age-related macular degeneration.

\(\dagger\)lod Scores given at α value.

Identification of ARMD pedigrees of sufficient size to allow direct linkage studies within 1 family has been difficult primarily because of its late age at onset, variable expressivity, and lack of reliable early diagnostic criteria.\(^13^,35-39\) Therefore, genetic investigation of ARMD has focused on nonparametric approaches using sibling pairs or small families\(^26\) or candidate gene approaches based on macular dystrophies with earlier ages at onset.\(^17^,18,37,39-44\)

We identified a large ARMD pedigree that exhibits autosomal-dominant inheritance. Genetic analysis of this family produced significant evidence for linkage of the disease gene to a region of approximately 9 cM on chromosome 1q. The linkage model that we used is similar to that previously proposed by Gorin et al.\(^3^6\)

Diagnostic classification of individuals with ARMD in this family was accomplished by using a scheme based on the well-established Wisconsin Age-Related Maculopathy Grading System.\(^3^6\) Two individuals (patients 2405 and 2403) seem to be nonpenetrant, ie, they exhibit the disease haplotype but not the ARMD phenotype. They were the youngest family members analyzed, 44 and 48 years, and may manifest the disease at a later age. In this family, the average age at diagnosis of ARMD was 65 years, and the earliest age at onset of symptoms was 52 years.

Variability of fundus appearance among affected family members included amount and size of drusen, degree of drusen confluence, extent and severity of pigment epithelial atrophy, and presence of hyperpigmentation. However, there was considerable consistency in the phenotypic expression of ARMD within this family. In the sixth and seventh decades of life, clinical findings were predominantly characterized by the appearance of large, soft confluent drusen. In 1 individual (patient 2005), serous pigment epithelial detachments developed and subsequently spontaneously flattened, leaving nongeographic retinal pigment epithelial atrophy. In the seventh and eighth decades of life, large, confluent drusen with early geographic atrophy were accompanied by the onset of vision loss, whereas the 8th to 10th decades of life were characterized by fading of drusen, advanced geographic atrophy, and more severe central visual impairment. There was no definite evidence of choroidal neovascularization in any affected family members.

This predominantly dry ARMD phenotype is consistent with previous descriptions of geographic atrophy and large drusen.\(^3^5-48\) Geographic atrophy is the most severe manifestation of dry age-related maculopathy, which has been reported to account for approximately 12% of legal blindness from ARMD.\(^1\) In population-based studies, geographic atrophy has been found in approximately one third of eyes with late age-related maculopathy.\(^3^,26,27\)

Age-related macular degeneration is often thought to be inherited as a complex trait, with probable involvement of multiple genes and environmental factors.\(^3^2,35\) However, dominant inheritance has been recognized in some familial cases.\(^10,12,36\) Our findings suggest that at least a subset of ARMD is inherited as an autosomal-dominant trait.

We conclude that the disease-causing gene for ARMD (ARMD1) in this family is located within approximately a 9-cM region on chromosome 1q25-1q31, defined by microsatellite markers D1S240 and D1S412. The localiza-
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REFERENCES