Exclusion of TIMP3 as a Candidate Locus in Age-Related Macular Degeneration

Monica A. De La Paz,* Margaret A. Pericak-Vance,*† Felicia Lennon,† Jonathan L. Haines,§ and Johanna M. Seddon¶

**Purpose.** Age-related macular degeneration (AMD) is a genetically complex disorder. Tissue inhibitor of metalloproteinases-3 (TIMP3) on chromosome 22 has been identified as a gene that is mutated in Sorsby's fundus dystrophy, an autosomal-dominant macular dystrophy that phenotypically resembles AMD. The purpose of this study was to determine whether TIMP3 is a major susceptibility gene for the AMD phenotype.

**Methods.** Thirty-eight multiplex families with AMD were identified in Massachusetts and North Carolina. The macular findings were graded according to a modification of the grading system used in the Age-Related Eye Disease Study, and persons with extensive intermediate drusen, any large drusen, geographic atrophy, or evidence of exudative maculopathy were coded as affected for the purpose of the analysis. Linkage analysis was performed using both model-dependent (lod score) and model-independent (sibpair) methods. For the lod score analysis, both autosomal-dominant as well as recessive low penetrance "affecteds only" analyses were examined. Three markers, D22S280, D22S529, and D22S268, linked tightly and flanking the TIMP3 locus, were chosen for the analysis. Association studies were performed by examining one randomly chosen affected person per family and comparing the patients with AMD with a series of age, gender, and ethnically matched control subjects with no known history of AMD.

**Results.** Lod score analysis excluded linkage in these data for an approximately 10-cm interval surrounding the TIMP3 gene for all models tested. In addition, no significant findings were observed with either the sibpair or the association study.

**Conclusions.** No evidence of linkage or association or both was found between AMD and TIMP3 in these 38 families. These data suggest that although clinically similar, the genetic defect in Sorsby's fundus dystrophy is of a different cause than the majority of the genetic causes of AMD. Invest Ophthalmol Vis Sci. 1997; 38:1060–1065.
TIMP3 in Age-Related Macular Degeneration

TABLE 1. Characteristics of Study Participants

<table>
<thead>
<tr>
<th></th>
<th>Boston</th>
<th>Duke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of families</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>Number of affected sibpairs</td>
<td>54</td>
<td>14</td>
</tr>
<tr>
<td>Number of participants</td>
<td>60</td>
<td>48</td>
</tr>
<tr>
<td>Total number stage 1 or 2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total number stage 3</td>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td>Total number stage 4</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Total number stage 5</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>Mean age (years) of affected persons (±SD)</td>
<td>71.5 ± 10.2</td>
<td></td>
</tr>
</tbody>
</table>

Affected sibpairs were calculated as follows by using all possible combinations of affected individuals in a sibship: Number of sibpairs = $n(n - 1)/2$ where $n$ equals the number of affected siblings within a sibship. For example, a sibship with 3 affected individuals would generate 3 sibpairs. Additional unaffected individuals, including siblings and (rarely) parents, were included when possible. Stage in the more severely affected eye is shown.

Recently, mutations in the gene encoding for the tissue inhibitor of metalloproteinases-3 (TIMP3) have been identified in patients with Sorsby's fundus dystrophy. TIMP3, which maps to 22ql3-qter, is one member of a family of enzymes involved in the regulation of extracellular matrix remodeling. Although the exact role of TIMP3 in the degenerative process is unknown, it has been proposed that impaired activity of this enzyme results in increased activity of the target metalloproteinase and, thus, progressive changes in Bruch's membrane. Bruch's membrane is composed of basement membrane material and extracellular matrix components. The changes in this tissue layer may cause the visually significant complications, including choroidal neovascularization and chorioretinal atrophy.

In view of the phenotypic and histopathologic similarities between Sorsby's fundus dystrophy and AMD, it may be possible that genes involved in the regulation of extracellular matrix turnover are involved in the pathogenesis of AMD. In this report, we have investigated whether the TIMP3 gene plays a major role in the genetic cause of AMD.

METHODS

Family Ascertainment

Families showing evidence of age-related maculopathy were identified in Massachusetts and North Carolina. Probands were identified within the clinic population of the authors (MAD and JMS), who are based within academic institutions (Duke University Medical Center [DUMC]; Massachusetts Eye and Ear Infirmary, Harvard Medical School [MEEI]), or through referral to the academic sites from local ophthalmologists.

Twenty-one families representing 54 affected sibpairs were identified from the Massachusetts region, and 17 families representing 14 affected sibpairs were from North Carolina. The study was conducted in accordance with the rules and regulations at the Institutional Review Board of each institution and with the tenets of the Declaration of Helsinki. Informed consent was obtained after the nature of the study was explained. All participating family members were evaluated either at the academic sites or at local ophthalmologists' offices, or medical records or fundus photographs were obtained from referring ophthalmologists for review, or both. Dilated ophthalmoscopic examination, when performed, included biomicroscopy with a hand-held 90-diopter lens or fundus contact lens and indirect ophthalmoscopy of the macula and periphery. For those persons who resided too distant from the examination site, study examinations were performed according to a standardized protocol, or copies of their medical records from the local ophthalmologist, including fundus photographs when possible, were obtained for review. All fundus photographs were reviewed by the authors (MAD, JMS), and all were considered to be of suitable quality for grading macular findings.

Macular findings were graded by the authors (MAD, JMS) according to a modification (by JMS) of the grading system used in the Age-Related Eye Disease Study, a multicenter study to evaluate the effect of antioxidant supplementation in AMD. Macular examination of an area encompassing a 2-disc diame-
FIGURE 2. Pedigrees showing chromosomal haplotypes between affected sibpairs. Marker order from top to bottom is D22S268, D22S280, and D22S529. Parental haplotypes are inferred and assigned arbitrarily. Haplotype, stage of maculopathy, and age of examination are included. In each family depicted, at least one affected sibpair fails to show a consistent affected haplotype. Stage of maculopathy (right eye, left eye) and age at examination in years are shown for affected persons.

ter radius, centered on the foveal center, was graded for each eye as follows:

- stage 1 = no drusen or small (<63 µm) nonextensive drusen, without retinal pigment epithelium (RPE) abnormalities;
- stage 2 = extensive small drusen or nonextensive intermediate drusen (drusen greater than 63 µm but not greater than 125 µm in size), and/or hyperpigmentation, focal hypopigmentation, pigment clumping, and focal retinal pigment epithelial atrophy not large enough to be considered geographic atrophy (see stage 4);
- stage 3 = extensive intermediate drusen or any large soft drusen (>125 µm in size), including drusenoid retinal pigment epithelial detachments;
- stage 4 = geographic atrophy (area of RPE atrophy with sharp margins, usually with visible chorioidal vessels, minimum diameter 175 µm or approximately 1/2 disc area);
- stage 5 = exudative AMD, including nondrusenoid pigment epithelial detachments, chorioidal neovascularization, serous or hemorrhagic retinal detachments, subretinal or sub-RPE hemorrhage or fibrosis, or photocoagulation scar consistent with treatment of AMD.

A summary of the families and sporadic persons ascertained is listed in Table 1. For purposes of statistical analysis, persons with stage 3 or higher in at least one eye were considered to be affected.

The control subjects for the association studies (N = 79) were ascertained through the clinic populations of DUMC and MEEI (many of whom were spouses of patients with AMD) and were age, gender, and ethnically matched to the patient population with AMD. Examination results of the fundus in each of these persons showed no evidence of age-related maculopathy.

**DNA Analysis**

Blood samples were collected from participants, and DNA was extracted by standard techniques. Genotyping was performed using either a semi-automated fluorescence scanning system (Molecular Dynamics, Duke University, Durham, NC) or silver staining (Massachusetts General Hospital, Boston, MA).

**Statistical Analysis**

Table 1 describes the family data included in the analyses. Because the genetic model for AMD is not known with certainty, linkage analysis was performed using both model-dependent (lod score) and model-independent (sibpair) methods. The computer program, VITESSE, was used in the lod score calculations. For the lod score analysis, both autosomal-dominant and autosomal-recessive low penetrance "affecteds only" models were tested. A low penetrance analysis incorporates phenotypic data on only affected family members, whereas genotypic (marker) data are included on all family members regardless of their clinical status. Low penetrance analysis is a conservative approach when using standard lod score analyses in a complex trait. This approach precludes exclusion of linkage based on normal at-risk persons who actually may carry the disease gene in question. A gene frequency of 0.001 was used for the AMD locus. Analysis using a variety of disease allele estimates, however, did not significantly alter the lod score results (data not shown). The markers (D22S268, D22S280, and D22S529) used in the analysis were chosen because they were linked tightly and flanked the TIMP3 gene on chromosome 22 (Collaborative Human Linkage Center, http://www.cbil.upenn.edu/HGC22.htm). Marker allele frequencies were generated from a series of 100 white unrelated control subjects available through the Center for the Study of Inherited and Neurological Disease (Ftp site: dnadoc.mc.duke.edu in the /pub/ALLELE FREQ directory). Both two-point and multipoint lod scores were calculated. The map used in the multipoint analysis was generated from published sources (Collaborative Human Linkage Center, http://www.cbil.upenn.edu/HGC22.htm). Sibpair analysis was performed using the ASPEX Computer Package. Association studies were performed as described previously. One affected person per family was chosen randomly for the association studies, and the marker allele frequencies for these persons were compared to the set of gender, age, and ethnically matched control subjects with no known history or symptoms of AMD.

Haplotype analysis was performed to identify dis-
cordant chromosomal configurations between affected sibpairs. Chromosomal haplotypes were constructed using the SIMWALK computer program as described previously.

RESULTS

Results of the lod score (z) analysis for both the low penetrance dominant and recessive analysis showed no evidence of linkage to the TIMP3 region of chromosome 22. Figure 1 shows the multipoint location scores (log_{10}m) for the TIMP3 region clearly excluding linkage (z ≤ −2) 5 cm on either side of the TIMP3 gene. Results of the sibpair analysis were not significant (P > 0.3) for all markers tested, indicating no significant excess of allele sharing among the affected sibpairs tested. In addition, there was no evidence of significant association for any of the markers examined (P > 0.5).

Figure 2 depicts 8 of the 38 families included in the analysis. As shown in Figure 2, persons 3 and 5 in family 1 have severe AMD (grade 5) in both eyes and represent an affected sibpair with discordant chromosomal haplotypes for the TIMP3 region. All persons who were diagnosed had unequivocal signs of age-related maculopathy as outlined above. Families 3 through 8 show a similar lack of cosegregation with the TIMP3 chromosomal region. The results of the haplotype analysis confirm the findings of the linkage and association studies showing no involvement of the TIMP3 locus with AMD.

DISCUSSION

Little is known about the exact cause of AMD. In this complex disorder, there is evidence suggesting involvement of genetic factors, including a twofold to threefold higher risk of disease among first-degree relatives of patients with AMD compared with that of relatives of control subjects without AMD. However, the exact role of genetics in disease development and progression is unknown. Sorsby's fundus dystrophy is a phenotypically similar rare hereditary macular dystrophy that shares certain histopathologic features with AMD. Both are associated with changes involving Bruch's membrane, and both are associated with degenerative changes of the macula, including choroidal neovascularization and chorioretinal atrophy. However, age of onset is early in Sorsby's fundus dystrophy with symptoms developing in the third to fourth decade. In this report, we investigated whether mutations in the TIMP3 locus are associated with AMD. We found no evidence for linkage or association between AMD and TIMP3 in our analysis of these 38 families. These data suggest that although clinically similar, the genetic variation found in these two disorders is substantially different.

Difficulties arise in evaluating candidate genes in complex diseases. These factors include heterogeneity, multiple underlying trait loci, misclassification, and potential phenocopies. For this reason, it is impossible to exclude with certainty a candidate gene as a potential susceptibility risk factor. However, rigorous evaluation of the TIMP3 gene in these 38 families using a variety of approaches for evidence of linkage, and based on our system of AMD classification, showed no evidence or suggestion of linkage or association to AMD. Thus, it is highly unlikely that TIMP3 plays a major role in defining genetic susceptibility to AMD based on the data in these 38 representative families. However, we cannot exclude the possibility that a subset of cases could be caused by the TIMP3 locus.

Changes at the level of Bruch's membrane are a prominent feature in AMD. The lack of association of TIMP3 with AMD also does not rule out involvement of the extracellular matrix in the disease process. Matrix metalloproteinases and inhibitors are a large class of enzymes involved in extracellular matrix structure and function. It may be possible that a different component of this enzyme system is involved in AMD. Alternatively, it is possible that more than one gene is involved or there may be an interaction of an environmental or biologic factor with a mutation that leads to disease expression. Clearly, additional research is needed to elucidate more fully the role of genetic factors in this common disorder.

Key Words

age-related macular degeneration, Sorsby's fundus dystrophy, tissue inhibitor of metalloproteinases-3

Acknowledgments

The authors thank the study participants and their families for their involvement in this project. The authors also thank Ruth Heinis (Clinical Coordinator, Duke Medical Center), Peggy Pate, and Helen Harbett (Duke Medical Center) for data entry and Barbara Rosi (Duke Medical Center) for assistance with genotyping. Karen Clements (Clinical Coordinator, MEEI, Harvard Medical School), Desiree Jones (MEEI, Harvard Medical School) for assistance with initiation and design of the age-related macular degeneration family study, and Carole Tucker (MEEI, Harvard Medical School) for assistance with design of standardized clinical data forms and off-site clinical and blood collection protocols, Stacey Lange (data entry) and Arthur Kristiansen (genotyping) of the Molecular Neurogenetics Unit, Massachusetts General Hospital.

References

1. National Advisory Eye Council. Vision Research, a Na-