Estrogen Receptor Alpha and Matrix Metalloproteinase 2 Polymorphisms and Age-related Maculopathy in Older Women

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In this study, the authors sought to determine whether single nucleotide polymorphisms in the estrogen receptor alpha (ESR1) and matrix metalloproteinase 2 (MMP2) genes are associated with age-related maculopathy (ARM) in older women. Subjects comprised a random sample of Caucasian women aged ≥74 years participating in the Study of Osteoporotic Fractures year 10 follow-up (n = 906) in 1997–1998. Fundus photographs were graded for ARM using a modification of the Wisconsin Age-Related Maculopathy Grading System. The prevalences of early ARM and late ARM were 46% and 4%, respectively. The MMP2 rs2287074 single nucleotide polymorphism (G→A) was associated with ARM. The A allele was present in 47%, 43%, and 30% of subjects with no, early, and late ARM, respectively (p = 0.01), and was associated with lower odds of any ARM (for AG vs. GG, odds ratio = 0.80, 95% confidence interval: 0.65, 0.99; for AA vs. GG, odds ratio = 0.64, 95% confidence interval: 0.42, 0.98). An interaction with use of postmenopausal hormone therapy was significant (p = 0.02). The MMP2 rs2287074 A allele may be associated with a lower likelihood of ARM in older Caucasian women, particularly those who have never used hormone therapy. The role of MMP2 rs2287074 in ARM should be further elucidated.

estrogen receptor alpha; macular degeneration; matrix metalloproteinase 2; polymorphism, single nucleotide

Abbreviations: ARM, age-related maculopathy; ESR1, estrogen receptor alpha; MMP2, matrix metalloproteinase 2; PCR, polymerase chain reaction; RPE, retinal pigment epithelium; SNP, single nucleotide polymorphism; SOF, Study of Osteoporotic Fractures.

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Age-related maculopathy (ARM), a disease characterized by progressive loss of central vision, is the leading cause of severe vision loss in persons aged 65 years or older in the developed world (1). It is estimated that over nine million people in the United States have ARM (2), and the numbers are expected to grow, as the population of Americans aged 65 years or older is projected to double between 2000 and 2030 (3). ARM may be classified as either early- or late-stage. Early ARM is characterized by the presence of large drusen and pigmentary abnormalities. Late ARM, or age-related macular degeneration, includes the presence of geographic atrophy and/or choroid neovascularization.

The cause of ARM is unknown; however, research suggests that the etiology is probably multifactorial, due to a combination of environmental and genetic risk factors. Recent research has shown common variants in the complement factor H (4–7), LOC38775 (8, 9), Htra serine peptidase 1 (10, 11), complement component 2 (12), and complement factor B (12) genes to be strongly associated with ARM. Although most of these findings have been independently replicated, these variants do not account for all cases. It is therefore important to study additional candidate genes that might affect disease risk, which may lead to more effective preventive and treatment measures.

Several (but not all) observational studies have shown a protective effect of postmenopausal hormone replacement therapy and/or markers of lifetime endogenous estrogen exposure on ARM (13–22). The effects of estrogen are mediated through estrogen receptors alpha and beta. The genes for both estrogen receptor subtypes (ESR1 and ESR2, respectively) are found on chromosomes 6q25 and 14q, respectively, and both estrogen receptor subtypes are expressed functionally active in the human retinal pigment epithelium (RPE) (23). Among other functions, they regulate the expression of matrix metalloproteinase 2 (MMP2). The MMP2 gene, found on chromosome 16q13, is important for extracellular matrix turnover (23, 24), and it has been hypothesized that extracellular matrix dysregulation is involved in the development of ARM (25). Both in vitro and in vivo studies have shown that estrogen regulates MMP2 expression in the RPE (23, 24, 26), which has been shown to be associated with changes characteristic of ARM (26–31).

A recent cohort study showed the T-A haplotype of estrogen receptor alpha (ESR1) PvuII-XbaI polymorphisms to be associated with greater risk of late ARM (32). To our knowledge, no epidemiologic studies to date have investigated the association of MMP2 polymorphisms with ARM. The MMP2 rs243865 single nucleotide polymorphism (SNP) (C → T) has been well characterized, and the C allele is associated with increased gene expression (33). Therefore, it is of interest to study this SNP with regard to ARM.

Although neither region 16q13 nor region 6q25 has been linked to ARM in previously published genome-wide scans, the evidence suggests a potential association of estrogen exposure with ARM and the lack of ARM association studies in humans involving the ESR1 and MMP2 polymorphisms warranted the present study, in which we sought to determine whether three SNPs in the ESR1 gene (rs2234693 (PvuII), rs9340799 (XbaI), and rs1801132) and two SNPs in the MMP2 gene (rs243865 (−1306) and rs2287074) were associated with ARM in women aged 74 years or older. Study participants had been genotyped previously for these ESR1 and MMP2 SNPs for candidate gene studies of osteoporosis. Thus, data for only these ESR1 and MMP2 SNPs were available for the present analysis.

MATERIALS AND METHODS
Subjects

Subjects consisted of a random sample of participants engaging in the year 10 clinic visit of the Study of Osteoporotic Fractures (SOF), a multicenter prospective study designed to determine risk factors for osteoporotic fracture. SOF participants were recruited in 1986–1988 from community-based listings at four clinical centers in different areas of the United States. There were 9,704 Caucasian women aged 65 years or older enrolled in the SOF. Approximately 7,672 women survived and were eligible for the year 10 follow-up visit in 1997–1998, with 4,820 (63 percent) attending the clinic.

Fundus photographs were taken at year 10 and were graded for a subset of subjects for studies of eye disease and osteoporotic fracture. This subset included 699 subjects who had incurred incident fractures during the previous 5-year interval and 1,123 Caucasians randomly sampled at baseline who attended the year 10 clinic visit without such incident first fractures. This latter group was eligible for the present analysis. Of the 1,123 eligible subjects, fundus photographs were gradable in one or both eyes for 1,065 (95 percent). Of those subjects, 906 had data for at least one of the five ESR1 or MMP2 SNPs. Persons included in the analysis and persons in the random sample who were not included did not differ in terms of baseline demographic or estrogen-related variables.

The SOF protocol was approved by the institutional review board at each institution, and all participants gave written informed consent. The University of California, Los Angeles, Medical Institutional Review Board approved the present study.

Measurement of ARM

Forty-five-degree stereoscopic fundus photographs were graded for ARM by two independent graders in a masked fashion, employing a modification of the Wisconsin Age-Related Maculopathy Grading System (34) used in the Third National Health and Nutrition Examination Survey (35, 36). ARM characteristics were summarized with the six-level graphic atrophy or exudative ARM (level 50 or 60) in at least one eye. Early ARM was defined as the presence of at least one of the following conditions in at least one eye, with or without pigmentary abnormalities (level 30 or 40).

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backed sheets. Following stringent hybridization of the site and immobilized in linear arrays on nylon membrane-oligonucleotide probes were designed for each biallelic and distinguish between the single nucleotide variants, two and a final extension step of 68

60 in either eye; late ARM

primers for each SNP were modified at the 5

probes in linear arrays, as described previously (38). Briefly, specific SNP detection with immobilized oligonucleotide

ace reaction (PCR) amplification followed by allele-

Systems (Alameda, California) using a multiplex polymer-

reaction volume containing 10–50 ng of purified human
genomic DNA. The GeneAmp PCR System 9600 thermal
cycler (PE Applied Biosystems, Foster City, California) was

used with the following thermal cycling profile: an initial
hold of 94°C for 12.5 minutes; then 33 cycles of 96°C for
15 seconds, 60°C for 1 minute, and 72°C for 1.25 minutes;
and a final extension step of 68°C for 5 minutes. To detect
and distinguish between the single nucleotide variants, two
oligonucleotide probes were designed for each biallelic
site and immobilized in linear arrays on nylon membrane-
backed sheets. Following stringent hybridization of the

biotinylated PCR products to the immobilized sequence-
specific probes, allelic variants were distinguished by chro-
mogenic detection using a Profiblot II T24 (Tecan U.S., Inc.,
Research Triangle Park, North Carolina). Linear arrays
were scanned on an Epson Perfection 1670 scanner (Epson
America, Inc., Long Beach, California), and genotypes
were assigned using software designed by Roche Molecular
Systems.

**Potential confounders**

Potential confounders included age at year 10, age at
menopause, surgical menopause, history of postmenopausal
hormone therapy, bone mineral density, smoking history
(ever vs. never), body mass index, hypertension, diabetes
mellitus, alcohol consumption, years of education, and
self-rated health status. Age at menopause, surgical meno-
pause status, and years of education were ascertained
from clinic and self-administered questionnaire completed at the
SOF baseline visit in 1986–1988. History of postmenopausal
hormone therapy was determined from clinic and
self-administered questionnaires completed at baseline, year
6, and year 10. In addition, participants were asked to bring
prescription and nonprescription medications to the clinic
visit for verification of use. Total hip bone mineral density
(g/cm²) was measured at year 10 by dual-energy x-ray

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**TABLE 1. Six-level age-related maculopathy severity scale used in a random sample of subjects participating in the year 10 follow-up of the Study of Osteoporotic Fractures, 1997–1998**

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>No drusen, or hard drusen or small drusen &lt;95 μm in diameter only, regardless of area of involvement, and no pigmentary abnormalities present</td>
</tr>
<tr>
<td>20</td>
<td>Hard drusen or small drusen &lt;95 μm in diameter, regardless of area of involvement, with increased retinal pigment, but no RPE† depigmentation; or soft drusen (≥95 μm) with drusen area &lt;960 μm and no pigmentary abnormalities present</td>
</tr>
<tr>
<td>30</td>
<td>Soft drusen (≥95 μm) with drusen area &lt;960 μm and RPE depigmentation present; or soft drusen with drusen area ≥960 μm with or without increased retinal pigment but and without RPE depigmentation</td>
</tr>
<tr>
<td>40</td>
<td>Soft drusen (≥95 μm) with drusen area ≥960 μm and RPE depigmentation present with or without increased retinal pigment</td>
</tr>
<tr>
<td>50</td>
<td>Geographic atrophy under the fovea</td>
</tr>
<tr>
<td>60</td>
<td>Exudative macular degeneration with or without the presence of geographic atrophy</td>
</tr>
</tbody>
</table>

* No age-related maculopathy (ARM) = level 10 or 20 in both eyes; early ARM = level 30 or 40 in at least one eye, but neither level 50 nor level 60 in either eye; late ARM = level 50 or 60 in at least one eye. 
† RPE, retinal pigment epithelium.

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**TABLE 2. Characteristics of the estrogen receptor alpha (ESR1) and matrix metalloproteinase 2 (MMP2) single nucleotide polymorphisms**

<table>
<thead>
<tr>
<th>Gene and single nucleotide polymorphism</th>
<th>Alternative name(s)</th>
<th>Chromosome</th>
<th>Location</th>
<th>Alleles</th>
<th>Codon</th>
<th>Amino acid substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR1</td>
<td></td>
<td>6q25.1</td>
<td>Intron 1</td>
<td>T/C</td>
<td></td>
<td>Proline → proline</td>
</tr>
<tr>
<td>rs2234693</td>
<td>IVS1 –401, Pvull</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs9340799</td>
<td>IVS1 –354, Xbal</td>
<td></td>
<td></td>
<td>A/G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1801132</td>
<td></td>
<td>16q13</td>
<td>Exon 4</td>
<td>C/G</td>
<td>325</td>
<td>Proline → proline</td>
</tr>
<tr>
<td>MMP2</td>
<td></td>
<td></td>
<td>Promoter</td>
<td>C/T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs243865</td>
<td></td>
<td></td>
<td>Exon 9</td>
<td>A/G</td>
<td>460</td>
<td>Threonine → threonine</td>
</tr>
<tr>
<td>rs2287074</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
absorptiometry using the Hologic QDR 1000 (Hologic, Inc., Bedford, Massachusetts). Body mass index was determined at year 10 by dividing each woman’s weight in kilograms by her height in meters squared, using the subject’s self-reported height at age 25 years because of the loss of height experienced by women with low bone mass. Hypertension and diabetes status were ascertained with the year 10 questionnaire by asking whether the subject had ever been told by a doctor that she had hypertension or diabetes, respectively. Alcohol consumption was defined by the number of alcoholic beverages consumed per week, reported at year 10. Smoking history was determined by asking subjects at baseline whether they were current smokers or had a history of smoking, as well as asking whether they were current smokers at year 10. Self-rated health status was determined at year 10 by asking subjects to rate their health as excellent, good, fair, poor, or very poor in comparison with other persons their own age.

Statistical analysis

Testing of Hardy-Weinberg equilibrium and linkage equilibrium and comparison of allele frequencies between subjects with early, late, and no ARM for each SNP were conducted using MENDEL 6.0 software (39, 40). SAS statistical software, version 9.1 (SAS Institute, Inc., Cary, North Carolina), was used for all other analyses. The gene counting method was used to estimate allele frequencies. Fisher’s exact test of independence was implemented using Mendel 6.0 to test each locus for Hardy-Weinberg equilibrium, as well as to test the hypothesis of linkage equilibrium among the ESR1 loci and MMP2 loci, respectively. Tests of homogeneity of allele frequencies among subjects with no, early, and late ARM were performed with likelihood ratio tests. Differences in the frequency of combinations of alleles among the ESR1 loci and the MMP2 loci, respectively, were also tested with likelihood ratio tests.

Comparisons of baseline variables by ARM status were made with chi-square tests and Wilcoxon rank-sum tests. Chi-square tests and Kruskal-Wallis tests were used to determine whether baseline variables differed among subjects with different genotypes. Because the MMP2 rs2287074 SNP was the only locus with significant differences in allele frequencies for each of the three outcomes, this was the only SNP that was further analyzed in multiple logistic regression analysis to control for potentially confounding factors and to investigate potential interactions. Because we assumed a log-additive model, the odds ratio for the minor allele homozygote relative to the major allele homozygote was equal to the square of the odds ratio for the heterozygote relative to the major allele homozygote. Because there were relatively few late ARM cases (n = 33), the primary outcome was “any ARM.”

Variables selected for inclusion in the multivariable model included those that have previously been shown to be associated with MMP2, estrogen exposure, or ARM in

<table>
<thead>
<tr>
<th>Gene, single nucleotide polymorphism, and allele</th>
<th>No ARM</th>
<th>Early ARM</th>
<th>Late ARM</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2234693</td>
<td>908</td>
<td>802</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>478</td>
<td>420</td>
<td>33</td>
<td>50.0</td>
</tr>
<tr>
<td>C</td>
<td>430</td>
<td>382</td>
<td>33</td>
<td>50.0</td>
</tr>
<tr>
<td>rs9340799</td>
<td>916</td>
<td>796</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>588</td>
<td>510</td>
<td>45</td>
<td>68.2</td>
</tr>
<tr>
<td>G</td>
<td>328</td>
<td>286</td>
<td>21</td>
<td>31.8</td>
</tr>
<tr>
<td>rs1801132</td>
<td>916</td>
<td>794</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>707</td>
<td>623</td>
<td>50</td>
<td>75.8</td>
</tr>
<tr>
<td>G</td>
<td>209</td>
<td>171</td>
<td>16</td>
<td>24.2</td>
</tr>
<tr>
<td>rs243865</td>
<td>912</td>
<td>802</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>678</td>
<td>607</td>
<td>53</td>
<td>80.3</td>
</tr>
<tr>
<td>T</td>
<td>234</td>
<td>195</td>
<td>13</td>
<td>19.7</td>
</tr>
<tr>
<td>rs2287074</td>
<td>912</td>
<td>802</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>485</td>
<td>461</td>
<td>46</td>
<td>69.7</td>
</tr>
<tr>
<td>A</td>
<td>427</td>
<td>461</td>
<td>46</td>
<td>69.7</td>
</tr>
</tbody>
</table>

* Likelihood ratio test of the homogeneity of allele frequencies among subjects with no ARM, early ARM, and late ARM.
† Number of alleles.
the literature and those associated with MMP2 rs2287074 genotypes or ARM in bivariate analyses at a significance level of \( p \leq 0.25 \). The final multivariable model included the following covariates: age, smoking, surgical menopause, age at menopause, history of postmenopausal hormone therapy, bone mineral density, body mass index, alcohol consumption, and hypertension. With the exception of age at year 10 and body mass index, interactions of these covariates with MMP2 rs2287074 genotype were explored because of biologic plausibility, given their associations with MMP2 and/or estrogen exposure and ARM either in this population or in the literature. Interactions were examined by inclusion of interaction terms in a logistic regression model and examination of their effects while controlling for potentially confounding factors.

Given that there were missing values for several predictor variables in the multiple logistic regression model, the main analysis was repeated after missing values were imputed using the multiple imputation method (41) as a means of examining the potential for bias due to systematic patterns in the missing data. Five imputations of each missing value were drawn using a Markov chain Monte Carlo method available in the PROC MI procedure in SAS (42). Each of the five complete data sets was analyzed with logistic regression, and the parameter estimates were then combined using the PROC MIANALYZE procedure in SAS to obtain an overall inference about the model parameters.

Although the number of subjects with late ARM in this sample was small, a secondary analysis was conducted to estimate the effect of MMP2 rs2287074 genotype on late ARM while controlling for only age in a logistic regression model in order to preserve the sample size.

**RESULTS**

Of the 906 subjects with gradable fundus photographs and SNP data for at least one of the five SNPs, 33 (3.6 percent) had late ARM and 415 (45.8 percent) had evidence of early ARM only; however, 14 of the latter subjects had a gradable fundus photograph for only one eye. Thus, these 14 subjects were included in the analysis involving any ARM, whereas they were excluded from the comparison of allele frequencies among subjects with early, late, and no ARM due to the uncertainty of ARM status in the fellow eye.

The hypothesis of Hardy-Weinberg equilibrium could not be rejected for each of the five SNPs. Furthermore, there was evidence of strong linkage disequilibrium among the three ESR1 SNPs (\( p < 0.00001 \)) and the two MMP2 SNPs (\( p < 0.00001 \)), respectively. Allele frequencies among each of the three outcomes for each SNP are shown in table 3.
The only SNP for which there was a significant difference in allele frequencies was MMP2 rs2287074, where the A allele was less prevalent in subjects with late ARM than in those with early or no ARM ($p = 0.01$). When combinations of multilocus alleles were considered for the three ESR1 SNPs and the two MMP2 SNPs, respectively, frequencies of allelic combinations did not differ between subjects with early, late, and no ARM (data not shown).

Because MMP2 rs2287074 was the only SNP for which there was a difference in allele frequencies between the three outcomes, this SNP was analyzed further with logistic regression to control for potential confounders and explore interactions. Baseline characteristics are shown by MMP2 rs2287074 genotype in table 4. Subjects with at least one A allele were significantly less likely to be surgically menopausal than subjects with the GG genotype. There were no significant differences in any other baseline variables among the genotypes.

In multiple logistic regression analyses for any ARM, the complete-case model ($n = 719$) and the multiple-imputation model ($n = 906$) gave similar results. Thus, only results from the complete-case model are presented. After controlling for potential confounders and assuming a log-additive model, there were significantly lower odds of any ARM associated with the A allele. AG heterozygotes had 20 percent lower odds of having any ARM than GG homozygotes (odds ratio = 0.80, 95 percent confidence interval: 0.65, 0.99, and, as expected because of the log-additive allelic effects assumption, the AA homozygotes had approximately 35 percent lower odds (odds ratio = 0.64, 95 percent confidence interval: 0.42, 0.98) (table 5). Furthermore, there was a significant interaction of the MMP2 rs2287074 variant with history of postmenopausal hormone therapy, suggesting lower odds of any ARM associated with the A allele among women who had never used postmenopausal hormone therapy, whereas the effect of the A allele was negated among users of postmenopausal hormone therapy. The A allele was also significantly associated with a lower likelihood of late ARM. There was no significant difference in allele frequencies for either ESR1 polymorphism among persons with no, early, or late ARM.

### DISCUSSION

This study found evidence to support an association between a common variant in the MMP2 gene and ARM. The MMP2 rs2287074 A allele was less prevalent among subjects with late ARM than among those with early or no ARM. After controlling for potential confounders and assuming a log-additive model, the A allele was associated with significantly lower odds of any ARM. Furthermore, there was a significant interaction of the MMP2 rs2287074 variant with history of postmenopausal hormone therapy, suggesting lower odds of any ARM associated with the A allele among women who had never used postmenopausal hormone therapy, whereas the effect of the A allele was negated among users of postmenopausal hormone therapy. The A allele was also significantly associated with a lower likelihood of late ARM. There was no significant difference in allele frequencies for either ESR1 polymorphism among persons with no, early, or late ARM.

### TABLE 5. Association of matrix metalloproteinase 2 (MMP2) rs2287074 genotype with any age-related maculopathy (ARM) in a random sample of subjects participating in the year 10 follow-up of the Study of Osteoporotic Fractures, 1997–1998

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No ARM (n = 369)</th>
<th>Any ARM (n = 380)</th>
<th>Odds ratio*</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>121 29.8</td>
<td>123 35.1</td>
<td>1 Referent</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>175 47.4</td>
<td>166 47.4</td>
<td>0.8</td>
<td>0.65, 0.99</td>
</tr>
<tr>
<td>AA</td>
<td>84 22.8</td>
<td>61 17.4</td>
<td>0.64</td>
<td>0.42, 0.98</td>
</tr>
</tbody>
</table>

* Logistic regression model with adjustment for age, smoking, surgical menopause status, age at menopause, history of postmenopausal hormone therapy, bone mineral density, body mass index, alcohol consumption, and hypertension.

### TABLE 6. Age-adjusted association of matrix metalloproteinase 2 (MMP2) rs2287074 genotype with late age-related maculopathy (ARM) in a random sample of subjects participating in the year 10 follow-up of the Study of Osteoporotic Fractures, 1997–1998

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No ARM (n = 456)</th>
<th>Late ARM (n = 33)</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>129 28.3</td>
<td>14 42.4</td>
<td>1 Referent</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>227 48.8</td>
<td>18 54.6</td>
<td>0.48</td>
<td>0.27, 0.85</td>
</tr>
<tr>
<td>AA</td>
<td>100 21.9</td>
<td>1 3.0</td>
<td>0.23</td>
<td>0.07, 0.72</td>
</tr>
</tbody>
</table>

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MMP2, also known as gelatinase A, is a membrane-bound protein that is important for extracellular matrix turnover (25). It has proteolytic activity against components of the basement membrane, preferentially cleaving collagen types IV, V, VII, and XI and gelatin (33, 43). MMP2 plays a pivotal role in angiogenesis and tumor progression and has been studied widely in cancer research (44, 45). To our knowledge, this is the first epidemiologic study to date that has evaluated the association between MMP2 polymorphisms and ARM; however, several laboratory studies have implicated a role for MMP2 in ARM pathogenesis. Exudative ARM, one of the two advanced forms of ARM, is characterized by choroidal neovascularization, an angiogenic process by which new blood vessels grow from the choroid through Bruch’s membrane and into the subretinal space, often resulting in leakage of blood, scarring, and severe vision loss (46). MMP2 expression has been shown to be up-regulated in experimental choroidal neovascularization in animal models (29) as well as in human donor eyes with ARM in comparison with controls (30). Furthermore, mouse models of experimental choroidal neovascularization have shown a significant decrease in choroidal neovascularization in MMP2-deficient mice compared with wild-type mice (31).

In contrast to the positive association observed between MMP2 expression and choroidal neovascularization, laboratory data paradoxically suggest a potentially protective role for MMP2 in dry ARM. As noted above, estrogen depletion in ovariectomized mice resulted in a loss of MMP2 expression and subsequent changes associated with dry ARM, such as sub-RPE deposit formation and Bruch’s membrane thickening (26). Furthermore, estrogen up-regulates MMP2 expression in vitro (23) and in vivo (26), and RPE cells treated with MMP2 showed a reduction in sub-RPE deposits (27).

Although previous laboratory data and the findings from this study support a potential role for MMP2 in ARM in humans, the specific function of the MMP2 rs2287074 SNP in ARM is unknown. This SNP is synonymous, resulting in the same amino acid (threonine) at codon 460 regardless of the allele present. Although this variant does not appear to create a new splice site or alter an existing one, it has been shown that variation at synonymous sites could result in allele-specific structural differences in mRNA that could affect mRNA structure-dependent mechanisms, which could have biologic consequences (47). Furthermore, the MMP2 rs2287074 SNP may not have a direct effect on ARM but rather may be physically linked to nearby genetic regions that might. Interestingly, this SNP is located in close proximity to a nonsynonymous SNP that results in a glycine-to-serine amino acid substitution at codon 456 (33). The functionality of this SNP is unknown; however, it is believed to be important in the targeting of substrates (33). Alternatively, it is possible that the associations observed are chance findings or a result of confounding by unknown factors. The nature of the association of this polymorphism with ARM needs to be further elucidated.

This study had several limitations. The women participating in the SOF may not be a representative sample of older women in the United States. They are healthier and largely community-dwelling, and in the present analysis they were all Caucasian; therefore, the results of this study may not be generalizable to less healthy, non-community-dwelling, non-White, or male populations. The mean age of participants in this analysis was 80 years. Therefore, the results may not be generalizable to younger postmenopausal women. In addition, because this was an exploratory study, we did not control for multiple comparisons. Thus, it is possible that the significant findings may have been due to chance.

Disease or exposure misclassification is a potential concern; however, the grading of fundus photographs by two independent graders in a masked fashion reduced the likelihood of ARM misclassification. Furthermore, ARM graders were masked to all subjects’ personal data, including ESR1 and MMP2 genotypes. Hence, misclassification would probably have been nondifferential and more likely to bias the relation toward the null. The study may have suffered from selection bias if, for example, a given allele or genotype is associated with mortality or with the loss of otherwise-eligible subjects differentially for ARM cases and noncases. Lastly, confounding by unknown factors or factors we were not able to control for in the analysis could also have resulted in biased estimates of effect.

In summary, in this study, we found the A allele of the MMP2 rs2287074 SNP to be inversely associated with any ARM and late ARM. There was a significant interaction with history of postmenopausal hormone therapy, such that the lower odds of any ARM associated with the A allele among never users were negated among women with a history of using hormone therapy. These results need to be replicated in future studies, particularly with sufficient sample sizes to confirm the association with late ARM and to determine whether the association differs between dry ARM and wet ARM. In addition, whether this SNP plays a direct role in ARM pathogenesis or is merely linked to nearby loci with a functional role in ARM should be further elucidated.

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REFERENCES


