Single-Nucleotide Polymorphisms in the Promoter Region of Matrix Metalloproteinase-1, -2, and -3 in Japanese with High Myopia

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PURPOSE. Polymorphisms in the promoter regions of matrix metalloproteinase (MMP) genes can cause variations in the expression of the MMP genes in the sclera that can lead to a greater susceptibility to axial elongation of the eye. The purpose of this study was to determine whether functional single-nucleotide polymorphisms (SNPs) in the MMP1, -2, and -3 promoter regions are associated with high myopia in the Japanese.

METHODS. Seven hundred twenty-five unrelated Japanese patients with high myopia (axial length of ≥26.0 mm in both eyes, or refractive error ≥−6.0 D in phakic cases) and ≥40 years of age were studied. Five hundred forty-six healthy, unrelated Japanese who were ≥40 years of age served as population-based control subjects. All the subjects were genotyped for the four functional SNPs MMP1 C−1607 1G/2G, MMP2 C−1506T, MMP2 C−735T, and MMP3 −1612 5A/6A with an SNP assay. The distribution of the genotypes in the cases and control subjects was compared by the χ2 test for trend.

RESULTS. No significant difference was detected in the distribution of the four SNPs MMP1 C−1607 1G/2G (P = 0.92), MMP2 C−1506T (P = 0.83), MMP2 C−735T (P = 0.10), and MMP3 −1612 5A/6A (P = 0.62), between the high myopia cases and the general-population controls.

CONCLUSIONS. The four functional SNPs in the MMP1, -2, and -3 promoter regions do not play critical roles in the development of high myopia in the Japanese population. (Invest Ophthalmol Vis Sci. 2010;51:4432–4436) DOI:10.1167/iovs.09-4871

Myopia is one of the most common ocular disorders worldwide, and the most important contributor to myopia, is longer axial lengths.1,2 The subset of myopic eyes with very long axial lengths (≥26 mm) or a high degree of myopic refractive error (≥−6 D) is classified as having high myopia.3 High myopia is associated with various ocular complications4,5 and is one of the major causes of blindness in individuals in developed countries.6-9

Myopia is a complex disease that is caused by genetic and environmental factors. Although several epidemiologic and genetic studies have been performed to determine the possible environmental risk factors and genetic susceptibilities,10 the definitive cause of high myopia has not been identified. The results of recent studies have indicated that the excessive axial elongation in highly myopic eyes is the result of active remodeling of the sclera. Scleral remodeling is a dynamic process that involves continual synthesis and degeneration of the extracellular matrix.11,12

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that degrade extracellular matrix proteins; more than 20 members of the MMP family have been identified in humans.13 Among them, MMP1, -2, -3, -9, and -14 have been shown to be expressed in the human sclera14-17 and are potential participants in scleral remodeling. In fact, earlier animal studies have shown that the level of the mRNA of MMP2 in the tree shrew sclera was temporally up- or downregulated during the development of induced myopia and its recovery.18,19

The expressions of many MMPs are regulated mainly at the transcription level, and single-nucleotide polymorphisms (SNPs) in the promoter region of several MMP genes have been shown to be transcriptional regulators.20 Such functional SNPs have been shown to convey a susceptibility to several diseases (e.g., atherosclerosis21 and cancers).22 For high myopia, variations in the expression of the MMP genes in the sclera due to polymorphisms in the promoter regions can cause variations in scleral remodeling activity, a key factor in axial elongation of the eye. Among the MMP genes that are expressed in human sclera, MMP1, -2, and -3 have been shown to have such functional SNPs in the promoter regions—namely, rs1799750 (MMP1 C−1607 1G/2G),23 rs243865 (MMP2 C−1506T),24 rs2285053 (MMP2 C−735T),25 and rs3025058 (MMP3 −1612 5A/6A).26 In a report of a recent study, an MMP3 −1612 5A/6A polymorphism was significantly associated with common myopia in the
elderly in the United Kingdom. However, this SNP was not significantly associated with high myopia in young Taiwanese individuals. The role played by MMPs in the development of high myopia in the Japanese population has not been examined.

Thus, in a case–control association study, we sought to determine whether four functional SNPs in the promoter region of the \textit{MMP1}, -2, and -3 genes—rs1799750 (\textit{MMP1} −1607 1G/2G), rs243865 (\textit{MMP2} C−1306T), rs2285053 (\textit{MMP2} C−735T), and rs3025058 (\textit{MMP3} −1612 5A/6A)—are associated with high myopia in the Japanese. We showed that these functional SNPs were not significantly associated with high myopia.

**METHODS**

All procedures adhered to the tenets of the Declaration of Helsinki. The Institutional Review Board and the Ethics Committee of each participating institute approved the protocols. All the patients were fully informed of the purpose and procedures of the study, and a written consent was received from each patient.

**Patients and Control Subjects**

Seven hundred twenty-five unrelated Japanese patients with high myopia who were ≥40 years of age were recruited from the Kyoto University Hospital, Tokyo Medical and Dental University Hospital, Fukushima Medical University Hospital, Kobe City Medical Center General Hospital, and Ozaki Eye Hospital. All the patients had a comprehensive ophthalmic examination, including dilated indirect and contact lens slit lamp biomicroscopy, automatic objective refraction, and measurements of the axial length by applanation A-scan ultrasonography or partial coherence interferometry (IOLMaster; Carl Zeiss Meditec, Dublin, CA). The high-myopia cases had to have an axial length of ≥26 mm in both eyes or a spherical equivalent refractive error of −6.0 D or more in the phakic eyes. For the general-population control, 546 healthy unrelated Japanese individuals who were ≥40 years of age were recruited from the Aichi Cancer Center Research Institute.

**Genotyping**

Genomic DNAs were prepared from the leukocytes of the peripheral blood. We genotyped the four functional SNPs in the promoter regions of \textit{MMP1}, -2, and -3: rs1799750 (\textit{MMP1} −1607 1G/2G), rs243865 (\textit{MMP2} C−1306T), rs2285053 (\textit{MMP2} C−735T), and rs3025058 (\textit{MMP3} −1612 5A/6A). The \textit{MMP1} −1607 2G allele has been shown to have higher transcriptional activity than the 1G allele. In addition, the \textit{MMP1} −1607 2G/2G and 2G/1G genotypes have been shown to have significantly higher mechanical force–induced \textit{MMP1} expression than the 1G/1G genotype. The \textit{MMP2} −1306 C and −735 C alleles have been reported to have higher promoter activity than the −1306 T and −735 T alleles, respectively. The \textit{MMP3} −1612 5A allele has higher promoter activity than 6A allele. The public dbSNP database build 129 (www.ncbi.nlm.nih.gov/projects/SNP; provided in the public domain by the National Center for Biotechnology Information [NCBI], Bethesda, MD) was used to extract the genotype information for these SNPs. All the SNPs were genotyped with SNP assays (Taqman; Applied Biosystems [ABI], Foster City, CA, with the Prism 7700 system; ABI), according to the manufacturer’s instructions.

**Statistical Analyses**

Hardy-Weinberg equilibrium (HWE) for genotypic distribution was determined with the HWE exact test for each group. Differences in the demographic features between the high-myopia and the control groups were tested for statistical significance by the \( \chi^2 \) test for dichotomous data and by the unpaired \( t \)-tests for continuous data. Differences in the observed genotypic distribution between the high-myopia group and the control group were tested by the \( \chi^2 \) test for trend. Logistic regression analysis was performed for age- and sex-adjusted data. These statistical analyses were performed with software R (http://www.r-project.org/). Because \textit{MMP1} rs1799750 and \textit{MMP3} rs3025058 are at 11q22.3, and rs243865 and rs2285053 are in \textit{MMP2} at 16q13-q21, their haplotypic effects were also evaluated with Haplovie 4.0. We used the HapMap database (http://hapmap.ncbi.nlm.nih.gov/index.html.en provided in the public domain by NCBI, Bethesda, MD), phase 3 release 2, to extract the haplotype and linkage disequilibrium (LD) blocks for the Japanese on SNPs harboring the \textit{MMP1}−\textit{MMP3} and \textit{MMP2} regions and compared them with the results of our study. The haplotypes and LD blocks were inferred by the solid spine of LD with a minimum D' of 0.5 in Haplovie 4.0. The significance of the differences in the estimated haplotype frequencies between the high-myopia group and the control group was examined by the \( \chi^2 \) test. The level of statistical significance was set at \( P < 0.05 \) and \( P_{\text{corr}} \) corrected \( P \) value after age and sex adjustments < 0.05. The statistical power calculation was performed with the case-control for discrete traits module of the Genetic Power Calculator (http://pngu.mgh.harvard.edu/purcell/gpc/). For the calculation, the type 1 error rate was set at 0.05, and the unselected controls (i.e., control subjects are random population samples) mode was used.

**RESULTS**

The demographics of the study population are shown in Table 1. The high-myopia group was significantly older (\( P < 0.0001 \)) and had a significantly lower male-to-female ratio (\( P < 0.0001 \)) than did the population-based control group. The axial lengths of the 1450 eyes of the 725 high myopia cases ranged from 26.00 to 36.32 mm, with a mean ± SD of 29.24 ± 1.85 mm. Among these eyes, 1011 (69.7%) were phakic and had not had corneal refractive surgery. The mean refractive error of the 1011 phakic eyes was −13.35 ± 4.38 D.

The genotype counts, associations, and odds ratios (ORs) for the four SNPs in the high-myopia and the control groups are shown in Table 2. The distributions of the genotypes for the four SNPs were in HWE (\( P > 0.05 \)). The difference in the genotypic distributions of the four SNPs between the high-myopia group and the control group was not statistically significant (\( P_{\text{corr}} > 0.05 \)). After correction for age and sex differences based on a logistic regression model, the difference in the genotypic distributions between the two groups was still not significant (\( P_{\text{corr}} > 0.05 \)). The power calculation results that were based on the additive model showed that the detectable ORs of

**Table 1. Characteristics of the Study Population (≥40 Years of Age)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>High Myopic Cases*</th>
<th>Population-Based Controls</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>725</td>
<td>546</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>323 (32.0)</td>
<td>279 (51.1)</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td>60.4 ± 10.2</td>
<td>57.9 ± 10.5</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Women</td>
<td>495 (68.0)</td>
<td>427 (48.9)</td>
<td></td>
</tr>
<tr>
<td>Axial length, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right eye</td>
<td>29.34 ± 1.89</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Left eye</td>
<td>29.14 ± 1.80</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Refractive error of phakic eyes, D‡</td>
<td>−13.51 ± 4.34</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Right eye</td>
<td>−13.19 ± 4.42</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Left eye</td>
<td>−13.19 ± 4.42</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

* Axial length of ≥26.00 mm in both eyes, or spherical equivalent refractive error of −6.0 D or more in the phakic eyes.
† Unpaired \( t \)-test.
‡ \( \chi^2 \) test.
§ For the calculations of refractive error, 439 (30.3%) eyes that had undergone cataract surgery or corneal refractive surgery were excluded.
a risk allele with 80% probability ranged from 1.27 (when the risk allele frequency in general population was 0.32, i.e., rs1799750 allele 1G) to 1.62 (when the risk allele frequency in general population was 0.93, i.e., rs243865 allele C) in a population in HWE.

The estimated haplotype frequencies in the high-myopia group and the control group are shown in Table 3. In the subjects of our study, MMP1 rs1799750 and MMP3 rs3025058 at 11q22.3 were in strong LD (D’ = 0.86, r² = 0.26), as were MMP2 rs243865 and rs2285053 at 16q13-q21 (D’ = 1.00, r² = 0.03). With the Japanese results of the HapMap project, we generated an LD block that extended a 46-kb region containing MMP1 rs1799750 and MMP3 rs3025058, as well as an LD block that extended a 20-kb region containing MMP2 rs243865 and rs2285053. The haplotype frequencies were not significantly different between the two groups (P > 0.05).

**DISCUSSION**

We hypothesized that variations in the expression of the MMP genes in the human sclera are associated with high myopia. To test this hypothesis, we focused on functional SNPs in the promoter regions that have been found to influence the expression of these genes. Among the MMP genes that have been shown to be expressed in human sclera, MMP1, -2, and -3, have been shown to have such functional SNPs in the promoter regions: rs1799750 (MMP1 -1607 1G/2G),23 rs243865 (MMP2 C-1306T C-1612 5A/6A),26 SNP analyses showed that these four functional promoter SNPs were not significantly associated with high myopia in our study group. Our sample size had an 80% power to detect an association of a risk allele with an OR as low as 1.62 in a population in HWE. Our results showed a strong LD between the MMP1 -1607 1G/2G and the MMP3 -1612 5A/6A as well as between the MMP2 C-1306T and MMP2 C-735T, which were consistent with the results of the HapMap project in the Japanese. The results of our study indicated that the MMP1 -1607 2G allele tended to be linked to the MMP3 -1612 6A allele and the MMP2 -1306 C allele tended to be linked to the MMP2 -735 T alleles. We also performed haplotype analysis; however, no new significant association was found.

We defined high myopia mainly by the axial length. High myopia is most commonly defined by the refractive error; however, multiple factors such as corneal curvature and a crystalline lens can affect refractive error. The axial length has

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**Table 2.** Genotype Counts, Associations, and ORs in the High-Myopia Cases and the Population-Based Controls

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Case*</th>
<th>HWE</th>
<th>Control</th>
<th>HWE</th>
<th>Nominal</th>
<th>OR (95% CI)</th>
<th>Adjusted</th>
<th>Adjusted OR§ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1799750</td>
<td>2G/2G</td>
<td>329</td>
<td>1.00</td>
<td>255</td>
<td>0.38</td>
<td>0.92</td>
<td>1.00 (ref)</td>
<td>0.99</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>(MMP1-1607)</td>
<td>2G/1G</td>
<td>313</td>
<td></td>
<td>227</td>
<td></td>
<td>1.07</td>
<td>0.84 (0.81-1.35)</td>
<td>1.06 (0.83-1.35)</td>
<td></td>
</tr>
<tr>
<td>1G/2G</td>
<td>1G/1G</td>
<td>74</td>
<td></td>
<td>60</td>
<td></td>
<td>0.96</td>
<td>0.66 (0.61-1.39)</td>
<td>0.96 (0.65-1.41)</td>
<td></td>
</tr>
<tr>
<td>rs243865</td>
<td>CC</td>
<td>621</td>
<td>0.57</td>
<td>467</td>
<td>0.76</td>
<td>0.83</td>
<td>1.00 (ref)</td>
<td>0.91</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>(MMP2)</td>
<td>CT</td>
<td>100</td>
<td></td>
<td>77</td>
<td></td>
<td>0.98</td>
<td>0.71 (0.75-1.35)</td>
<td>1.03 (0.74-1.44)</td>
<td></td>
</tr>
<tr>
<td>C-1306T</td>
<td>TT</td>
<td>2</td>
<td></td>
<td>2</td>
<td></td>
<td>0.75</td>
<td>0.51 (0.51-0.53)</td>
<td>0.75 (0.51-0.53)</td>
<td></td>
</tr>
<tr>
<td>rs2285053</td>
<td>CC</td>
<td>369</td>
<td>0.53</td>
<td>292</td>
<td>0.74</td>
<td>0.10</td>
<td>1.00 (ref)</td>
<td>0.15</td>
<td>1.07 (0.84-1.36)</td>
</tr>
<tr>
<td>(MMP3)</td>
<td>CT</td>
<td>290</td>
<td></td>
<td>210</td>
<td></td>
<td>0.19</td>
<td>0.86 (0.84-1.36)</td>
<td>1.07 (0.84-1.36)</td>
<td></td>
</tr>
<tr>
<td>C-735T</td>
<td>TT</td>
<td>64</td>
<td></td>
<td>34</td>
<td></td>
<td>1.49</td>
<td>0.96 (0.96-2.32)</td>
<td>1.47 (0.93-2.31)</td>
<td></td>
</tr>
<tr>
<td>rs3025058</td>
<td>5A/5A</td>
<td>15</td>
<td>1.00</td>
<td>14</td>
<td>0.20</td>
<td>0.62</td>
<td>1.00 (ref)</td>
<td>0.78</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>(MMP3-1612)</td>
<td>5A/6A</td>
<td>180</td>
<td></td>
<td>121</td>
<td></td>
<td>1.39</td>
<td>0.65 (0.96-2.98)</td>
<td>1.38 (0.63-2.99)</td>
<td></td>
</tr>
<tr>
<td>5A/6A</td>
<td>5A/6A</td>
<td>528</td>
<td></td>
<td>404</td>
<td></td>
<td>1.22</td>
<td>0.58 (2.56)</td>
<td>1.26 (0.59-2.70)</td>
<td></td>
</tr>
</tbody>
</table>

95% CI, 95% confidence interval.

* Axial length of ≥26.00 mm in both eyes, or spherical equivalent refractive error of −6.0 D or more in the phakic eyes.

† Differences in the estimated haplotype frequency were examined by χ² test for trend.

‡ Differences in the genotypic distribution were examined by HWE exact test.

§ Adjustment for age and sex was performed based on a logistic regression model.

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**Table 3.** Association of Haplotypes with High Myopia

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Case*</th>
<th>Control</th>
<th>Nominal P†</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP1 rs1799750-MMP3 rs3025058</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2G-6A</td>
<td>0.666</td>
<td>0.666</td>
<td>0.96</td>
<td>1.00 (0.85-1.19)</td>
</tr>
<tr>
<td>1G-6A</td>
<td>0.188</td>
<td>0.196</td>
<td>0.62</td>
<td>0.95 (0.78-1.16)</td>
</tr>
<tr>
<td>1G-5A</td>
<td>0.133</td>
<td>0.124</td>
<td>0.50</td>
<td>1.08 (0.86-1.37)</td>
</tr>
<tr>
<td>2G-5A</td>
<td>0.012</td>
<td>0.014</td>
<td>0.67</td>
<td>0.86 (0.44-1.70)</td>
</tr>
<tr>
<td>MMP2 rs243865-rs2285053</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-C</td>
<td>0.639</td>
<td>0.666</td>
<td>0.15</td>
<td>0.89 (0.75-1.08)</td>
</tr>
<tr>
<td>C-T</td>
<td>0.289</td>
<td>0.259</td>
<td>0.10</td>
<td>1.16 (0.97-1.38)</td>
</tr>
<tr>
<td>T-C</td>
<td>0.072</td>
<td>0.074</td>
<td>0.83</td>
<td>0.97 (0.72-1.31)</td>
</tr>
</tbody>
</table>

95% CI, 95% confidence interval.

* Axial length of ≥26.00 mm in both eyes, or spherical equivalent refractive error of −6.0 D or more in the phakic cases.

† Differences in the estimated haplotype frequency were examined by χ² test.
been shown to be the most important contributor to refractive error,\textsuperscript{1,2} and axial elongation is attributable to active scleral remodeling.\textsuperscript{11,12} We hypothesized that variations in the expression of the MMP genes in the sclera due to functional promoter polymorphisms result in variations in scleral remodeling and therefore are associated with the susceptibility of axial elongation. Thus, we propose that axial length is a more appropriate parameter than refractive error when assessing the association between the functional promoter SNPs of MMP genes and high myopia.

The cases of high myopia and the general population controls were all selected to be ≥40 years of age because we believed that the impact of genetic factors on the development of high myopia is different between younger and older individuals. Recent epidemiologic studies showed that the incidence of high myopia is rapidly increasing, especially in East Asia.\textsuperscript{3,4,11,12} Although genetic factors are important in the development of myopia, especially high myopia,\textsuperscript{10} the large increase in incidence over the past 50 years suggests a much stronger impact of environmental factors in the younger generation, probably due to changes in lifestyle (e.g., urbanization, education, and increasing near work than for the older generation).\textsuperscript{33} When we enrolled cases and controls of ≥20 years of age to check the results for another inclusion criteria, 840 highly myopic cases with axial length ≥26.00 mm (56.7 ± 13.5 years; male-female, 32.6%:67.4%), and 843 general-population–based controls (48.1 ± 16.1 years; male-female, 50.8%:49.2%) were recruited. Although the increased sample size brought higher detection power, SNP analyses and haplotype analyses found no new significant differences (data not shown) between these redefined case and control groups. The other age inclusion criteria (≥30 or ≥50 years of age) also showed no significant results (data not shown).

This study has some limitations. First, only four functional SNPs in the promoter region of \textit{MMP1}, -2, and -3 were tested. These four SNPs were not associated with Japanese high myopia; however, there are many other polymorphisms in these genes. The results of our study do not conclusively determine whether these genes play a role in high myopia. Second, the cases and controls were not age- and sex-matched. To minimize the possible influence of these differences, we adjusted for age and sex, using logistic regression analyses, and found no new significant association. Finally, we used general population-based control subjects and had no information on eye phenotype, including axial length or refractive data. It is possible that some of the eyes in the control group had an axial length ≥26 mm; this greater length would be a possible explanation for the negative results. A recent Japanese population-based study\textsuperscript{33} showed that the prevalence of high myopia in the elderly in Japan is estimated to be higher than that in the other countries\textsuperscript{25–30}; however, high myopia was defined by refractive error in that study,\textsuperscript{34} and there are no published data about the distribution of axial length in the Japanese general population. To check the results for a more strict axial length–based definition of high myopia, we also performed a subset analysis on cases with much longer axial lengths (≥27, 28, and 29 mm in both eyes). However, no new significant differences in the genotypic distributions were found (data not shown).

Thus, we conclude that the four functional SNPs in the MMP promoters do not play critical roles in the development of high myopia in the Japanese. To circumvent the limitations mentioned, future studies on the other SNPs of these genes with age- and sex-matched control subjects who have information on eye phenotype are necessary.

In summary, we have shown that the four functional SNPs in the promoter region of the MMP genes rs17997750 (\textit{MMP1} −1607 1G/2G), rs243865 (\textit{MMP2} C−1306T), rs2285055 (\textit{MMP2} C−735T), and rs3025058 (\textit{MMP3} −1612 5A/6A) were not significantly associated with high myopia in the Japanese population.

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**References**


