Myopia and Polymorphisms in Genes for Matrix Metalloproteinases

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Purpose. To investigate the relation between myopia and variations in three genes coding for matrix metalloproteinases, enzymes that degrade matrix proteins and modulate scleral extensibility.

Methods. Three hundred sixty-six men and women, from Sheffield, United Kingdom, were genotyped for the 1G/2G polymorphism in the *MMP-1* gene, the 5A/6A polymorphism in the *MMP-3* gene and the Arg→Gln polymorphism in exon 6 of the *MMP-9* gene and assessed for refractive error.

Results. Risk of myopia was increased in people homozygous for the 5A allele of the *MMP-3* gene (odds ratio [OR], 3.1; 95% confidence interval [CI], 1.1–9.0) compared to those who were homozygous for the 6A allele, and in people homozygous for the Gln allele in exon 6 of the *MMP-9* gene (OR, 2.8; 95% CI, 1.1–7.0) compared to those who were homozygous for the Arg allele. People who were homozygous for the 2G allele of the *MMP-1* gene had an odds ratio for myopia of 2.3 (95% CI, 0.9–6.1), compared with those who were homozygous for the 1G allele, although this relation did not reach statistical significance. Risk of myopia increased progressively with the dose of these three alleles, showing a greater than 10-fold difference across the range.

Conclusions. The results suggest that common variations in three of the genes that control breakdown of matrix proteins in the sclera may contribute to the development of simple myopia. (*Invest Ophthalmol Vis Sci.* 2009;50:2632–2636) DOI: 10.1167/iovs.08-2427

For the eye to form a sharp image on the retina, the length of its optical axis must correspond to the refractive power of the lens and cornea. During postnatal growth of the mammalian eye, a mechanism responsive to visual feedback operates to achieve this matching.[1] The experimental placement of a diffuser or minus power lens in front of the developing eye causes an increase in rate of axial elongation,[2] accompanied by remodeling of scleral extracellular matrix and controlled by changes in gene expression of matrix metalloproteinases—enzymes that degrade matrix proteins and modulate scleral extensibility.[3–5]

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Excessive axial growth of the eye results in myopia. Apart from the need for optical correction, myopia predisposes to several diseases including retinal detachment,[6] myopic maculopathy,[7] and glaucoma.[8,9] Environmental causes have been implicated by studies showing associations between myopia and higher levels of close work by children. But there are also indications that myopia is genetically determined. Children with two myopic parents are at greater risk of myopia than children with emmetropic parents[10] and estimates of heritability from twin studies are high.[11] We investigated variations in three genes coding for enzymes involved in matrix protein remodelling and risk of myopia in 366 men and women taking part in an epidemiologic study in Sheffield, United Kingdom.

Methods

Participants

In recent years, the Medical Research Council (MRC) Epidemiology Resource Centre has conducted several studies on cohorts of people born in the Jessop Hospital for Women, Sheffield, United Kingdom, whose recorded birth measurements are still available. The members of these birth cohorts were traced through the National Health Service Central Register, and those still living in the city were invited to take part in research into the processes by which environment in early life influences adult disease.

We took the opportunity to examine the relation between risk of myopia and variations in three genes coding for enzymes involved in matrix protein remodelling in a group of men and women aged 66 to 75 years who had taken part in one of these studies in Sheffield. One of the aims of this study had been to examine the relation between size at birth and risk of age-related macular degeneration. The study has been described previously.[12] Briefly, we asked the Office for National Statistics to trace all 4793 people whose births were recorded between 1922 and 1930. Only those still living in Sheffield were eligible to take part in the study. A stratified sample of 746 people, comprising all 236 subjects from the highest and lowest fifths of birthweight and 85 randomly chosen subjects of each sex from each of the three intervening fifths of birthweight, was selected. After obtaining permission from their general practitioners, we wrote to 660 people asking whether we could interview them at home; 412 (62%) agreed to be interviewed by a fieldworker. Of these, 392 (95%) were willing to attend a clinic.

Measurements

An ophthalmologist determined the participants' refractive error by measuring their usual distance glasses with a lensmeter. Visual acuity was assessed for each eye with a Bailey-Lovie logMAR chart with participants wearing their usual distance glasses. Retinoscopy and subjective refraction were performed on all eyes failing to read at logMar 0.2 or better. The spherical equivalent for each eye was calculated by adding the spherical error to half the cylindrical component of the distance correction. The ophthalmologist also asked about history of lens extraction and assessed nuclear lens opacities at slit lamp examination, according to the Lens Opacities Classification System III.[13]

A fasting venous blood sample was taken for DNA extraction. All samples were stored at −80°C for later analysis. Participants' genotypes for the matrix metalloproteinase-1 (*MMP-1*) gene −1607 insG,

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TABLE 1. Characteristics of the 366 Study Participants

Characteristics	Men (n = 201)	Women (n = 165)
Age (y)*	69.7 (1.86)	70.0 (2.20)
Left school aged >14 years, n (%)	34 (17.0)	30 (18.2)
Average spherical equivalent (D), n (%)		
Emmetropia (−0.99 to +0.99)	77 (38.3)	46 (27.9)
Hypermetropia (≥ +1.00)	99 (49.3)	93 (56.4)
Myopia (≤ −1.00)	25 (12.4)	26 (15.8)
Nuclear cataract, n (%)	40 (21.3)	57 (35.4)

* Mean (SD).

MMP-3 gene −1612 insA, and *MMP-9* gene exon 6 Arg→Gln polymorphisms were determined by standard methods. In brief, for each polymorphism studied, a DNA sequence containing the polymorphic site was amplified by polymerase chain reaction (PCR) and the amplicon digested with an appropriate restriction endonuclease that cleaved only one of the two alleles. The digests were then subjected to gel electrophoresis, stained with a fluorescent nucleic acid stain (Vistra Green; Amersham, Buckinghamshire, UK) and scanned in a fluorimager.

The research followed the tenets of the Declaration of Helsinki. The study was approved by the South Sheffield Local Research Ethics Committee, and all participants gave written informed consent.

Statistical Analysis

ANOVA and χ^2 test were used to examine the characteristics of the participants. Logistic regression was used to examine the relation between the 1G/2G polymorphism in the *MMP-1* gene, the 5A/6A polymorphism in the *MMP-3* gene, and the Arg→Gln polymorphism in exon 6 of the *MMP-9* gene and risk of myopia, defined as a refractive error of −1 D or worse. Probabilities are given for the trend in the odds ratios across the groups. The analysis is based on the 366 (93%) participants with complete data on all three genotypes.

RESULTS

Of the 392 men and women who attended a clinic for ophthalmic examination, 366 had data on *MMP-1*, *-3*, and *-9* genotypes. The characteristics of these participants are shown in Table 1. In total, 51 (14%) of the participants were myopic. Refractive error in the myopic participants ranged from −1.00 to −11.3 average spherical equivalent.

Table 2 shows logistic regression analyses of risk of myopia according to *MMP-1*, *-3*, and *-9* genotypes. In logistic regression analysis of the1G/2G polymorphism in the *MMP-1* gene, the 5A/6A polymorphism in the *MMP-3* gene and the Arg→Gln polymorphism in exon 6 of the *MMP-9* gene, risk of myopia was highest in participants who were homozygous for the 5A allele in the promotor region of the *MMP-3* gene or the Gln allele in exon 6 of the *MMP-9* gene. These associations remained statistically significant after adjustment for education, a risk factor for myopia, and the presence of nuclear cataract, which may cause index myopia. Compared with those who were homozygous for the 6A allele, people who were homozygous for the 5A allele of the *MMP-3* gene had an odds ratio for myopia of 3.1 (95% confidence interval [CI], 1.1–9.0; P = 0.03). Compared with those who were homozygous for the Arg allele of the *MMP-9* gene, people who were homozygous for the Gln allele had an odds ratio for myopia of 2.8 (95% CI, 1.1–7.0; P = 0.03). Risk of myopia was also increased in people who possessed the 2G allele of the *MMP-1* gene. People who were homozygous for the 2G allele had an odds ratio for myopia of 2.3 (95% CI, 0.9–6.1), compared with those who were homozygous for the 1G allele, though this relation was not statistically significant (P = 0.08).

To explore how the risk changed with increasing dose of these alleles, we classified participants in seven groups according to how many of these three alleles they possessed. For example, individuals who were homozygous for the 1G allele of *MMP-1*, the 6A allele of *MMP-3*, and the Arg allele in exon 6 of the *MMP-9* gene were classified as having an allele dose of 0, and those who were homozygous for 2G allele of *MMP-1*, the 5A allele of *MMP-3*, and the Gln allele in exon 6 of the *MMP-9* gene were classified as having an allele dose of 6. Table 3 and Figure 1 show the odds ratios for myopia according to allele dose, with the category with three alleles used as the reference group. Rising allele dose was associated with a progressive increase in prevalence and risk of myopia. Prevalence of myopia rose from 7% among those with no higher risk allele to 50% among those who had all six higher risk alleles. Relative risk, as estimated by odds ratios, increased by a factor of more than 10 across the range of allele dose. The strength of this relation changed little after adjustment for education and for nuclear cataract.

TABLE 2. Risk of Myopia According to *MMP-3, -9,* and *-1*

Genotype and Polymorphism	n	Subjects with Myopia n (%)	Odds Ratio (95% CI)	
			Unadjusted	Adjusted*
MMP-3				
6A/6A	73	7 (9.6)	1.0	1.0
5A/6A	196	24 (12.2)	1.3 (0.5–3.2)	1.6 (0.6–4.4)
5A/5A	97	20 (20.6)	2.4 (1.0–6.2)	3.1 (1.1–9.0)
			P for trend = 0.026	P for trend = 0.015
MMP-9				
Arg/Arg	165	19 (11.5)	1.0	1.0
Arg/Gln	164	21 (12.8)	1.1 (0.6–2.2)	1.2 (0.6–2.4)
Gln/Gln	37	11 (29.8)	3.3 (1.4–7.6)	2.8 (1.1–7.0)
			P for trend = 0.005	P for trend = 0.026
MMP-1				
2G/2G	93	16 (17.2)	1.8 (0.8–4.2)	2.3 (0.9–6.1)
1G/2G	176	25 (14.2)	1.4 (0.7–3.1)	1.8 (0.8–4.5)
1G/1G	97	10 (10.3)	1.0	1.0
			P for trend = 0.260	P for trend = 0.188

* Adjusted for education and presence of nuclear cataract.

TABLE 3. Risk of Myopia According to Allele Dose

Alleles (*n*)	*n*	Prevalence of Myopia (%)	Odds Ratio (95% CI)	
			Unadjusted	Adjusted*
None	14	7	0.5 (0.1–4.5)	0.5 (0.1–4.1)
1	66	11	0.8 (0.3–2.2)	0.8 (0.3–2.1)
2	84	11	0.8 (0.3–2.1)	0.7 (0.3–1.8)
3	96	13	1.0 (reference)	1.0 (reference)
4	68	15	1.2 (0.5–3.0)	1.2 (0.5–3.1)
5	32	28	2.7 (1.0–7.3)	2.7 (1.0–7.4)
6	6	50	7.0 (1.3–38.7)	6.1 (1.1–35.2)
			P for trend = 0.005	*P* for trend = 0.002

* Adjusted for education and presence of nuclear cataract.

DISCUSSION

In this study of older adults, we found an association between simple myopia and genetically determined variations in three enzymes involved in extracellular matrix remodelling. Risk of myopia was increased in subjects who possessed the 5A allele of the *MMP-3* gene, the Gln allele of the *MMP-9* gene, and the 2G allele of the *MMP-1* gene (though the latter association was not statistically significant). Those subjects homozygous for the polymorphisms associated with myopia were at greatest risk.

Matrix metalloproteinases are essential for remodelling of the extracellular matrix (ECM). They act by breaking down components of the ECM. They are essential for growth of the eye. Animal studies have shown that MMP gene expression is associated with experimental myopia. Changes in refractive power and vitreous chamber depth coincide with alterations in MMP mRNA levels in the sclera which increase or decrease the rate of axial elongation according to the presence of a myopiagenic stimulus.[4] In our study, we found that those alleles in the promoter regions which were more transcriptionally active were associated with a higher risk of myopia. For example, the 2G allele of the *MMP-1* gene contains an additional guanine at position 1067 bp. This single-nucleotide polymorphism occurs in the *MMP-1* promoter and affects an Ets binding site. The 2G allele results in a 2- to 10-fold increased transcription of *MMP-1* compared with the 1G variant—an effect that has been demonstrated in both normal and tumor cell lines.[14] The more active 2G allele in *MMP-1* would also be expected to increase ECM remodelling and was associated with higher risk of myopia in our study.

The promoter region of the *MMP-3* gene (stromelysin 1) also contains a polymorphism that affects regulation of gene expression. *MMP-3* is a crucial enzyme in tissue remodelling; it not only directly degrades ECM proteins but it activates other MMPs such as *MMP-1* and *-9*. It has been shown that the 5A allele in the promoter region of *MMP-3* has an approximate twofold increased gene product compared with the 6A allele.[15] This finding suggests that individuals homozygous for the 6A allele would have lower levels of *MMP-3* in the scleral wall, which is in keeping with our finding of a lower risk of myopia among these subjects. The 5A/6A alleles are common variants in the population and were evenly distributed among our study participants. They might therefore be biologically important in the pathogenesis of simple myopia in the general population. In a case control study of high myopia in young Taiwanese men, there was a nonsignificant trend toward higher frequency of the 5A/5A genotype in the cases which is consistent with our finding.[16] However, subjects with low myopia (between −1.5 D and −6 D) were excluded from this study, whereas we included the whole range of myopia.

The third variant explored in our subjects is a polymorphism within the catalytic domain of the *MMP-9* enzyme (gelatinase B). This R279Q polymorphism occurs within the coding region (exon 6) of the *MMP-9* gene and results in the substitution of a positively charged amino acid (arginine) by an uncharged amino acid (glutamine). It is suggested that this substitution will affect binding affinity of the enzyme for its substrate elastin. As this polymorphism occurs at a ratio of approximately 0.65/0.35 for the two alleles, it could also represent a biologically important variability within populations. An alternative explanation for the association we found with *MMP-9* is that the 279glutamine allele in the coding region of the *MMP-9* gene is in strong linkage disequilibrium with the −1562T allele in the promoter region of the same gene—these two alleles being preferentially associated.[17] The −1562T promoter and 279Q coding alleles are associated with higher plasma levels of the *MMP-9* enzyme.[18] Our findings of a higher risk of myopia being associated with the glutamine allele is consistent with the general hypothesis of higher levels of MMPs with increased risk of myopia. A major substrate for this enzyme is elastin, and higher levels of *MMP-9* could reduce scleral elastin content and thus reduce its distensibility. This reduction would give it a lower yield point, making it more likely to deform plastically against a given intraocular pressure (hence increasing the tendency toward myopia). This possibility is supported by findings showing that large artery stiffness was increased in carriers of the −1562T allele (which had greater *MMP-9* mRNA and protein levels).[19]

A similar argument of linkage disequilibrium in the case of the two *MMP-9* polymorphisms could also be applied to explain the associations we found between *MMP-3* and *-1* and risk of myopia. The *MMP-1* gene is found on chromosome 11 (11q22.3) at chromosome position 102.17 cM. The *MMP-3* gene is at an adjacent locus on the same chromosome

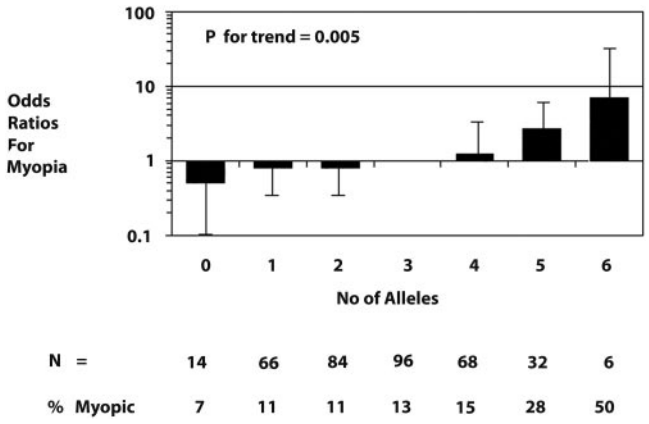

FIGURE 1. Odds ratios (95% CIs) for myopia according to allele dose. The category with three alleles is used as the reference group.

(11q22.3) at chromosome position 102.24 cM. A genomewide linkage study was performed for simple myopia among 221 dizygotic twin pairs by screening for 737 highly polymorphic microsatellite markers.[20] A linkage peak was present on chromosome 11 (11q23-24; position 125 cM) which is close to the position of the *MMP-3* and *-1* genes, but at LOD score 2.9, it was below the level used to define significant linkage (LOD >3.2). This proximity to the linkage signal means that either *MMP-3* or *-1* gene or both could be causally implicated in myopia development or that they are linked with a third gene or genes in this region responsible for the phenotypic association with myopia. No significant linkage peak was found in this study corresponding to the position of the *MMP-9* gene on chromosome 20 (20q11.2-q13.1).

In our study, we defined myopia as being −1.0 D or more extreme average spherical equivalent. We used logistic regression to assess the strength of association with different MMP polymorphisms. This approach is consistent with a biological definition of myopia as a failure of the normal emmetropization process. Most individuals achieve emmetropia when growth of the eye is complete and the population distribution of refractive error is therefore non-normally distributed. This distribution makes myopia more suited to analysis as a categorical variable. High myopia (defined as a refractive error of −6.00 D or more extreme spherical equivalent) is often autosomally dominantly inherited[21] and is not considered in this analysis due to the small number of participants in this category.

The potential difficulties of studying refractive error in an elderly population have been considered by the investigators of the Salisbury Eye Evaluation study, whose study participants had a mean age of 70 years.[22] They found that heredity explained 62% of the variance in refractive error in these participants. They comment that "nongenetic influences, especially those related to cumulative age effects, would have had a relatively large effect on refractive error in this age group, increasing the total phenotypic variance and weakening the estimated genetic effect." In other words, refractive status might be misclassified in studies of elderly people. There is an overall trend toward increasing hypermetropia in later life. In a cohort of people aged 50 and over, there was a hyperopic shift of +0.41 D over 5 years among those aged 50 to 59 years.[23] The same study found a myopic shift of −0.02 D over 5 years in people over the age of 70 with the shift being −0.65 D in those with significant nuclear lens opacity at baseline. In our study, very few participants who were myopic would have been misclassified as emmetropic or hyperopic, because we chose a cutoff of −1.00 D or more extreme to define myopia. Also any misclassification of emmetropes or hypermetropes as myopes due to nuclear sclerotic lens opacity has been adjusted for in the analysis using LOCS III. A similar method of adjustment was adopted in the Beaver Dam Eye Study.[24]

Polymorphisms in MMP genes affect susceptibility to cardiovascular disease and so have the potential to introduce survivor bias in an elderly population.[25] We found the 5A polymorphism of the *MMP-3* gene to be associated with myopia. It is also associated with a 1.5- to 2-fold increased risk of myocardial infarction.[26] If participants with the 5A allele were less likely to participate due to decreased survival then we would expect to see an association between the 6A allele and myopia. In fact, we found myopia to be associated with the 5A allele, which would tend to suggest that differential mortality in the population with this polymorphism did not result in significant bias.

We would be cautious about drawing any firm conclusions about associations between simple myopia and MMP polymorphisms from this study. The study was designed to investigate causes of age-related eye diseases rather than myopia. Although participants were not selected by refractive error it is possible that subjects with myopia would be more likely to take part, although this would only introduce bias if the likelihood of participation were also associated with MMP allele variants, which seems unlikely. This study was performed on a single sample, and our results must be replicated in other populations. The number of people in some categories of allele frequency is small and risk estimates are imprecise.

Simple myopia is a common trait that is strongly heritable, albeit with additional environmental determinants. An animal model suggests an important role for MMPs in the development of experimental myopia. Our findings suggest that variations in the activity of three matrix metalloproteinase enzymes caused by distinct functional polymorphisms might play a role in the development of myopia in humans. Risk of myopia was greatest among our study participants when polymorphisms in the promoter regions of *MMP-1* and *-3* led to more abundant enzyme production. The polymorphisms we examined occur at high frequency in the general population and could be among the possible candidates that explain genetic susceptibility to myopia. Polymorphisms in other genes that regulate scleral protein turnover may also be worth investigating. However, because of the possibility that our findings are the result of type 1 error, further studies are needed to confirm our results.

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