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Matrix metalloproteinases and educational attainment in refractive error: evidence of gene-environment interactions in the AREDS study

Robert Wojciechowski, OD, PhD^{1,2}, Stephanie S. Yee, MS³, Claire L. Simpson, PhD², Joan E. Bailey-Wilson, PhD², and Dwight Stambolian, MD, PhD³

¹Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

²Statistical Genetics Section, Inherited Disease Research Branch, National Human Genome Research Institute, Baltimore, MD

³Department of Ophthalmology and Genetics, University of Pennsylvania, Philadelphia, PA

Abstract

Purpose—A previous study of Old Order Amish families has shown association of ocular refraction with markers proximal to matrix metalloproteinase (MMP) genes MMP1 and MMP10 and intragenic to MMP2. We conducted a candidate gene replication study of association between refraction and single nucleotide polymorphisms (SNPs) within these genomic regions.

Design—Candidate gene genetic association study.

Participants—2,000 participants drawn from the Age Related Eye Disease Study (AREDS) were chosen for genotyping. After quality control filtering, 1912 individuals were available for analysis.

Methods—Microarray genotyping was performed using the HumanOmni 2.5 bead array. SNPs originally typed in the previous Amish association study were extracted for analysis. In addition, haplotype tagging SNPs were genotyped using TaqMan assays. Quantitative trait association analyses of mean spherical equivalent refraction (MSE) were performed on 30 markers using linear regression models and an additive genetic risk model, while adjusting for age, sex, education, and population substructure. Post-hoc analyses were performed after stratifying on a dichotomous education variable. Pointwise (*P-emp*) and multiple-test study-wise (*P-multi*) significance levels were calculated empirically through permutation.

Main outcome measures—MSE was used as a quantitative measure of ocular refraction.

Results—The mean age and ocular refraction were 68 years (SD=4.7) and +0.55 D (SD=2.14), respectively. Pointwise statistical significance was obtained for rs1939008 (*P-emp*=0.0326). No SNP attained statistical significance after correcting for multiple testing. In stratified analyses, multiple SNPs reached pointwise significance in the lower-education group: 2 of these were statistically significant after multiple testing correction. The two highest-ranking SNPs in Amish

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Corresponding author: Robert Wojciechowski, OD, MSc, PhD Department of Epidemiology Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, E6516 Baltimore, MD 21205 Tel: 410-614-5041 rwojciec@jhsph.edu.

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families (rs1939008 and rs9928731) showed pointwise $P\text{-emp} < 0.01$ in the lower-education stratum of AREDS participants.

Conclusions—We show suggestive evidence of replication of an association signal for ocular refraction to a marker between MMP1 and MMP10. We also provide evidence of a gene-environment interaction between previously-reported markers and education on refractive error. Variants in MMP1- MMP10 and MMP2 regions appear to affect population variation in ocular refraction in environmental conditions less favorable for myopia development.

Keywords

refraction; refractive error; myopia; association study; gene-environment interaction; matrix metalloproteinase; MMP; genetics

Myopia is characterized by a progressive elongation of the posterior pole of the eye relative to its optical power. This lengthening occurs via a remodeling of the sclera, a fibrous connective tissue made-up primarily of extracellular collagen. Collagen undergoes cycles of active breakdown and synthesis by fibroblasts during experimentally induced myopization. This degradation of scleral extracellular matrix can be initiated by matrix metalloproteinases (MMP), which are a family of calcium-dependent proteinases that play important roles in the modulation of connective tissue remodeling. In humans, the MMP gene family consists of 23 distinct genes.

Several experimental studies with animal models have implicated MMPs in myopization.¹⁻⁵ Specifically, differential MMP2 mRNA expression has been reported in experimental myopia studies in tree shrew¹⁻⁴ and chicks.⁵ In these studies, form deprivation causes increased MMP2 mRNA expression in occluded eyes compared to normally-seeing eyes, leading to collagen degradation, scleral remodeling and posterior segment elongation. A similar mechanism may be involved in refractive error regulation in humans, albeit on a much longer time scale.

We and others have postulated that inherited genetic variations in MMP genes or their regulators may alter MMP proteolytic activity and lead to differences in susceptibility to myopia.^{6, 7} Hall et al⁶ reported higher odds of myopia among British participants who carried high-risk alleles in MMP1, MMP3 and MMP9 (although MMP1 did not reach statistical significance) compared to non-carriers. In a comprehensive family-based screening of 16 MMP and tissue inhibitors of matrix metalloproteinase (TIMP) genes, we⁷ reported significant genetic associations between ocular refraction and variants within MMP2 and in the intergenic region of MMP1 and MMP10. Although these effects were seen in Old Order Amish families, no evidence of genetic association to ocular refraction was found in Orthodox Ashkenazi Jewish pedigrees. We pointed out that considerable differences in putatively myopiagenic environmental and behavioral risk factors between these cultural and genetic isolates may account for discrepancies in association signals. Other groups have not substantiated a role for MMP variants in the development of high myopia. In a case-control association study of high myopia among young Taiwanese men, Liang et al⁸ found little evidence of a genetic effect for 13 polymorphisms within MMP3 and TIMP1. Nakanishi et al showed that functional single nucleotide polymorphisms (SNPs) in promoter regions of MMP1, MMP2 and MMP3 were not significantly associated with high myopia in the Japanese.⁹ More recently, Leung et al.¹⁰ found no statistically significant associations between high myopia and polymorphisms in MMP2, TIMP2 and TIMP3 in a Chinese case-control study. It should be noted that studies showing significant association between MMP polymorphisms and refractive error have been found in European-derived populations whereas negative results have been reported for high myopia in East Asians. Hence, even though animal models have implicated a role for matrix metalloproteinases in

form-deprivation and lens-induced myopia through scleral remodeling, it remains unclear whether MMP polymorphisms contribute significantly to refractive variation in human populations.

We report results of a replication study of candidate genes for ocular refraction in the Age Related Eye Disease Study (AREDS). Candidate genes (MMP1, MMP2, and MMP10) were chosen based on our previous study which showed a statistically significant association between two SNPs (rs9928731 and rs1939008) and quantitative refractive phenotypes in a group of large Amish extended families ascertained for myopia.⁷ We found suggestive evidence of replication of the rs1939008 locus at chr11q22.2 proximal to MMP1 and MMP10 in the AREDS sample. Post-hoc analyses revealed interactions between educational attainment and rs1939008 and rs9928731 on ocular refraction.

Materials and Methods

Participants

This study was conducted according to the principles expressed in the Declaration of Helsinki. All study subjects were participants in phase I of the original AREDS study. AREDS is a multi-center prospective cohort study that primarily focused on evaluating the clinical course and risk factors for the development of age-related macular degeneration (AMD) and cataract.¹¹ Written informed consent was obtained from all study participants as part of enrollment, and the study was approved by the local Institutional Review Board of each participating site.

The AREDS study enrolled 4,757 persons aged 55 to 80 years beginning in 1992. Participants had to be free of any illness or condition that would make compliance with study medication or long-term follow-up difficult. During the randomization visit, participants underwent manifest refraction with visual acuity measurement, were given a baseline interview, and donated a blood sample. Analyses reported in this manuscript were conducted on data from the first participant visit (i.e., at the randomization visit). In addition to ophthalmic data, demographic characteristics were collected at the randomization visit. Blood samples were collected for over 3,700 participants for genetic research. These subjects consented to their genetic specimen being used in eye disease research.

Selection and exclusion criteria—At baseline enrollment, the AREDS participants were classified into one of several AMD categories. Category 1 was a group of participants who were free of age related macular changes. Category 2 was made up of participants with mild or borderline age-related macular changes (multiple small drusen or intermediate drusen and/or pigment abnormalities) in one or both eyes. To qualify for either of these categories, both eyes had to have visual acuity of 20/32 or better (75 or more letters read correctly) measured by a standard protocol, media clear enough for good quality fundus photographs and absence of any ocular disorder that might interfere with assessment of either AMD or lens opacities. In the current study, a subset of 2,000 AREDS participants was selected from the Category 1 or 2 groups for genotyping in the current study. Exclusion criteria for the current analysis included ophthalmic conditions that may have precluded an accurate measurement of refractive error including significant cataract, retinitis pigmentosa, color blindness, other congenital eye problems, LASIK, artificial lenses, and other eye surgery and advanced AMD. Refractive error at baseline enrollment into the AREDS study was analyzed.

Marker selection and genotyping

Markers for genotyping were selected based on a previous study of Amish families that showed statistically significant association of ocular refraction to markers within or near MMP1, MMP10 and MMP2⁷. Markers from regions outside MMP1–MMP10 and MMP2 were not queried in the association analysis. A total of 18 haplotype tagging SNPs were selected for genotyping as follows. For MMP2, 9 tagging SNPs (HapMap data release 24, NCBI-B36 Assembly) covering the entire MMP2 gene were selected for genotyping. Additional tagging SNPs around rs1939008 (HapMap data release 24, NCBI-B36 Assembly) were chosen 27,130 bp 3' and 45,862 bp 5' to this SNP. All 18 SNPs were genotyped using a customized TaqMan SNP genotyping assay (Applied Biosystems [ABI], Foster City, CA). All PCR amplifications were performed with the following thermal cycling conditions: 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. PCR reactions were performed with TaqMan Genotyping Master Mix (ABI) in either a GeneAmp PCR System 9700 (ABI) or a 7900HT Fast Real-Time PCR System (ABI). All pre- and post-PCR plate readings were performed on a 7900HT Fast Real-Time PCR System (ABI), and the allele types were confirmed by the system's software (7900HT Fast Real-Time PCR System SDS software version 2.3; ABI). In addition, 12 haplotype tagging SNPs were added from a whole-genome microarray experiment to increase marker density within the MMP1–MMP10 and MMP2 candidate regions. Microarray genotyping was carried out at the Center for Inherited Disease Research (Johns Hopkins University) using the Illumina HumanOmni 2.5 bead chip (Illumina, Inc., San Diego, CA, USA) which assays approximately 2.44 million SNPs across the genome and includes over 1.7 million markers from the 1,000 Genomes Project pilot release (www.1000genomes.org Accessed June 29, 2012). All 30 analyzed SNPs were polymorphic in AREDS participants and none departed from Hardy-Weinberg expectations.

Quality assessment and control

We applied a series of quality control filters at the population, the individual, and at the marker levels to assure high data quality. First, potential plate batch effects were explored (by testing for homogeneity of allele frequencies across plates) and were found to be absent. Individuals with genotyping call rates below 98% on the full marker set were excluded from further analyses. Participants who self-reported as being non-Caucasian (n=86) were also excluded. In addition, genotypes of one individual who self-identified as Caucasian were more consistent with an African American ancestry. This individual was removed from further analyses.

A SNP was removed from the association analysis set if its call rate was below 99%, its minor allele frequency was below 0.01, or if its distribution departed significantly from Hardy-Weinberg expectations ($p < 0.0001$). SNPs were also excluded if they showed more than one genotype inconsistency between: 1) HapMap control samples and the consensus genotype in the HapMap database (<http://hapmap.ncbi.nlm.nih.gov/> Accessed June 29, 2012) or 2) investigator-provided duplicate samples. SNPs were also removed if they showed more than 1 Mendelian inconsistency in HapMap control family trios.

Population structure and cryptic relatedness

Population stratification was assessed using a subset of approximately 40,000 independent, polymorphic ($MAF > 0.01$), autosomal markers from the Human OMNI 2.5 panel. We utilized the principal components method used in the EIGENSTRAT¹² software and implemented in the EIGENSOFT package (version 3.0; <http://genepath.med.harvard.edu/~reich/Software.htm> Accessed June 29, 2012) for population stratification testing.

Cryptic relatedness was estimated by calculating pairwise identical by descent (IBD) coefficients using the software PLINK (version 1.07; <http://pngu.mgh.harvard.edu/~purcell/plink> Accessed June 29, 2012)¹³ and pseudo-kinship coefficients using EMMAX¹⁴ for all pairs of individuals in the study. Estimates of the average proportion of alleles shared IBD ($\bar{r}$) across all markers were almost identical in each pair of individuals using both methods. For each cryptically-related pair ($\bar{r}$, including 14 putative full sibling pairs), we excluded one individual from the analyses. The remaining subject was chosen based on genotype quality metrics and refractive error.

Statistical analysis

Genetic association was estimated by fitting a linear regression model with the PLINK (version 1.07) statistical software.¹³ The dependent variable was the spherical equivalent refraction, averaged between the eyes (or Mean Spherical Equivalent[MSE]). A general additive genetic model was used to code the SNP effect (i.e., SNPs were coded according to the number of minor alleles [0,1,2] for each person); covariates included: the first three principal components of the EIGENSTRAT analysis; age; sex; and the highest level of schooling achieved. This education variable was coded as a unit-spaced ordinal variable ranging from 1 to 5 using the following coding scheme: 1=grade 11 education or less; 2=high school graduate; 3=some college or associate degree; 4=bachelor's degree; and 5=post-graduate work. Nominal statistical significance (*P*-*asym*) was assessed using the asymptotic distribution of the test statistic. Empirical p-values were obtained using a permutation procedure implemented in PLINK wherein phenotypes were randomly permuted and the analyses repeated 10,000 times at all markers. The (non-permuted) test statistic is compared to the null distribution of test statistics under permutation to determine a pointwise *empirical* p-value (*P*-*emp*). *P*-*asym* and *P*-*emp* were nearly identical for all SNPs, showing that the asymptotic distribution is valid in this dataset. A similar procedure (comparison of the non-permuted test statistic to the null distribution of all SNPs combined over the 10,000 permutations) is used to determine a study-wise p-value (*P*-*multi*), which is adjusted for multiple testing. Since only phenotypes are permuted, the linkage disequilibrium structure within the sample population is maintained: correlations between SNPs are identical for each permutation. Hence, the permutation procedure yields valid estimates of experiment-wise p-values that take into account linkage disequilibrium.

Exploratory analyses

Exploratory analyses were conducted using the same phenotype, genotype and covariate information, but changing the implied genetic model to: dominant; recessive; and a general genotypic model which includes both additive and dominance terms in the statistical model. These genetic models were adjusted for the same covariates as the additive model (i.e., age, sex and the first three principal components of EIGENSTRAT results). In addition, we performed stratified analyses in order to test SNP-environment interaction. Participants were stratified into two educational categories: a higher education group which included individuals with a bachelor's degree or greater (n=754); and a lower education group which was comprised of all other levels of formal schooling (n=1,159). All stratified exploratory analyses were adjusted for age, sex, and population stratification. Asymptotic and empirical p-values for different genetic models as well as for stratified analyses were determined as described above. Hence, empirical study-wise p-values (*P*-*multi*) are adjusted for multiple correlated SNPs, and no additional multiple-testing correction was made to account for multiple statistical models.

Results

After removing non-Caucasian and related individuals, data from 1913 AREDS participants (784 men and 1129 women) were available for analysis. The mean age was 68 years (SD=4.7; range=55–81) and the average MSE was +0.55 D (SD=2.14). Additional details are given in Table 1. Association testing was conducted on 30 SNPs, all of which passed our quality control filters. The mean minor allele frequency was 0.246, the average genotyping call rate was 99.16%, and none of the SNPs departed significantly from HWE (Table 2, online at <http://aaojournal.org>).

Results of genetic association testing of MSE under the general additive model, while adjusting for age, sex, education level, and population substructure are shown in Table 2 (online at <http://aaojournal.org>). No SNP in the MMP2 region showed a pointwise statistical significance of association with MSE at the $p = 0.05$ level (minimum $P\text{-}asym=0.0725$; minimum $P\text{-}emp=0.0686$ for rs857403). One marker in the MMP1–MMP10 region, rs1939008, showed both pointwise $P\text{-}asym$ and $P\text{-}emp$ below 0.05 ($P\text{-}asym=0.0332$ and $P\text{-}emp=0.0326$). However, this marker did not achieve study-wide statistical significance after adjusting for multiple testing ($P\text{-}multi = 0.4593$). This marker is located in an intergenic region ~4 Kb downstream of MMP1 and ~5Kb upstream of MMP10. SNP rs1939008 was the most highly associated marker in a candidate gene set analysis of ocular refraction among Amish families ($p=0.00016$).⁷

Post-hoc exploratory analyses tested for association under a variety of genetic models (dominant, recessive and genotypic) using different SNP coding schemes. None of these alternative genetic models produced a more highly significant minimum $P\text{-}asym$ or $P\text{-}emp$ than under the additive genetic model (results not shown).

Results of stratified analyses, conducted separately for two educational classes under a dominant genetic model, are presented in Table 3 (see online material at <http://aaojournal.org>). Associated SNPs showed a significant increase in statistical significance in the lower educational category (minimum $P\text{-}emp=0.0003$ for rs12272341) compared to the combined analyses ($P\text{-}emp=0.0632$ for rs12272341; minimum $P\text{-}emp=0.0326$ for rs1939008). In the lower education analysis, two SNPs, rs12272341 and rs1939008, showed statistically significant association after empirical multiple-testing correction ($P\text{-}multi=0.00057$ and 0.0298, respectively). SNP rs12272341 is located in an intron of the MMP10 gene. The greatest evidence of association in the MMP2 region among the lower education stratum was observed for rs9928731 ($P\text{-}emp=0.00067$, $P\text{-}multi=0.12$). This SNP corresponded to the most significant signal in the MMP2 region in Amish families.⁷

In contrast to the lower education group, we saw no evidence of statistical association of MSE with MMP1–MMP10 SNPs in the higher education group. Only one SNP (rs243866 5' of MMP2) showed nominally significant association ($P\text{-}emp=0.0357$; $P\text{-}multi=0.5051$) in the higher education stratum. Mean, standard error, minimum and maximum values of MSE and age at baseline examination in all analyzed subjects and in the lower and higher educational subgroups are shown in Table 1. In addition, this table shows the correlation of these traits with the ordinal educational attainment variable. A negative correlation is observed in the complete sample but this correlation is much attenuated in the two subsets, as expected.

Discussion

We tested 30 SNPs in regions of MMP1–MMP10 and MMP2 for association to ocular refraction (MSE) in an elderly cohort of 1913 people sampled from the AREDS study. These regions were chosen because the most highly significant association signals for ocular

refraction in a candidate gene set study of Amish families were found for SNPs in the intergenic region between MMP1 and MMP10 (rs1939008), and in the 6th intron of MMP2 (rs9928731). Our results show that rs1939008 was again the most strongly associated marker in AREDS subjects; the pointwise association *P-emp* for rs1939008 was 0.033. Moreover, the direction of the observed association was the same in both the AREDS and Amish studies.

No marker met a stringent multiple-testing threshold for statistical significance (minimum $P\text{-multi}=0.45$ for rs1939008) in the entire dataset. Hence, after accounting for multiple testing, definite replication could not be confirmed, even though the asymptotic and empirical pointwise *p*-values met nominal significance for replication at rs1939008. Given the marginal evidence for replication, we believe that this locus requires additional evidence from independent studies to confirm its role in refractive error regulation. However, the most highly significant SNPs in the lower education subset, rs12272341 and rs1939008, were statistically significant after correcting for the multiple SNPs tested in the current study ($P\text{-multi}=0.00057$ and $P\text{-multi}=0.0298$).

SNP rs1939008, if truly associated with refraction in all AREDS participants, was predicted to have a small effect on MSE: under the additive model, each additional minor (A) allele was associated with an average +0.16 D change in refractive error. After adjusting for age, sex and population substructure, rs1939008 accounted for less than 1% of the variation of MSE. This is considerably less than the ~4.8% variance explained by this SNP in Amish families.⁷

There are several differences between the AREDS cohort and Amish participants in our previous study which may explain discrepancies between the respective association results. First, AREDS participants (mean age=68 years; SD=4.7) were older than subjects in the Amish study (mean age=36.7; SD=17), which was composed of multigenerational families. Older subjects, having been exposed to environmental factors longer than younger individuals, are likely to differ in the relative amount of environmental variance contributing to the total phenotypic variance. Second, AREDS was a large, multicenter study whose participants were drawn from across the United States. Hence, considerable genetic and environmental heterogeneity was likely to exist across geographic regions. In contrast, the Old Order Amish are a culturally isolated and genetically endogamous religious group whose environment is comparatively homogeneous. Third, AREDS participants were highly educated, with only 6% not having completed high school and almost 40% having at least a Bachelor's degree. The Amish, on the other hand, receive no formal schooling beyond age sixteen. In our previous study, we speculated that differences in evidence for association between Amish and Ashkenazi Jewish families could have been due to differing environmental and behavioral influences. Orthodox Ashkenazi Jews, who place a strong emphasis on religious scholarship and devotional study thought to be risk factors for myopia development^{15, 16}, showed no evidence of association of MSE to 127 SNPs in 8 candidate regions which covered 11 MMP and 4 TIMP genes. We conjectured that, if differences in association signals could be attributed to disparities in environmental factors, "we would not expect replication of our findings in populations, such as South Asian Chinese and Japanese, with high prevalences of environmentally induced myopia".⁷ Indeed, Leung et al.¹⁰ found no evidence of association between MMP2 and high myopia among Southern Han Chinese. Moreover, in a study conducted in Japan, Nakanishi et al.⁹ concluded that four functional SNPs in MMP1, MMP2 and MMP3 promoter regions did not play critical roles in high myopia development. The current study lends credence to this hypothesis, wherein the effect of polymorphisms on ocular refraction is mediated by the environment. Our results show that strong statistical evidence of replication of SNPs originally identified in the Amish (rs1939008 and rs9928731) was present only in the lower education AREDS group. Little-

to-no evidence of association of candidate SNPs was seen in the more highly educated stratum. Hence, a statistical gene-environment interaction can explain our results, as well as findings from previous studies in different populations.^{7, 8, 10}

In comparison to the genetic effects explained by an association to rs1939008, intrinsic biological and environmental factors had more substantial effects on ocular refraction in the AREDS cohort. Women were on average +0.34 D more hyperopic than men ($p=0.001$); and, within the age range in AREDS, each decade was accompanied by a corresponding mean hyperopic shift of +0.42 D ($p=0.0005$). It should be noted, however, that the cross-sectional nature of the data does not allow us to distinguish between an age effect and a cohort effect (or secular trend). Nevertheless, our results with respect to age are consistent with previous reports of aging effects in longitudinal studies of older cohorts.^{17, 18}

The most significant predictor of MSE in our study was level of education, which was coded as an ordinal variable. Our linear model predicted that each incremental increase in degree of education was accompanied by a -0.24 D shift towards myopia (p for trend= 3.0×10^{-9}). Hence, it is clear that refractive development in this sample was mediated by environmental and behavioral main effects. These environmental effects, moreover, can be detected well beyond school age. Nevertheless, our measure of educational achievement explained only 2.5% of the total variance of ocular refraction, leaving a large fraction of the variation unexplained. It is likely that our crude surrogate for environmental factors does not fully capture the underlying environmental and complex behavioral influences on refractive development. However, the older age of the participants and the limited number of individuals with low educational achievement in the sample may also have played a role in this finding. Ocular refraction is known to be affected by a number of intrinsic biological factors, as well as by behavioral and environmental dynamics, the specifics of which are yet to be fully understood.¹⁹ Future investigations of myopia and refraction, including genome wide association (GWAS) and next-generation sequencing studies, should take these known risk factors and potential effect modifiers into account in the analyses of the effects of genetic variants.

Even though we find evidence of environmental interaction with rs1939008 and rs9928731 on ocular refraction, the exact mechanism through which the environment modifies the effect of variants localized to the MMP1–MMP10 intergenic or MMP2 regions on refraction remains unexplored. Though neither SNP represents a coding variant, both are located near regions significantly enriched for histone H3 lysine 27 acetylation (H3K27ac), a reliable chromatin marker of active enhancers.²⁰ Non-coding variants in these regions can disrupt regulatory function and, in some cases, have been postulated to cause disease phenotypes.²¹ Moreover, epigenetic regulation of gene expression via histone modification is cell-type specific. In our associated regions, H3K27ac is highly enriched in human fibroblasts (MMP2 region) and human keratinocytes (MMP1–MMP10 intergenic region). It is also possible that SNPs associated with MSE in this study are not causal, but are in linkage disequilibrium with the true causal variants. Marker rs1939008 tags a small haplotype block, but is also in longer-range linkage disequilibrium with intragenic MMP1 and MMP10 SNPs (Figure 1). In fact, our most significant association signal in the stratified analysis was located in an intronic SNP within MMP10 (rs12272341; $P_{\text{emp}}=0.0003$; $P_{\text{multi}}=0.0057$). MMP2 intronic SNP rs9928731, on the other hand, was not found to be in strong linkage disequilibrium with other tested markers (Figure 2) (although CEU HapMap data show that rs9928731 lies in a haplotype block that spans most of the MMP2 gene).

We conducted a replication study of genetic association of ocular refraction in an elderly cohort nested within the AREDS study. We found suggestive evidence of replication to an intergenic SNP (rs1939008) between MMP1 and MMP10. In a follow-up analysis, we found

that SNPs proximal to MMP1–MMP10 and MMP2 showed statistical evidence of interaction with educational attainment on refraction. Two SNPs (rs1939008 and rs9928731) which were first found to be associated with refraction in Amish families were also associated in a lower education AREDS group, but not in the higher education group. Our results, combined with data from previous studies, suggest that variants in MMP1–MMP10 and MMP2 genes may play a role in refractive variation in individuals not exposed to an environment favorable for myopia development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Linkage disequilibrium (LD) plot of single nucleotide polymorphisms (SNPs) in the MMP1–MMP10 region (chr11q22.2) for 1,912 participants from the Age Related Eye Disease Study (AREDS). Top: LD plot showing D-prime values. Bottom: LD plot showing r-squared values. The marker highlighted in blue represents a SNP found to be in association with ocular refraction in a study of Amish families. Highlighted green areas show approximate locations of MMP1 and MMP10 genes.

Figure 2. Linkage disequilibrium (LD) plot of single nucleotide polymorphisms (SNPs) in the MMP2 region (chr16q12.2) for 1,912 participants from the Age Related Eye Disease Study (AREDS). Top: LD plot showing D-prime values. Bottom: LD plot showing r-squared values. The marker highlighted in blue represents a SNP found to be in association with ocular refraction in a study of Amish families. Highlighted green areas show approximate locations of the MMP2 gene.

Table 1

Ocular refraction and age in all individuals and in the lower and higher educational attainment groups

	All Individuals					Lower Education Group					Higher Education Group				
	Mean	SD	Min	Max	Corr	Mean	SD	Min	Max	Corr	Mean	SD	Min	Max	Corr
RE Left Eye	0.56	2.17	-12.12	9	-0.15	0.81	2.07	-12.12	9	-0.04	0.16	2.26	-10.38	6.75	-0.06
RE Right Eye	0.54	2.21	-10.75	8.5	-0.16	0.82	2.1	-10.75	8.5	-0.05	0.12	2.3	-9.75	7.38	-0.06
RE Mean	0.55	2.14	-11.44	8	-0.16	0.82	2.04	-11.44	8	-0.05	0.14	2.23	-10.06	6.94	-0.06
Age	68.07	4.7	55.3	81.19	-0.08	68.34	4.69	55.3	81.19	-0.03	67.66	4.68	55.66	80	-0.04

RE= Refractive error; SD=Standard Deviation; Min=Minimum value, and Max=Maximum value; Corr=Correlation of each trait with the ordinal educational attainment variable.