Genetic Association between *MMP9* and Choroidal Neovascularization in Age-Related Macular Degeneration

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Purpose: To evaluate the first association specific to exudative age-related macular degeneration (AMD) located near the matrix metalloproteinase 9 (*MMP9*) gene.

Design: Genetic association study.

Participants: One thousand seven hundred twelve patients with AMD (672 nonexudative, 1040 exudative) of predominantly northern European descent seeking treatment at the University of Iowa Hospitals and Clinics.

Methods: We reanalyzed the International AMD Genetics Consortium (IAMDGC) data to validate the association of polymorphisms near *MMP9* with exudative AMD and to identify additional associated single nucleotide polymorphisms (SNPs), especially *MMP9* coding sequence SNPs. We genotyped a cohort of 1712 AMD patients from Iowa with 3 SNPs identified with our analysis of the IAMDGC cohort using commercially available real-time quantitative polymerase chain reaction (PCR) assays. Firth regression was used to measure the association between *MMP9* SNP genotypes and exudative AMD in our cohort of patients from Iowa. In addition, we developed a PCR-based assay to genotype the Iowa cohort at a short tandem repeat polymorphism (STRP) at the *MMP9* locus.

Main Outcome Measures: Odds ratios and *P* values for exudative compared with nonexudative AMD patients in the Iowa cohort for *MMP9* SNPs (rs4810482, rs17576, and rs17577) and STRP.

Results: We identified 3 SNPs in the *MMP9* locus (rs4810482, rs17576, and rs17577) that are highly associated with exudative AMD in patient cohorts of the IAMDGC. These *MMP9* SNPs also are associated with exudative AMD in the cohort of 1712 AMD patients from Iowa (rs4810482: odds ratio [OR], 0.82; *P* = 0.010; rs17576: OR, 0.86; *P* = 0.046; and rs17577: OR, 0.80; *P* = 0.041). We also genotyped the cohort of AMD patients from Iowa at rs142450006, another *MMP9* polymorphism that previously was associated with exudative AMD. We detected a 4bp STRP, (TTTC)$_n$, at the rs142450006 locus that is highly polymorphic and associated significantly with exudative AMD (OR, 0.78; *P* = 0.016).

Conclusions: This study independently confirms and expands an association between the *MMP9* locus and exudative AMD, further implicating a role for extracellular matrix abnormalities in choroidal neovascularization. *Ophthalmology Science 2021;1:100002 © 2020 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Age-related macular degeneration (AMD) is highly prevalent in elderly individuals living in the West and is one of the most feared diseases because of the risk of irreversible central vision loss involving the macula. Vision loss resulting from AMD occurs through 1 of 2 mechanisms: (1) geographic atrophy, whereby retinal photoreceptors, supporting retinal pigment epithelium, and choroidal vasculature slowly degenerate through a nonexudative process, and (2) choroidal neovascularization (CNV) resulting in relatively rapid vision loss resulting from new vessel growth under the retina in an exudative manner.

Age-related macular degeneration is a heterogeneous disease that is caused by the combined action of environmental and genetic risk factors. Smoking, diet (e.g., consumption of leafy green vegetables), and to a lesser degree, obesity and hypertension have been identified as environmental factors that influence the risk of AMD.[1–5] More than 30 genetic risk factors for AMD have been discovered with large genome-wide association studies, including polymorphisms in the complement factor H (*CFH*) gene and the age-related maculopathy susceptibility 2 (*ARMS2*) locus.[6–11] Many of these factors function in pathways that are important in the pathogenesis of macular degeneration, including complement biology (*CFH*, *C2/CFB*, *CFI*, *C3*, and *C9*) and extracellular matrix biology (*ADAMTS9*, *MMP9*, *MMP19*, and *TIMP3*).

Although many risk factors for AMD (of any type) development have been identified, only 1 gene, *MMP9*, has been associated specifically with development of exudative AMD. In 2015, the International AMD Genetics Consortium (IAMDGC) reported that a single nucleotide polymorphism (SNP), rs142450006, near the *MMP9* gene is

associated with exudative AMD.[12] An association between rs142450006 and progression to exudative AMD also was shown in the Age-Related Eye Disease Study cohort,[13] a subset of the full IAMDGC cohort used in the initial report.[12] Notably, a 2014 study of a Han Chinese patient population failed to detect an association between several SNPs near *MMP9* and either exudative AMD or polypoidal choroidal vasculopathy.[14] Thus, additional studies are needed to confirm the initial report of an association between *MMP9* and exudative AMD.

The genetic association between *MMP9* and exudative AMD has a plausible biological basis because of several features of this enzyme. First, *MMP9* encodes a protease that remodels extracellular matrix and basement membranes and interacts with collagens I and IV, elastin, and fibrinogen.[15] These substrates of MMP9 comprise Bruch's membrane,[16−18] a structure whose injury is well known to predispose individuals to CNV.[17,18] Second, both risk of AMD and activity of MMP9 in Bruch's membrane increase with age,[19] which suggests that increased MMP9 function and increased risk for AMD may be related. Third, MMP9 interacts with tissue inhibitor of metalloproteinases 3 (TIMP3),[20] which is encoded by the gene known to cause Sorsby fundus dystrophy, which almost uniformly results in CNV in patients at an early age.[21] Fourth, MMP9 is elevated in the plasma[22] and aqueous humor[23] of AMD patients with CNV. Finally, MMP9 is produced in lesions associated with mouse models of experimental CNV,[24] and mice that are *MMP9* deficient produce smaller CNV lesions than wild-type control mice.[24] Together, these biological data suggest that *MMP9* is involved in the pathogenesis of exudative AMD. In this study, we sought to identify an association between *MMP9* and exudative AMD (compared with nonexudative AMD) in an independent cohort of patients from Iowa and to confirm the initial discovery in the IAMDGC cohort.

Methods

Reanalysis of Initial Genetic Association

Data from the initial IAMDGC study,[12] including the complete imputed set of genotypes, clinical grades, and metadata for datasets phg000783.v1.p1 and phs001039.v1.p1, were downloaded from the Database of Genotypes and Phenotypes.[25] Sample- and patient-level metadata were retained (e.g., DNA_SOURCE and age). These data were converted to a Plink-compatible dataset, and Plink2 software (www.cog-genomics.org/plink/2.0)[26] was used to remove any variants with minor allele frequency less than 1% or for which more than 10% of genotypes were missing. To help correct for population stratification, principal components were computed using principal component analysis in Plink2 for use as population structure covariates. This final dataset then was analyzed similarly to the strategy used in Fritsche et al.[12] Specifically, comparisons among the IAMDGC participants that were classified as (1) no AMD, (2) intermediate AMD, and (3) exudative AMD were evaluated using a Firth regression in Plink2, with DNA_SOURCE, age, AMD risk alleles, and the first 3 population-level principal components included as covariates.

Selection of Proxy Single Nucleotide Polymorphisms

To confirm the original association between risk of exudative AMD developing and *MMP9*, we identified a set of SNPs in the *MMP9* locus that were associated with exudative AMD in the reanalysis of the IAMDGC dataset. We used a significance level of $\alpha = 10^{-5}$ to capture a broad sampling of the genetic variance. These SNPs then were evaluated using the LDLink's LDHap tool[27] based on European populations to remove redundant SNPs sequentially. The final set of rs4810482, rs17576, and rs17577 encompass the set of associated genotypes in the *MMP9* locus.

Identification and Phenotyping of the Iowa Age-Related Macular Degeneration Cohort

All patients provided written informed consent for this research study, which was approved by the University of Iowa Institutional Review Board and adhered to the tenets of the Declarations of Helsinki. The study included 1712 patients with AMD who were treated at the Retina Clinic of the University of Iowa Hospitals and Clinics. All patients underwent a complete eye examination, including measurement of Snellen visual acuity, slit-lamp bio-microscopy of the anterior segment and fundus, binocular indirect ophthalmoscopy, and spectral-domain OCT. Many patients underwent fundus photography, fluorescein fundus angiography, indocyanine green angiography, or OCT angiography performed when a diagnosis of neovascular AMD was in question. Careful history was obtained for all patients that included whether they had a history of intravitreal injections or laser therapy (thermal or photodynamic therapy[28]) for AMD. Two board-certified retina fellowship-trained specialists (E.H.S. and I.C.H.) reviewed all ophthalmic medical records and imaging data for each patient to determine whether they had any history of CNV, intravitreal injections, or laser treatment consistent with a current or past diagnosis of exudative AMD. Patients needed to have had CNV resulting from AMD in 1 eye to be categorized as having exudative AMD and were maintained in this category even if the CNV resolved with treatment. Patients with pigment epithelial detachment but no history of subretinal or intra-retinal fluid, injections, or laser therapy for CNV were classified as having nonexudative AMD. Patients with nonexudative AMD had to have at least multiple small or medium drusen and must never have been told they had any form of macular degeneration before 50 years of age. Patients with geographic atrophy but no evidence of fibrosis or a history of CNV treatment were classified as having nonexudative AMD. Patients with a diagnosis of polypoidal choroidal vasculopathy or retinal angiomatous proliferation were excluded from the study.

Genotyping

We genotyped cohorts of AMD patients at 3 SNPs (rs4810482, rs17576, and rs17577) in the *MMP9* locus using commercially available real-time quantitative polymerase chain reaction (PCR) assays following the manufacturer's protocol (TaqMan; Applied BioSystems) using a CFX96 PCR machine (BioRad), as we have described previously.[29] In the process of genotyping rs142450006, we determined that this SNP, which initially was described as a 4-bp insertion-deletion, actually lies within a short tandem repeat polymorphism (STRP) that is highly polymorphic. We discovered a tetranucleotide repeat sequence, $(TTTC)_n$, at this locus. Consequently, we developed a PCR-based assay to genotype AMD patients at this locus by determining the number of TTTC repeats. We used a standard PCR reaction (with forward primer

AAGTATGGGCTCTGGAGTAGGTTT and reverse primer AGAGGGAGACTCTGTCTGAAAAA) to amplify a DNA fragment containing the STRP. Next, we determined the number of tandem repeats at the *MMP9* locus in each patient's DNA sample using polyacrylamide gel electrophoresis and silver staining, as we have described previously.[30] The data that support the findings of this study are available on request from the corresponding author.

Confirmation of Association between *MMP9* and Exudative Age-Related Macular Degeneration in a Patient Cohort from Iowa

We compared the allele frequencies for each *MMP9* SNP between Iowa patients with exudative AMD and nonexudative AMD using a Firth regression in Plink2[26] to calculate the *P* value. A significance level of 0.017 was used to account for testing 3 hypotheses, which serves as a very conservative significance level because all 3 of the SNPs are in moderately high linkage disequilibrium with each other (pairwise $R^2 = 0.28$, 0.34, and 0.91, respectively; $D' > 0.90$).

Results

Reanalysis of International Age-Related Macular Degeneration Genetics Consortium Data Confirms That *MMP9* Alleles Confer Risk for Exudative Age-Related Macular Degeneration

To confirm the association between markers in the *MMP9* locus and exudative AMD first reported in Fritsche et al,[12] the full IAMDGC dataset was downloaded from the Database of Genotypes and Phenotypes and analyzed using the control (no AMD), nonexudative AMD (intermediate), and exudative AMD subsets. The results of this analysis are presented in Table 1 and Figure 1. As expected, no association was detected when comparing *MMP9* variations between control participants and nonexudative AMD patients, but strong associations were identified when comparing the control participants and exudative AMD patients ($P < 10^{-8}$), and the dry and wet AMD groups ($P < 10^{-7}$). The associated SNPs in the *MMP9* locus were distilled to 3 SNPs (rs4810482, rs17576, and rs17577) that are a nonredundant set of SNPs capable of reproducing the genetic variance in the *MMP9* locus. The association data for these 3 SNPs are shown in Table 1. These SNPs are not independent of each other, with genotype correlations (R^2) ranging from 0.28 to 0.91, and hence they represent a single association of genotype in the *MMP9* locus to development of exudative AMD. Based on the findings of Fritsche et al[12] and our reanalysis of the IAMDGC data, we focused our studies of the *MMP9* locus on comparing patients with nonexudative and exudative AMD to investigate and confirm the association between *MMP9* and exudative AMD further.

Construction of an Independent Age-Related Macular Degeneration Cohort

We assembled an independent cohort of AMD patients from the University of Iowa Hospitals and Clinics to validate the association between *MMP9* and exudative AMD reported by Fritsche et al.[12] A total of 1712 study participants with either exudative AMD (n = 1040) or nonexudative AMD (n = 672) were studied. The Iowa cohort showed a prevalence of exudative disease (60.7%), gender distribution (61.9% female), and average age at the most recent examination (80.5 years) that were similar to those features in the IAMDGC cohort. As shown in Table 2, the relative prevalence of women in the 2 cohorts is similar in the exudative AMD (62%) and nonexudative AMD (61%) populations in the Iowa cohort. The average age of study participants in the Iowa AMD cohort with exudative disease was slightly older.

rs142450006 Is a Tetrameric Short Tandem Repeat Polymorphism (TTTC)$_n$

We obtained Sanger sequence of a DNA segment spanning the SNP rs142450006 that is associated with exudative AMD in the IAMDGC cohort. We detected a repetitive sequence at the rs142450006 locus that consisted of numerous tandem repeats of the tetranucleotide sequence TTTC, which are known as STRPs. Moreover, we discovered that the number of tetranucleotide repeats in this STRP was highly polymorphic. When we typed 1117 of the 1712 AMD patients at this STRP, we identified 12 distinct alleles that each had a different number of tandem TTTC repeats, an example of which is shown in Figure 2.

Validation of the Association between the *MMP9* Locus and Exudative Age-Related Macular Degeneration in an Independent Cohort

We investigated the association of the 3 SNPs (rs4810482, rs17576, and rs17577), identified above as associated with exudative AMD in the IAMDGC dataset by genotyping the Iowa cohort of 1712 AMD patients (Table 3) for these SNPs. A statistical analysis of these SNPs provided nominal *P* values for an association between exudative AMD and 2 *MMP9* SNPs (rs17576 and rs17577) with a *P* value of less than 0.05 (uncorrected threshold for significance), whereas analysis of 1 *MMP9* SNP, rs4810482, produced a significant association, with a *P* value of less than 0.017 (Bonferroni-corrected threshold for significance). Thus, although not sufficient for genome-wide association, these results confirm the originally published association between exudative AMD and SNPs at the *MMP9* locus in an independent cohort of well-characterized AMD patients. We also genotyped a subset of Iowa AMD patients (nonexudative AMD, n = 427; exudative AMD, n = 690) at the tetranucleotide STRP at the rs142450006 locus, and we detected a significant association between the most common allele of the STRP and exudative AMD (OR, 0.78; 95% CI, 0.64−0.95; *P* = 0.016).

In summary, all 3 SNPs (rs4810482, rs17576, and rs17577) demonstrated association with exudative AMD ($P < 0.05$). The rs4810482 SNP met a Bonferroni-corrected significance level ($P < 0.017$), as did the previously undescribed STRP at the rs142450006 locus. All 4 markers (3 SNPs and 1 STRP) are in linkage disequilibrium with each other, confirming a single, robust association between the *MMP9* locus and exudative AMD.

Table 1. Summary of Associations to Exudative Age-Related Macular Degeneration in the International Age-Related Macular Degeneration Genomics Consortium Cohort

| | International Age-Related Macular Degeneration Genomics Consortium Cohort | | | | | |
| | Control Participants vs. Nonexudative AMD Patients | | Control Participants vs. Exudative AMD Patients | | Nonexudative vs. Exudative AMD Patients | |
Marker	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value
rs4810482 (C) 20:44,634,550	1.01 (0.96−1.15)	0.82	0.88 (0.84−0.92)	5.8×10^{-9}	0.87 (0.83−0.91)	1.3×10^{-7}
rs17576 (G) 20:44,640,225	1.02 (0.97−1.07)	0.44	0.88 (0.84−0.92)	3.5×10^{-9}	0.86 (0.81−0.90)	3.8×10^{-9}
rs17577 (A) 20:44,643,111	0.97 (0.91−1.04)	0.44	0.82 (0.77−0.88)	7.2×10^{-10}	0.83 (0.77−0.89)	1.7×10^{-7}

AMD = age-related macular degeneration; CI = confidence interval.
A summary of association results from reanalysis of the full International Age-Related Macular Degeneration Genomics Consortium dataset are presented for several single nucleotide polymorphisms (SNPs) in the MMP9 locus. Marker names are followed by their effect allele. Associations with these SNPs were reevaluated to verify that the associations are specific to exudative forms of AMD. All 3 SNPs are found not to be associated when comparing control individuals with patients with intermediate AMD. In contrast, comparisons between control participants or intermediate AMD patients with those with exudative AMD both yielded significant results. Chromosome positions are noted for each marker in the GRCh37 human reference genome.

Discussion

It previously was established that SNPs near the MMP9 gene are associated with AMD.[12,31] Some studies also suggest that variants at the MMP9 locus are associated with exudative AMD.[12−14] This study found compelling supportive evidence for an association between SNPs in the MMP9 locus and exudative AMD, providing confirmation of an association between the MMP9 locus and exudative AMD in an independent cohort. To achieve this, we reanalyzed the original dataset of the IAMDGC and confirmed the prior report[12] that a polymorphism in the MMP9 locus
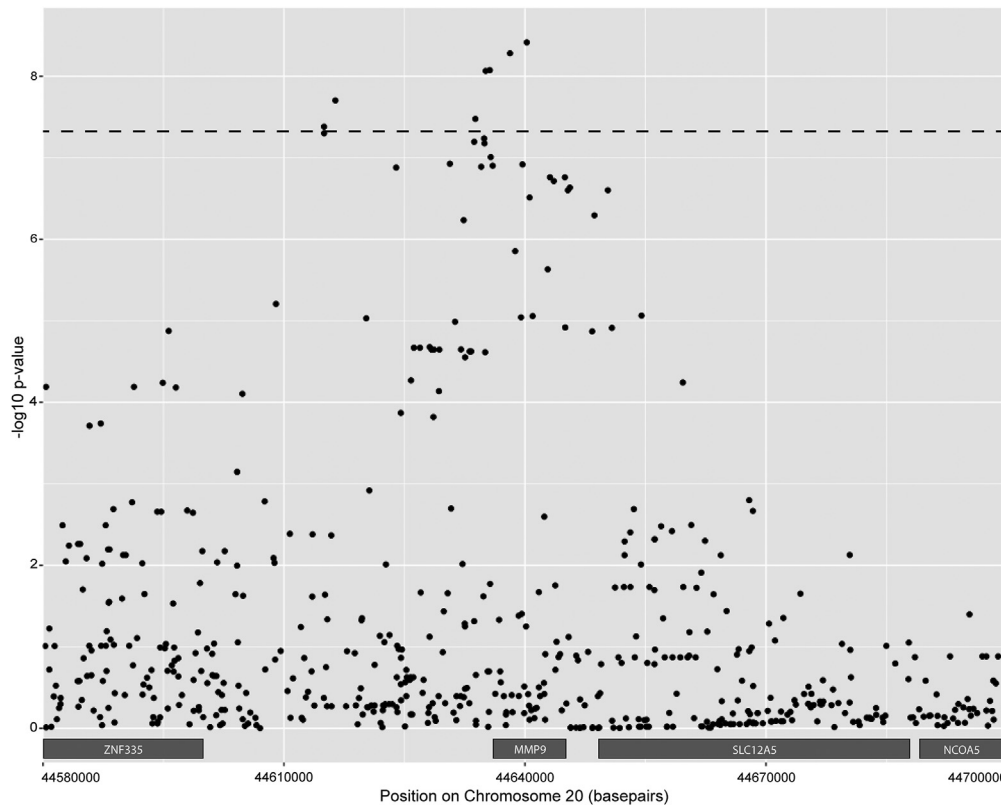

Figure 1. Associations to exudative age-related macular degeneration (AMD) at the MMP9 locus. The association of single nucleotide polymorphisms to exudative AMD is presented as the log-transformed P value versus position within the locus, with a dashed line indicating the threshold of genome-wide significance (5×10^{-8}). The positions of the genes in the immediate vicinity of MMP9 also are shown.

Table 2. Demographic Characteristics of the Iowa Age-Related Macular Degeneration Cohort

Age-Related Macular Degeneration Form	Patient Gender		Age (yrs)	
	Female	Male	Average	Standard Deviation
Nonexudative	408	264	77.1	10.95
Exudative	651	389	82.7	8.47

This table presents a summary of demographic information for the Iowa age-related macular degeneration cohort for the exudative and non-exudative forms.

(rs142450006) is associated with exudative AMD. We selected 3 other SNPs in *MMP9* (rs4810482, rs17576, and rs17577) that also are associated with exudative AMD in the IAMDGC cohort. Next, we analyzed a large, independent cohort of 1712 AMD patients from Iowa. We determined that the DNA sequence variation at this locus (rs1452450006) includes at least 12 different alleles that consist of different numbers of tandemly repeated tetranucleotide sequences, (TTTC)$_n$, rather than the previously described 2 alleles (T/TTTTC).[12] When we genotyped our cohort of AMD patients from Iowa at this STRP, we demonstrated that alleles of this marker at the rs142450006 locus are associated with exudative AMD. Furthermore, we report that 3 additional SNPs in *MMP9* (rs17577, rs17576, and rs4810482, all of which are in linkage disequilibrium with the imputed SNP rs142450006 originally reported[12]) also are associated with exudative AMD. Replication is an essential step in establishing true genetic associations. Our report provides the first independent replication of the association between the *MMP9* locus and exudative AMD and confirms the validity of this important discovery.

The *MMP9* gene encodes matrix metallopeptidase 9, also known as 92-kDa type IV collagenase, 92-kDa gelatinase, or gelatinase B, which is part of a family of zinc metalloproteinases associated with degradation of the extracellular matrix. The breakdown of the extracellular matrix is a critical component in a variety of physiologic processes, including wound healing, innate immune defense, and angiogenesis.[32–38] Gene expression studies show that the most prominent cellular source of *MMP9* RNA in the aging eye is choroidal macrophages.[39] *MMP9* has several known substrates in Bruch's membrane (elastin and collagen IV) that form an angiogenic barrier, which suggests that excess *MMP9* expression may promote angiogenesis and exudative AMD. Several observations and prior investigations support this hypothesis. First, increased levels of pro-MMP9 have been detected in choroid and retinal pigment epithelium preparations from human eyes with exudative AMD.[40] Second, increased abundance of elastin-derived peptides have been detected in the sera of patients with exudative AMD, which is consistent with increased MMP9 activity as well as degradation of elastin and potentially Bruch's membrane origin.[41,42] Third, transgenic mice with *MMP9* deficiency (knockout mice) exhibit reduced angiogenesis in nonocular tissues, suggesting that excess MMP9 may promote angiogenesis.[32] Fourth, mutation of *TIMP3*, which encodes a principal inhibitor of MMP9, causes Sorsby fundus dystrophy, an autosomal dominant maculopathy characterized by extensive
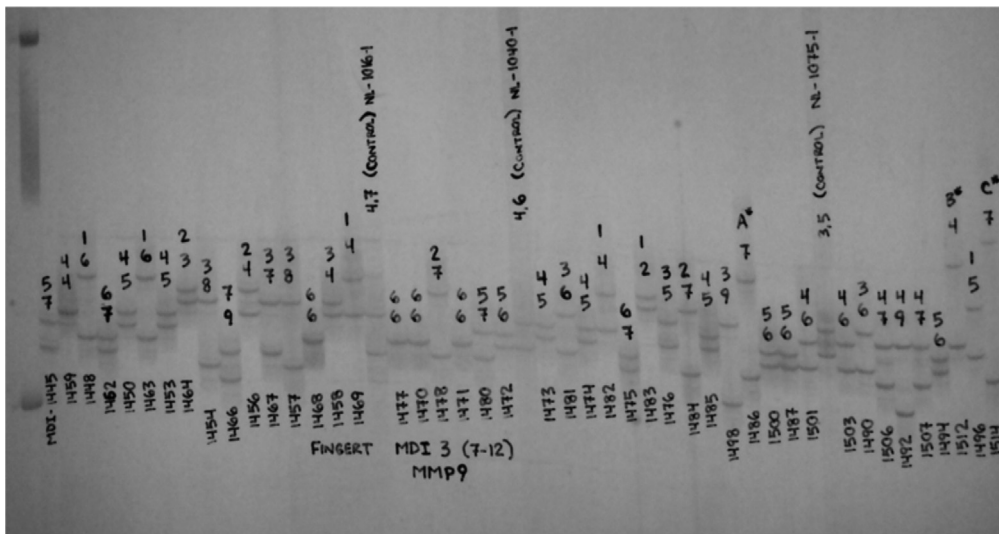

Figure 2. Short tandem repeat polymorphism (STRP) at the rs142450006 locus. The STRP at the rs142450006 locus consists of tandem repeats of the tetranucleotide sequence TTTC. We amplified the STRP at the rs142450006 locus using DNA samples from 48 individuals in a polymerase chain reaction (PCR) analysis. The length of the PCR product is proportional to the number of TTTC repeats in each individual's genome at the rs142450006 locus (1 number of repeats for each of an individual's 2 chromosomes). Polymerase chain reaction products were separated based on their length via polyacrylamide gene electrophoresis and silver staining. Polymerase chain reaction products with the fewest number of TTTC repeats migrate the fastest through the gel and have migrated furthest (toward the bottom of the gel), whereas PCR products with the most number of TTC repeats migrate the slowest through the gel and have migrated the shortest distance (toward the top of the gel). Twelve different numbers of TTTC repeats were identified that were numbered arbitrarily 1 through 9 and A, B, and C on this gel. One individual's amplified DNA was loaded into each of the 48 lanes in the gel. The 2 bands in each lane represent how many TTTC repeats are present in the genotype (unless the individual has the same number of repeats on both chromosomal copies of the rs142450006 locus).

Table 3. Summary of Single Nucleotide Polymorphism Associations to Exudative Age-Related Macular Degeneration

| | Minor Allele Frequency | | | Nonexudative vs. Exudative AMD in Iowa Cohort | |
Marker	gnomAD	Nonexudative	Exudative	Odds Ratio (95% Confidence Interval)	P Value
rs4810482 (C) 20:44,634,550	0.383	0.381	0.337	0.82 (0.71−0.95)	0.010
rs17576 (G) 20:44,640,225	0.356	0.375	0.341	0.86 (0.75−0.99)	0.046
rs17577 (A) 20:44,643,111	0.148	0.153	0.128	0.80 (0.67−0.99)	0.041

AMD = age-related macular degeneration.
This table presents a summary of the 3 single nucleotide polymorphisms (SNPs) validating the association of variants in the MMP9 locus to the exudative form of AMD in the Iowa cohort. Marker names are followed by their effect allele. Minor allele frequency is presented for the European (non-Finnish) population in gnomAD, as well as for the nonexudative and exudative AMD patients of the Iowa cohort. The odds ratios and P values from the association analysis of these 3 SNPs between exudative and nonexudative AMD in the Iowa cohort are presented. Chromosome positions are noted for each marker in the GRCh37 human reference genome.

neovascularization.[43] These observations are consistent with the assertion that variants in the MMP9 locus may confer risk for exudative AMD by increasing production of MMP9 and its proangiogenic activity.

However, it is possible that the association with exudative AMD is the result of polymorphisms in the MMP9 gene that alter the structure of the protein it encodes. A total of 4 non-synonymous cSNPs, rs1805088, rs17576, rs2250889, and rs17577, are located in the coding sequence of MMP9. Reanalysis of the IAMDGC data[12] shows that only rs17576 and rs17577 are associated with the neovascular phenotype ($P < 10^{-6}$). The effect of either SNP on MMP9 protein structure may be the cause for the increased risk of neovascular AMD developing. The rs17576 cSNP encodes an A-to-G change that alters the 279th amino acid in MMP9 from glutamine to arginine (Q279R). In our reanalysis of the IAMDGC dataset, rs17576 showed the strongest statistical association with exudative AMD ($P = 3.8 \times 10^{-9}$). Additionally, analysis of the Q279R variation with mutation algorithms (SIFT,[44] Polyphen-2,[45] and BLOSUM62[46]) all suggest that it is a relatively benign variant. The second cSNP, rs17577, encodes a G-to-A change that alters the 668th amino acid of MMP9 from arginine to glutamine (R668Q). In our reanalysis of the IAMDGC dataset, this SNP is strongly supportive of association with exudative AMD in the region, with an uncorrected P value of 1.7×10^{-7}. The rs17577 variation commonly is observed in gnomAD, ranging from 30% in the South Asian population to 6.9% in the Latino population. Analysis with SIFT, Polyphen-2, and BLO-SUM62 algorithms all suggest that the R668G variant is relatively benign. These 2 cSNPs are in linkage disequilibrium with each other, with R^2 of 0.34 in the European population and R^2 of 0.27 when assessed across all populations using the LDpair tool in the The National Cancer Institute's LDLink data resource.[27]
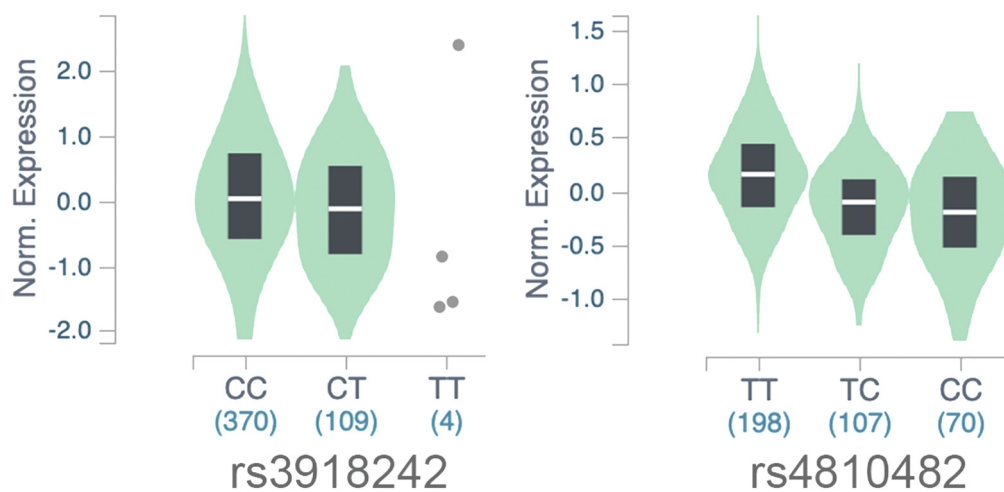
Figure 3. Expression QTLs for MMP9. The distribution of normalized expression values from Genotype-Tissue Expression's collection of cultured fibroblasts are presented as a violin plot. The expression values are stratified by genotype for rs3918242 and rs4810482 ($P = 8.7 \times 10^{-6}$ and $P = 3.3 \times 10^{-11}$, respectively). This figure shows that the expression of MMP9 is higher in samples with the homozygous reference (TT) genotype than for heterozygous (TC) and homozygous alternate (CC) genotypes.

Additional study of the Q279R and R668G variants are needed to judge their potential functional consequences and pathogenicity.

Of note, the alleles of the evaluated SNPs vary greatly across ethnic populations in gnomAD. For example, the minor allele frequency of rs17576 ranges from 22.6% in the Latino population to 74.5% in the East Asian population, which suggests that the contribution of this locus to exudative AMD varies among ethnic groups. Alternatively, the underlying causal variant of the association identified by the IAMDGC[12] and confirmed in this study may not be present in the general Han Chinese population. These are potential reasons that Zeng et al[14] did not find an association of *MMP9* polymorphisms with exudative AMD. In addition, the small change in allele frequency identified in this study (as noted in Table 3), coupled with the relatively small number of participants in the study by Zeng et al[14] (157 with exudative AMD and 204 control participants) provides limited statistical power and also may contribute to why they failed to identify an association between *MMP9* and exudative AMD.

In addition to amino acid changing variants, several of the CNV risk-associated SNPs are associated with changes in *MMP9* transcription. The most well known is rs3918242, which is a C-to-T change 1571 base pairs upstream of the transcription start site of the *MMP9* transcript in RefSeq (NM_004994.2). Although not the most significant expression quantitative trait loci (eQTL) association for *MMP9* in Genotype-Tissue Expression,[47] with a *P* value of 8.7×10^{-6} in cultured fibroblasts, it has been reported broadly as being associated with expression changes in *MMP9*. For example, Zhang et al[48] reported that rs3918242 has a functional effect on transcription and that it is associated with the severity of atherosclerosis in patients with coronary artery disease. In fact, several SNPs (e.g., rs6017721 and rs3848722) that are associated with increased risk of CNV in patients with AMD are in eQTLs with *MMP9* expression. Examples of the changes in expression based on rs3918242 and rs4810482 are presented in Figure 3.

The SNPs on chromosome 20 that are associated with exudative AMD are either within the *MMP9* gene or closer to *MMP9* than to other known genes, which suggests that the association is the result of altered expression or function of *MMP9*. However, we cannot fully exclude the possibility that the association is the result of alterations in a neighboring gene, rather than *MMP9*. From the Genotype-Tissue Expression resource, we know that SNPs in the *MMP9* locus alter expression of neighboring genes including *PLTP, SLC12A5, NEURL2, ZSWIM1, SNX21, RPL13P2, ZNF335, SPATA25, PCIF1,* and *CD40*.[47] Notably, 2 of these genes, *PLTP* and *CD40*, have been linked with neovascularization[49−52] and are plausible candidates for promoting exudative AMD.

We found the *MMP9* locus was associated significantly with exudative AMD when compared with control participants and with nonexudative AMD patients, that is, no genetic association was seen between control participants and nonexudative AMD patients. This contrasts with other AMD risk loci such as *CFH*[6−9] and *ARMS2/HTRA1*,[11,53,54] which have been confirmed by many groups to be associated with both exudative and nonexudative forms of AMD. Several investigators have found that the *ARMS2* locus has a stronger effect for polypoidal choroidal vasculopathy and choroidal neovascularization compared with nonexudative AMD.[55−60] That *ARMS2* is associated with both exudative and nonexudative AMD was highlighted by in vitro studies demonstrating increased proliferation and inhibition of cell migration with wild-type *ARMS2* and A69S mutants, but no difference was observed between the wild-type and mutant in tube formation in RF/6A cells. Thus, although *ARMS2* mutation increases the risk of having any form of AMD and brings a higher risk for exudative AMD and polypoidal choroidal vasculopathy, the mechanism for causing CNV alone is less clear than the mechanisms described above for *MMP9*. Similar to *MMP9*'s putative role in the eye, alterations in *HTRA1/ARMS2* also result in changes in extracellular matrix components.[61−63] It is possible that *ARMS2* is involved in 2 distinct pathways, one related to AMD development and another related to neovascularization. Alternatively, *ARMS2*'s increased risk for neovascular AMD may be caused by earlier disease onset, leading to increased amounts of advanced disease. Taken together, although extracellular matrix abnormalities are involved with *ARMS2* and the pathogenesis of AMD, *MMP9* seems to have a unique, specific role in exudative AMD that warrants further exploration.

Limitations of this study include the lack of robust data on environmental factors such as smoking and cardiovascular disease that could result in bias of our results. However, smoking status was accounted for when Yan et al[13] found that CNV progression was associated with *MMP9* in the Age-Related Eye Disease Study population, a subgroup of the IAMDGC cohort.[12] This study lacked the high-density SNP data required to impute haplotypes that may have increased the power to discriminate further the specific causal variation underlying the risk of exudative AMD developing.

In conclusion, we validated the association of the *MMP9* gene on chromosome 20 with the development of exudative AMD, including the description of a previously unappreciated STRP in the locus. The discovery that *MMP9* is a risk factor for exudative disease provides new insights into pathogenesis of AMD. Further studies of MMP9 and its retinal substrates may reveal new biological pathways and therapeutic targets that have the potential to prevent acute and catastrophic retinal damage from developing in AMD patients. However, additional experiments are necessary to characterize the specific DNA sequence changes better in the chromosome 20 locus that underlie the increased risk for exudative AMD.

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Footnotes and Disclosures

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