CrossMark

American Academy of Ophthalmology
The Eye M.D. Association

MMP20 and *ARMS2/HTRA1* Are Associated with Neovascular Lesion Size in Age-Related Macular Degeneration

*Yumiko Akagi-Kurashige, MD,[1,2] Kenji Yamashiro, MD, PhD,[1] Norimoto Gotoh, MD, PhD,[1,2] Masahiro Miyake, MD,[1,2] Satoshi Morooka, MD,[1] Munemitsu Yoshikawa, MD,[1,2] Isao Nakata, MD, PhD,[1,2] Kyoko Kumagai, MD, PhD,[1] Akitaka Tsujikawa, MD, PhD,[1] Ryo Yamada, MD, PhD,[2] Fumihiko Matsuda, PhD,[2] Masaaki Saito, MD, PhD,[3] Tomohiro Iida, MD, PhD,[4] Masako Sugahara, MD,[5] Yasuo Kurimoto, MD, PhD,[5] Ching-Yu Cheng, MD, PhD,[6,7,8,9] Chiea-Chuen Khor, MBBS, PhD,[6,10,11] Tien-Yin Wong, FRCS, PhD,[6,7,8,9] Nagahisa Yoshimura, MD, PhD,[1] on behalf of the Nagahama Cohort Research Group**

Purpose: Age-related macular degeneration (AMD) is the leading cause of severe visual impairment. Despite treatment, a central scotoma often remains. The size of the scotoma depends on the lesion size of the choroidal neovascular membrane and significantly affects the patient's quality of life, and the lesion size of neovascularization also affects response to treatments. The aim of this study was to identify genes associated with the neovascular lesion size in neovascular AMD.

Design: A genome-wide association study (GWAS).

Participants: We included 1146 Japanese patients with neovascular AMD.

Methods: We performed a 2-stage GWAS for the lesion size of AMD as a quantitative trait among 1146 (first stage: 727, second stage: 419) Japanese patients with neovascular AMD. Lesion size was determined by the greatest linear dimension measured with fluorescein angiography examination before treatment. We examined the association between the genotypic distribution of each single nucleotide polymorphism (SNP) and the trait using an additive model adjusted for age and sex. To evaluate the associations between AMD development and SNPs associated with lesion size, we also performed a case-control study by using the genotype data from these 1146 Japanese patients as case subjects and the fixed dataset from the Nagahama Study as control subjects.

Main Outcome Measures: Genes associated with the lesion size in neovascular AMD.

Results: In the discovery stage, rs10895322 in *MMP20* showed a genome-wide significant *P* value of 6.95×10^{-8}, and rs2284665 in *ARMS2/HTRA1* showed a *P* value of 1.55×10^{-7}. The associations of these 2 SNPs were successfully replicated in the replication stage, and a meta-analysis of both stages showed genome-wide significant *P* values (2.80×10^{-9} and 4.41×10^{-9}, respectively). In a case-control study using 3248 Japanese subjects as controls, we could not find contribution of *MMP20* rs10895322 for AMD development. Although MMP20 has been thought to be expressed only in dental tissues, we confirmed MMP20 expression in the human retina and retinal pigment epithelium/choroid with polymerase chain reaction.

Conclusions: The growth of choroidal neovascularization in AMD would be affected by 2 genes: *MMP20*, a newly confirmed gene expressed in the retina, and *ARMS2/HTRA1*, a well-known susceptibility gene for AMD. *Ophthalmology 2015;122:2295-2302 © 2015 by the American Academy of Ophthalmology.*

*Supplemental material is available at www.aaojournal.org.

Age-related macular degeneration (AMD) is the leading cause of severe visual impairment among people older than 50 years of age in industrialized countries. Although the cause of AMD is still unclear, it has been shown that the neural retina is damaged by atrophy or the intrusion of choroidal neovascularization (CNV) into the retinal tissue. The development of CNV depends on vascular endothelial growth factor (VEGF), which is similar to neovascularization occurring in other tissues. Therefore, neovascular AMD is currently treated with anti-VEGF drugs, which were originally developed as antitumor drugs.[1,2] However, visual acuity after anti-VEGF treatment is sometimes unsatisfactory, and a central blind spot (scotoma) corresponding to the CNV scar often remains. Further elucidation of mechanisms to control the growth of neovascular tissue will potentially define novel targets that may be used to improve neovascular AMD treatments and that may be applied for treating tumors.

Age-related macular degeneration is a complex disease that is caused by the combination of genetic and

http://dx.doi.org/10.1016/j.ophtha.2015.07.032
ISSN 0161-6420/15

environmental factors. In 2005, a genome-wide association study (GWAS) using 146 participants found that the *CFH* gene is strongly associated with AMD,[3] and *ARMS2/HTRA1* was identified as the second major susceptibility gene for AMD.[4,5] Since then, several genes have been reported to be associated with AMD, such as *C2/CFB*, *C3*, *CFI*, *CETP*, and *VEGFA*.[6] Recently, the AMD Gene Consortium performed a large-scale GWAS and found 7 new loci associated with AMD and confirmed 12 previously reported loci.[7]

After the discovery of strong genetic associations to AMD development, several intensive phenotype−genotype studies have been performed.[8−17] Among various phenotypes, the size of CNV is one of the most important phenotypes. Large CNV size leads to large scotoma, which strongly affects the patients' quality of life.[18] Furthermore, CNV lesions also affect the patient's response to anti-VEGF treatment, such that eyes with larger sized lesions generally have poorer therapeutic responses.[19,20] To examine the genetic determinants of CNV lesion size in neovascular AMD, previous studies have evaluated associations of lesion size with a number of AMD susceptibility genes, such as *CFH*, *ARMS2/HTRA1*, and *VEGFA*.[8−14,21] Among these genes, several studies have reported that *ARMS2/HTRA1* was significantly associated with the lesion size,[9,11,12] whereas one report negated this association.[14] So far, whether other genes affect the size of CNV lesions remain unknown. Therefore, we performed a GWAS for lesion size in a Japanese population to identify genes associated with lesion size in patients with neovascular AMD.

Methods

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

Patients and Study Methods

A 2-staged GWAS on neovascular lesion size was performed, using 1146 Japanese patients with AMD recruited from the Kyoto University Hospital, Fukushima Medical University Hospital, and Kobe City Medical Center General Hospital. All patients were treatment naïve. Patients from Kyoto University Hospital were examined for the discovery stage, and patients from Fukushima Medical University Hospital and the Kobe City Medical Center General Hospital were examined for the replication stage. For the diagnosis of AMD, all patients received a complete ophthalmic examination before treatment, including measurement of best-corrected visual acuity, indirect ophthalmoscopy and slit-lamp biomicroscopy with a contact lens by a retina specialist, and fluorescein and indocyanine green angiography (HRA-2; Heidelberg Engineering, Heidelberg, Germany). Two subtypes of AMD, typical AMD (tAMD) and polypoidal choroidal vasculopathy (PCV), were included for the analysis. Lesion size was determined by the greatest linear dimension (GLD) measured with fluorescein angiography examination before treatment. The GLD was determined as a lesion with any classic or occult CNV in eyes with AMD. When a contiguous retinal pigment epithelium (RPE) detachment or subretinal hemorrhage was observed, it was included in the lesion according to the Treatment of age-related macular degeneration with photodynamic therapy (TAP) Study protocol.[22] In patients with bilateral AMD, eyes with larger CNV size were chosen for the analysis. Patients displaying any of the following characteristics were excluded from the study: (1) high myopia (spherical equivalent <-6.00 diopters), (2) geographic atrophy or drusen alone, or (3) an old lesion without a clear diagnosis. All subjects in this cohort were unrelated and of Japanese ethnicity.

To evaluate the associations between AMD development and single nucleotide polymorphisms (SNPs) associated with lesion size, we performed a case-control study by using the genotype data from these 1146 Japanese patients as case subjects and the fixed dataset from the Nagahama Study as control subjects (Supplemental Information, available at www.aaojournal.org).

Genotyping and Imputation

Genomic DNA was prepared from peripheral blood using a DNA extraction kit (QuickGene-610L; Fujifilm, Tokyo, Japan). For the samples from Kyoto University, genome-wide genotyping was performed using HumanOmni2.5-8 or HumanOmniExpress (Illumina Inc, San Diego, CA). The SNPs genotyped on both platforms were included for the analysis. To ensure high-quality genotype data, a series of quality control filters were applied to the data, including minor-allele frequency cutoffs (>0.01), genotypic success rate ($>90\%$), individual call rate ($>90\%$), and estimated relatedness (PI-HAT <0.35). Quality control was performed using PLINK (version 1.07; http://pngu.mgh.harvard.edu/,purcell/plink; accessed April 11, 2015). The fixed dataset consisted of 580 936 SNPs in 727 individuals. For the replication stage, 419 samples from Fukushima Medical University Hospital or the Kobe City Medical Center General Hospital were genotyped using the Illumina HumanOmniExpress. We did not consider deviations in genotype distributions from the Hardy−Weinberg equilibrium because all samples were from patients with AMD.

To evaluate the association between lesion size and AMD susceptibility SNPs that were previously reported by the AMD Gene Consortium,[7] we performed a case-control study using imputed genotypes referencing 1000 genomes project cosmopolitan haplotypes (1092 samples from all over the world; available at IMPUTE2 website [https://mathgen.stats.ox.ac.uk/impute/impute_v2.html], accessed April 11, 2015) after stringent quality control. Among the previously reported AMD susceptibility SNPs by the AMD Gene Consortium, rs5749482 in *TIMP3* was not imputed because it could not be identified. In addition, we chose rs800292 in *CFH*, rs2241394 in *C3*, and rs3764261 in *CETP*, because these SNPs showed the strongest association in each gene in the Asian population,[23] instead of previously reported SNPs of rs10737680 in *CFH*, rs2230199 in *C3*, and rs1864163 in *CETP*.

Polymerase Chain Reaction for *MMP20*

Human retinal complementary DNA (cDNA) and RPE/choroid cDNA were obtained from ScienCell Research Laboratories (Carlsbad, CA) and BioChain Institute, Inc. (Newark, CA). The polymerase chain reaction (PCR) reactions were performed under the following conditions: initial denaturation at 94°C for 2 minutes, followed by 40 cycles at 94°C for 15 seconds, 52°C for 15 seconds, and polymerization at 72°C for 60 seconds.

The PCR primers were as follows: 5′-TTTCTGGAGACGGC AGGTTCAC −3′ (forward), 5′-GGCCTTTGGAAGAATCCCTC TC −3′ (reverse).

The PCR products were subjected to electrophoresis on 2.0% agarose gels and visualized by ethidium bromide staining. Glyceraldehyde 3-phosphate dehydrogenase was used as an internal control for cDNA quantification.