ceptors as well as an indirect effect via the recruitment of monocytes.

In conclusion, we demonstrate that TNFα upregulation after RD plays a critical role in photoreceptor degeneration. The neurotoxic effects of TNFα on photoreceptors are mediated through its chemotactic properties, which lead to monocyte recruitment and monocyte-generated oxidative stress and possibly by a direct effect of TNFα on the photoreceptors. Blockade of TNFα and/or its receptors may provide new therapeutic avenues to treat photoreceptor degeneration in the setting of RD and of other retinal disorders that share common features.

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References

1. Dunaief JL, Dentchev T, Ying GS, Milam AH. The role of apoptosis in age-related macular degeneration. *Arch Ophthalmol.* 2002;120:1435–1442.
2. Arroyo JG, Yang L, Bula D, Chen DF. Photoreceptor apoptosis in human retinal detachment. *Am J Ophthalmol.* 2005;139:605–610.
3. Chang CJ, Lai WW, Edward DP, Tso MO. Apoptotic photoreceptor cell death after traumatic retinal detachment in humans. *Arch Ophthalmol.* 1995;113:880–886.
4. Cook B, Lewis GP, Fisher SK, Adler R. Apoptotic photoreceptor degeneration in experimental retinal detachment. *Invest Ophthalmol Vis Sci.* 1995;36:990–996.
5. Barber AJ, Lieth E, Khin SA, Antonetti DA, Buchanan AG, Gardner TW. Neural apoptosis in the retina during experimental and human diabetes: early onset and effect of insulin. *J Clin Invest.* 1998;102:783–791.
6. Fulton AB, Hansen RM, Petersen RA, Vanderveen DK. The rod photoreceptors in retinopathy of prematurity: an electroretinographic study. *Arch Ophthalmol.* 2001;119:499–505.
7. Doonan F, Cotter TG. Apoptosis: a potential therapeutic target for retinal degenerations. *Curr Neurovasc Res.* 2004;1:41–53.
8. Zacks DN, Hanninen V, Pantcheva M, Ezra E, Grosskreutz C, Miller JW. Caspase activation in an experimental model of retinal detachment. *Invest Ophthalmol Vis Sci.* 2003;44:1262–1267.
9. Zacks DN, Zheng QD, Han Y, Bakhru R, Miller JW. FAS-mediated apoptosis and its relation to intrinsic pathway activation in an experimental model of retinal detachment. *Invest Ophthalmol Vis Sci.* 2004;45:4563–4569.
10. Hisatomi T, Nakazawa T, Noda K, et al. HIV protease inhibitors provide neuroprotection through inhibition of mitochondrial apoptosis in mice. *J Clin Invest.* 2008;118:2025–2038.
11. Nakazawa T, Hisatomi T, Nakazawa C, et al. Monocyte chemoattractant protein 1 mediates retinal detachment-induced photoreceptor apoptosis. *Proc Natl Acad Sci U S A.* 2007;104:2425–2430.
12. Nakazawa T, Matsubara A, Noda K, et al. Characterization of cytokine responses to retinal detachment in rats. *Mol Vis.* 2006;12:867–878.
13. Brouckaert P, Libert C, Everaerdt B, Takahashi N, Cauwels A, Fiers W. Tumor necrosis factor, its receptors and the connection with interleukin 1 and interleukin 6. *Immunobiology.* 1993;187:317–329.
14. Moss ML, Jin SL, Becherer JD, et al. Structural features and biochemical properties of TNF-alpha converting enzyme (TACE). *J Neuroimmunol.* 1997;72:127–129.
15. Tartaglia LA, Goeddel DV. Two TNF receptors. *Immunol Today.* 1992;13:151–153.
16. Shohami E, Ginis I, Hallenbeck JM. Dual role of tumor necrosis factor alpha in brain injury. *Cytokine Growth Factor Rev.* 1999;10:119–130.
17. El-Ghrably IA, Dua HS, Orr GM, Fischer D, Tighe PJ. Detection of cytokine mRNA production in infiltrating cells in proliferative vitreoretinopathy using reverse transcription polymerase chain reaction. *Br J Ophthalmol.* 1999;83:1296–1299.
18. Limb GA, Hollifield RD, Webster L, Charteris DG, Chignell AH. Soluble TNF receptors in vitreoretinal proliferative disease. *Invest Ophthalmol Vis Sci.* 2001;42:1586–1591.
19. Durrani K, Ahmed M, Foster CS. Adamantiades-Behcet disease: diagnosis and current concepts in management of ocular manifestations. *Compr Ophthalmol Update.* 2007;8:225–233.
20. Adan A, Sanmarti R, Bures A, Casaroli-Marano RP. Successful treatment with infliximab in a patient with diffuse subretinal fibrosis syndrome. *Am J Ophthalmol.* 2007;143:533–534.
21. Weiss K, Rieger R, Keitzer R, Pleyer U. Successful infliximab treatment of posterior scleritis in a 13-year-old child refractory to other immunosuppressive therapy. *Graefes Arch Clin Exp Ophthalmol.* 2007;245:1735–1737.
22. Japiassu RM, Brasil OF, Cunha AL, de Souza EC. Regression of vasoproliferative tumor with systemic infliximab. *Ophthalmic Surg Lasers Imaging.* 2008;39:348–349.
23. Theodossiadis PG, Liarakos VS, Sfikakis PP, Vergados IA, Theodossiadis GP. Intravitreal administration of the anti-tumor necrosis factor agent infliximab for neovascular age-related macular degeneration. *Am J Ophthalmol.* 2009;147:825–830, 830 e821.
24. Nakazawa T, Takeda M, Lewis GP, et al. Attenuated glial reactions and photoreceptor degeneration after retinal detachment in mice deficient in glial fibrillary acidic protein and vimentin. *Invest Ophthalmol Vis Sci.* 2007;48:2760–2768.
25. She H, Nakazawa T, Matsubara A, et al. Photoreceptor protection after photodynamic therapy using dexamethasone in a rat model of choroidal neovascularization. *Invest Ophthalmol Vis Sci.* 2008;49:5008–5014.
26. Stoll G, Jander S, Schroeter M. Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. *Adv Exp Med Biol.* 2002;513:87–113.
27. Brabers NA, Nottet HS. Role of the pro-inflammatory cytokines TNF-alpha and IL-1beta in HIV-associated dementia. *Eur J Clin Invest.* 2006;36:447–458.
28. Park KM, Bowers WJ. Tumor necrosis factor-alpha mediated signaling in neuronal homeostasis and dysfunction. *Cell Signal.* 2010;22:977–983.
29. Nakazawa T, Nakazawa C, Matsubara A, et al. Tumor necrosis factor-alpha mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. *J Neurosci.* 2006;26:12633–12641.
30. Figiel I. Pro-inflammatory cytokine TNF-alpha as a neuroprotective agent in the brain. *Acta Neurobiol Exp (Wars).* 2008;68:526–534.
31. Diem R, Meyer R, Weishaupt JH, Bahr M. Reduction of potassium currents and phosphatidylinositol 3-kinase-dependent AKT phosphorylation by tumor necrosis factor-(alpha) rescues axotomized retinal ganglion cells from retrograde cell death in vivo. *J Neurosci.* 2001;21:2058–2066.
32. Webster L, Stanbury RM, Chignell AH, Limb GA. Vitreous intercellular adhesion molecule 1 in uveitis complicated by retinal detachment. *Br J Ophthalmol.* 1998;82:438–443.
33. Fontaine V, Mohand-Said S, Hanoteau N, Fuchs C, Pfizenmaier K, Eisel U. Neurodegenerative and neuroprotective effects of tumor necrosis factor (TNF) in retinal ischemia: opposite roles of TNF receptor 1 and TNF receptor 2. *J Neurosci.* 2002;22:RC216.
34. Dopp JM, Mackenzie-Graham A, Otero GC, Merrill JE. Differential expression, cytokine modulation, and specific functions of type-1 and type-2 tumor necrosis factor receptors in rat glia. *J Neuroimmunol.* 1997;75:104–112.
35. Bohatschek M, Kloss CU, Hristova M, Pfeffer K, Raivich G. Microglial major histocompatibility complex glycoprotein-1 in the axotomized facial motor nucleus: regulation and role of tumor necrosis factor receptors 1 and 2. *J Comp Neurol.* 2004;470:382–399.
36. Dziewulska D, Mossakowski MJ. Cellular expression of tumor necrosis factor a and its receptors in human ischemic stroke. *Clin Neuropathol.* 2003;22:35–40.
37. Raivich G, Bohatschek M, Werner A, et al. Lymphocyte infiltration in the injured brain: role of proinflammatory cytokines. *J Neurosci Res.* 2003;72:726–733.

38. Dopp JM, Sarafian TA, Spinella FM, Kahn MA, Shau H, de Vellis J. Expression of the p75 TNF receptor is linked to TNF-induced NFkappaB translocation and oxyradical neutralization in glial cells. *Neurochem Res.* 2002;27:1535–1542.
39. Arnett HA, Mason J, Marino M, Suzuki K, Matsushima GK, Ting JP. TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. *Nat Neurosci.* 2001;4:1116–1122.
40. Besirli CG, Chinskey ND, Zheng QD, Zacks DN. Inhibition of retinal detachment-induced apoptosis in photoreceptors by a small peptide inhibitor of the fas receptor. *Invest Ophthalmol Vis Sci.* 2010;51:2177–2184.
41. Zacks DN, Boehlke C, Richards AL, Zheng QD. Role of the Fas-signaling pathway in photoreceptor neuroprotection. *Arch Ophthalmol.* 2007;125:1389–1395.
42. Elzey BD, Griffith TS, Herndon JM, Barreiro R, Tschopp J, Ferguson TA. Regulation of Fas ligand-induced apoptosis by TNF. *J Immunol.* 2001;167:3049–3056.

Polymorphisms in *ARMS2* (*LOC387715*) and *LOXL1* Genes in the Japanese With Age-Related Macular Degeneration

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• PURPOSE: To determine whether polymorphisms in the *ARMS2* (*LOC387715*) gene and the *lysyl oxidase–like 1* (*LOXL1*) gene are associated with age-related macular degeneration (AMD) in Japanese patients.

• DESIGN: Clinically relevant laboratory investigation.

• METHODS: Forty-one unrelated Japanese subjects with dry AMD, 50 subjects with exudative (wet) AMD, and 60 subjects with polypoidal choroidal vasculopathy (PCV) were studied. The single nucleotide polymorphisms (SNPs), p.Ala69Ser of the *ARMS2* gene and p.Arg141Leu of the *LOXL1* gene, were amplified by polymerase chain reaction, directly sequenced, and genotyped.

• RESULTS: For the *ARMS2* gene, the genotype frequency of the p.Ala69Ser single nucleotide polymorphism in eyes with dry AMD was not significantly different from that in the controls ($P = .04$), but the frequency was significantly higher in the exudative AMD group ($P = 3.1 \times 10^{-8}$) and PCV group ($P = 6.9 \times 10^{-3}$). For the *LOXL1* gene, the genotype frequency of the p.Arg141Leu single nucleotide polymorphism was not statistically higher in the dry AMD and PCV groups than in the control group (dry AMD, $P = .05$; PCV, $P = .16$), but was statistically higher in the exudative AMD group ($P = 6.8 \times 10^{-3}$). Regression analyses showed significant associations between the *ARMS2* gene and *LOXL1* gene in patients with exudative AMD.

• CONCLUSIONS: The p.Ala69Ser polymorphism of the *ARMS2* gene is strongly associated with exudative AMD and PCV and is associated marginally with dry AMD. The polymorphisms in the *LOXL1* gene did not predispose the individual to dry AMD and PCV. These findings suggest that there is a significant association between the *ARMS2* gene and *LOXL1* gene in exudative AMD. (Am J Ophthalmol 2011;151:550–556. © 2011 by Elsevier Inc. All rights reserved.)

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AGE-RELATED MACULAR DEGENERATION (AMD) IS the most frequent cause of irreversible blindness in the elderly in developed countries. AMD is a complex disorder that is genetically associated with multiple susceptibility loci. Both genetic predispositions[1] and environmental factors, such as smoking,[2–6] play important roles in the pathogenesis of AMD. AMD is broadly classified as either dry, nonneovascular or wet, or exudative neovascular AMD. The primary clinical sign of dry AMD is the presence of drusen with indistinct margins that are located between the retinal pigment epithelium and Bruch membrane. Drusen are small yellow or white accumulations of extracellular material. The proteins in drusen include apolipoproteins and members of the complement system. The primary clinical sign of exudative AMD is choroidal neovascularizations (CNVs) that develop from new blood vessels beneath the retina in the subretinal space.

Phenotypic and genetic heterogeneity makes the determination of the cause of AMD difficult. In the United States and Europe, approximately 85% to 90% of the patients diagnosed with AMD[7] have dry AMD, with a high prevalence of eyes with drusen. However, the exudative, or wet type, of AMD is more common in Japanese persons.[8] Earlier studies have shown that there are substantial differences in the phenotype and frequencies of single-nucleotide polymorphisms (SNPs) between the 2 ethnic groups.

Recently, 2 major genes, the complement factor H (*CFH*) gene on chromosome 1q31[9–11] and *ARMS2/HTRA1* gene on chromosome 10q26,[12–14] were shown to be significantly associated with a distinct component of the AMD phenotype in 2 different biological pathways. *ARMS2* is located in the ellipsoid, a mitochondria-concentrated part of human photoreceptor cells, and *HTRA1* is a serine protease gene. Polymorphisms in *ARMS2* were associated with a decrease in the stability of the mRNA of the *ARMS2* gene.[15] *HTRA1* seems to regulate the degradation of extracellular matrix proteoglycans. *CFH* is a component of an innate system that modulates inflammation through the C3 component, and it influences the formation of drusen that characterize dry AMD. However, *ARMS2/HTRA1* influences the formation of CNVs, the hallmark of exudative AMD.

Thorleifsson and associates used a genome-wide scan to show a strong association between SNPs in the *lysyl oxidase–like 1* (*LOXL1*) gene and pseudoexfoliation syndrome (XFS;

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OMIM:177650).[16] XFS is a generalized disorder of the extracellular matrix characterized by the pathologic accumulation of abnormal fibrillar material in the anterior segment of the eye.[17–19] The *LOXL1* gene is a member of the lysyl oxidase family of proteins that catalyzes the oxidative deamination of the lysine residues of tropoelastin,[20] and the homeostasis of elastic fibers requires the lysyl oxidase-like 1 protein.[21] Thus, the lysyl oxidase family of proteins plays important roles in elastogenesis.

In the pathogenesis of AMD, the age-related degradation of the elastic lamina of the Bruch membrane may permit the growth of CNVs. It recently was reported that the elastic lamina of the Bruch membrane in *LOXL1*-deficient mice was fragmented and less continuously than in controls, and these alterations led to more aggressive CNV growth after laser photocoagulation.[22] It was also suggested that the elastin gene (*ELN*) was a susceptibility gene for polypoidal choroidal vasculopathy (PCV) and that a different pathogenic process may be involved in the phenotypic expression of neovascular AMD and PCV.[23] However, we are not aware of any study that has examined the relationship between the common polymorphisms in the *LOXL1* gene and the presence of AMD.

The relative homogeneous and well-characterized Japanese population provides a unique opportunity to evaluate the possible association between AMD and the mRNA stability-related *ARMS2* gene and the elastin-related *LOXL1* gene. In addition, the factors that explain why some individuals develop the more aggressive exudative AMD, whereas others have the slowly degenerating dry AMD, have not been determined. Thus, the purpose of this study was to investigate the association between the SNPs of the *ARMS2* and *LOXL1* genes and the phenotypes of Japanese patients with dry AMD, exudative AMD, and PCV.

METHODS

WE STUDIED 41 UNRELATED JAPANESE PATIENTS WITH DRY or geographic atrophic AMD (grade 4[24]; 34 men and 7 women; mean age, 73.3 ± 9.2 years), 50 unrelated Japanese patients with exudative AMD (grade 5[24]; 40 men and 10 women; mean age, 71.3 ± 8.2 years), 60 unrelated Japanese patients with PCV (48 men and 12 women; mean age, 70.3 ± 9.2 years), and 138 Japanese controls who represent normal individuals of older age (101 men and 37 women; mean age, 68.6 ± 7.4 years) with no signs of macular disease (stage 0). All of the subjects were from the Ophthalmology Clinic of the Tohoku University Hospital, Miyagi, Japan, and standard ophthalmic examinations were performed on all subjects.

Genomic DNA was extracted from the leukocytes of peripheral blood and was purified with the Qiagen QIAamp Blood Kit (Qiagen, Valencia, California, USA), and the SNPs rs10490924 or p.Ala69Ser of the *ARMS2* gene and rs1048661 or p.Arg141Leu of the *LOXL1* gene were amplified by polymerase chain reaction, directly sequenced, and genotyped. The 2 primer sets for rs10490924 of *ARMS2* were 5′-TTC AAA TCC CTG GGT CTC TG-3′ and 5′-CTG CTG CTG CTC AGT TTC CT-3′, and the sequencing primer was 5′-GAC CTC TGT TGC CTC CTC TG-3′. For rs1048661 of exon 10f *LOXL1*, the 2 primer sets were 5′-CTC AGC GCT CCG AGA GTA G-3′ and 5′-ACA CGA AAC CCT GGT CGT AG-3′.

These primers were used under standard polymerase chain reaction conditions. The amplifications of the 2 SNPs were performed at 60 C annealing temperature. The polymerase chain reaction fragments were purified by ExoSAP-IT (USB, Cleveland, Ohio, USA) and were sequenced with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Foster City, California, USA) on an automated DNA sequencer (ABI PRISM 3100 Genetic Analyzer; Perkin-Elmer).

• OPHTHALMIC EXAMINATION, DEFINITIONS, AND SUBTYPE CLASSIFICATION OF AGE-RELATED MACULAR DEGENERATION: All of the subjects underwent a complete ophthalmic examination, including visual acuity measurement, slit-lamp biomicroscopy of the fundi, color fundus photography, optical coherence tomography, fluorescein angiography, and indocyanine green angiography. The type and status of the AMD was made by 2 retina specialists (R.W. and T.A.) before the genetic analyses.

The dry AMD subjects had many large, soft drusen, geographic atrophy with adjacent soft drusen without neovascularization, or both. The exudate AMD subjects had clear vascular CNV networks or diffuse staining of CNV membranes in the fluorescein angiography or indocyanine green angiography images, or both. The PCV patients had characteristic abnormal vascular network of choroidal vessels with polyp-like dilations at the terminals of the branches in the fluorescein angiography or indocyanine green angiography images, or both.[25]

The AMD subtypes were diagnosed and classified using the criteria of the Age-Related Eye Disease Study Research Group.[24] The inclusion criteria were: age 50 years or older, diagnosis of AMD in one or both eyes, and no other retinochoroidal diseases, for example, high myopia (> -6 diopters, spherical equivalent), angioid streaks, central serous chorioretinopathy, and possible ocular histoplasmosis. The control subjects were confirmed not to have clinical evidence of AMD by the findings in the same complete ophthalmologic examination that was used to identify the AMD patients.

• STATISTICAL ANALYSES: The genotypes were in Hardy–Weinberg equilibrium. The significance of differences in the genotype frequencies among the cases and controls was tested by the Fisher exact test, depending on the cell counts. P values and odds ratios (approximated to relative risk) were calculated as a measure of the association between the ARMS2 and LOXL1 genotype and the phenotype of AMD,

TABLE 1. *ARMS2* Allele Frequencies in Japanese Patients with Dry AMD, Exudative AMD, PCV, and in Controls

SNP	p.A69S (rs10490942 G/T)		*P* Value[a]	Odds Ratio (95% CI)
Allele	T	G		
Dry AMD (n = 41)	0.476	0.524	.01	0.53 (0.32 to 0.88)
Exudative AMD (n = 50)	0.680	0.320	8.1×10^{-10}	4.39 (2.69 to 7.17)
PCV (n = 60)	0.467	0.533	7.7×10^{-3}	1.80 (1.17 to 2.80)
Control (n = 138)	0.326	0.674		

AMD = age-related macular degeneration; CI = confidence interval; PCV = polypoidal choroidal vasculopathy; SNP = single nucleotide polymorphism.

[a]The significance of the association was determined by a contingency table analysis using the chi-square test.

TABLE 2. Frequency of Genotypes p.A69S of *ARMS2* Gene in Patients with Dry AMD, Exudative AMD, PCV, and Control Subjects

ARMS2 p.A69S Variant	Dry AMD (n = 41)	Exudative AMD (n = 50)	PCV (n = 60)	Control (n = 138)
T/T	8 (19.5%)	24 (48.0%)	18 (30.0%)	16 (11.6%)
T/G	23 (56.1%)	20 (40.0%)	20 (33.3%)	58 (42.0%)
G/G	10 (24.4%)	6 (12.0%)	22 (36.7%)	64 (46.4%)
P value[a]	.04	3.1×10^{-8}	6.9×10^{-3}	

AMD = age-related macular degeneration; PCV = polypoidal choroidal vasculopathy.

Data presented are number of patients, unless otherwise indicated.

[a]The significance of the association was determined by a contingency table analysis using the chi-square test.

with the effects of the mutant allele assumed to be dominant (wild/wild vs wild/mutant and mutant/mutant combined). For each odds ratio, the P values and 95% confidence intervals were calculated using the SNPAlyze program version 4.0 (Dynacom, Yokohama, Japan). The significance of associations was determined by contingency table analysis using the chi-square or Fisher exact test. The Hardy–Weinberg equilibrium was determined by using gene frequencies obtained by simple gene counting and the chi-square test with Yates' correction for comparing observed and expected values. Two-locus analyses were performed for the rs10490924 SNP of ARMS2 and the rs1048661SNP of LOXL1 by comparing each genotypic combination with the baseline of homozygosity for the common allele at both loci using logistical regression (JMP software version 7.0.2; SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

• DISTRIBUTION OF P.ALA69SER SINGLE-NUCLEOTIDE POLYMORPHISM OF ARMS2 IN DRY AGE-RELATED MACULAR DEGENERATION, EXUDATIVE AGE-RELATED MACULAR DEGENERATION, POLYPOIDAL CHOROIDAL VASCULOPATHY, AND CONTROL SUBJECTS: The allelic frequencies for the p.Ala69Ser SNP of the ARMS2 gene for dry AMD, exudative AMD, PCV, and control subjects are presented in Table 1. For the ARMS2 gene, the genotype frequency of the rs10490924 or p.Ala69Ser SNPs was significantly higher in the dry AMD and PCV groups (minor allele frequency, T = 0.476 in dry AMD and T = 0.467 in PCV)

TABLE 3. Allele Frequencies and Frequency of Genotypes p.R141L of the LOXL1 Gene in Patients with Dry AMD, Exudative AMD, PCV, Exfoliation Syndrome, and Control Subjects

SNP	p.R141L (rs1048661 G/T)		*P* Value[a]
Allele	T	G	
Dry AMD (n = 41)	0.585	0.415	.21
Exudative AMD (n = 50)	0.620	0.380	.05
PCV (n = 60)	0.517	0.483	.86
XFS[b] (n = 54)	0.947	0.053	1.5×10^{-12}
Control (n 26 138)	0.507	0.493	

AMD = age-related macular degeneration; PCV = polypoidal choroidal vasculopathy; XFS = exfoliation syndrome.

[a]The significance of the association was determined by a contingency table analysis using the chi-square test.

[b]Data reported by Fuse and associates.[31]

TABLE 4. Frequency of Genotypes p.R141L of the *LOXL1* Gene in Patients with Dry AMD, Exudative AMD, PCV, and Control Subjects

LOXL1 p.R141L Variant	Dry AMD (n = 41)	Exudative AMD (n = 50)	PCV (n = 60)	XFS[a] (n = 54)	Control (n = 138)
T/T	16 (39.0%)	22 (44.0%)	18 (30.0%)	49 (90.7%)	30 (21.7%)
T/G	16 (39.0%)	18 (36.0%)	26 (43.3%)	4 (7.4%)	80 (58.0%)
G/G	9 (22.0%)	10 (20.0%)	16 (26.7%)	1 (1.9%)	28 (20.3%)
P value[b]	0.05	6.8×10^{-3}	0.16	1.5×10^{-12}	

AMD = age-related macular degeneration; PCV = polypoidal choroidal vasculopathy; XFS = exfoliation syndrome.
Data presented are number of patients, unless otherwise indicated.
[a]Data reported by Fuse and associates.[31]
[b]The significance of the association was determined by a contingency table analysis using the chi-square test.

than in the controls (T = 0.326). However, the degree of significance was less in the dry AMD versus the control group ($P = .01$) and higher in the PCV versus control group ($P = 7.7 \times 10^{-3}$). The T allele of the rs10490924 SNP was detected at a significantly higher frequency in patients with exudative AMD than in control subjects ($P = 8.1 \times 10^{-10}$).

The genotype frequencies for the 2 *ARMS2* SNPs were compared among dry AMD, exudative AMD, PCV, and control groups (Table 2). For the *ARMS2* gene, the genotype frequency of the p.Ala69Ser SNP was significantly higher in the dry AMD ($P = .04$), the exudative AMD ($P = 3.1 \times 10^{-8}$), and PCV ($P = 6.9 \times 10^{-3}$) groups than in the control group (Table 2). The SNP adhered to the Hardy–Weinberg expectations ($P > .05$).

• **DISTRIBUTION OF P.ARG141LEU SINGLE-NUCLEOTIDE POLYMORPHISM OF *LOXL1* IN DRY AGE-RELATED MACULAR DEGENERATION, EXUDATIVE AGE-RELATED MACULAR DEGENERATION, POLYPOIDAL CHOROIDAL VASCULOPATHY, AND CONTROL SUBJECTS:** The allelic frequencies of the *LOXL1* SNP were compared among the dry AMD, exudative AMD, PCV, and control subjects (Table 3). The T allele of rs1048661 and Arg141Leu was not detected at a statistically higher frequency in patients with dry AMD ($P = .21$) and PCV ($P = .86$) than in control subjects. In exudative AMD, the frequency of the T variant was higher than that of controls, but the frequency was statistically marginal (major allele frequency T = 0.620 in exudative AMD and T = 0.507 in controls).

The genotype frequencies for the 2 *LOXL1* SNPs were compared among dry AMD, exudative AMD, PCV, and control subjects (Table 4). For the *LOXL1* gene, the genotype frequency of the p.Arg141Leu SNP was not statistically higher in the dry AMD ($P = .05$) and PCV ($P = .16$) groups than in the control group. However, genotype frequency of this SNP was statistically higher in the exudative AMD group than in the control group ($P = 6.8 \times 10^{-3}$). The SNP adhered to the Hardy–Weinberg expectations ($P > .05$).

TABLE 5. Two-Locus Odds Ratios for *ARMS2* p.A69S and *LOXL1* p.R141L in Exudative AMD Cases

		LOXL1 p.R141L Variant (rs1048661)	
ARMS2 p.A69S		G/G + T/G	T/T
variant	G/G	1	2.17
(rs10490924)	T/G	2.82	6.11
	T/T	13.5	29.4

AMD = age-related macular degeneration.
Interaction between *ARMS2* and *LOXL1* single-nucleotide polymorphisms is significant by multivariate logistic regression analysis ($P < .0001$).

• **TWO LOCUS ANALYSES:** Two locus analyses were performed for the rs10490924 SNP of *ARMS2* and for the rs1048661 SNP of *LOXL1* by comparing each genotypic combination with the baseline of homozygosity or heterozygosity for the common allele at both loci using logistical regression (JMP software version 7.0.2). Possible interactions between rs10490924 and rs1048661 for a susceptibility of dry AMD, exudative AMD, and PCV were evaluated. Multivariate logistic regression analyses showed a significant interaction between the SNPs of *ARMS2* and *LOXL1* SNPs in the rs10490924 SNP of *ARMS2* plus the rs1048661 SNP of *LOXL1* T/T homozygote ($P < .0001$) in the exudative AMD group. The odds ratio of the combined genotypes, *ARMS2* rs10490924 and *LOXL1* rs1048661, was 29.4 (Table 5). In other cases, there was no significant relationship between *ARMS2* and *LOXL1* polymorphisms.

DISCUSSION

• **GENETIC HETEROGENEITY AND OTHER AGE-RELATED MACULAR DEGENERATION LOCI:** The 2 major genetic variants, *CFH* [9–11] and *ARMS2/HTRA1*,[12–15] are independently associated with the progression of AMD. This is important because the progression leads to visual impairment and blindness. Their presence does not necessarily lead to the development of AMD, because some individuals with

AMD who progress have the nonrisk genotype and others without AMD progression carry the risk genotype. Thus, phenotypic and genetic heterogeneities complicate the cause of AMD, and other modifier loci could exist.

• DISTRIBUTION OF *ARMS2* SINGLE NUCLEOTIDE POLYMORPHISMS IN AGE-RELATED MACULAR DEGENERATION SUBJECTS: Several studies have used refined linkage disequilibrium mapping and case-control association studies to probe the most susceptible alleles, rs10490924 of *ARMS2* and rs11200638 of *HTRA1*, in AMD patients.[12–14,26] It has been shown that the *HTRA1* variant confers similar risks to dry AMD and exudative AMD. The rs11200638 SNP is located in the promoter of *HTRA1* and has been shown to be strongly associated not only with exudative AMD,[12,13] but also with dry AMD in white persons and Chinese persons.[27] Our findings are in agreement with these findings. One thing that we need to consider is that the mean age of the control group was 68.6 ± 7.4 years, which is younger than the ages of the dry AMD, exudative AMD, and PCV groups. In addition, there is a possibility that in these control subjects, AMD or PCV would develop as they age.

The differentiation of PCV from exudative AMD is very important because of differences not only in the prognosis of the disease, but also in the response to treatments, including photodynamic therapy.[28] It is interesting that PCV exists more frequently in Asians, Hispanics, and African Americans. This suggests that some genetic factors are involved in PCV.[29] It has been reported that a polymorphism of the elastin gene is associated with PCV and that different pathogenic processes are involved in the development of exudative AMD than in PCV.[23]

• DISTRIBUTION OF *LOXL1* SINGLE NUCLEOTIDE POLYMORPHISMS IN AGE-RELATED MACULAR DEGENERATION SUBJECTS: XFS is a generalized disorder of the extracellular matrix and is characterized by the pathologic accumulation of abnormal fibrillar material in the anterior segment of the eye.[17–19] This then predisposes the eye to glaucomatous optic neuropathy. The prevalence of XFS increases with age and is highest in those 70 and 80 years of age.[30] Thus, XFS and AMD both are age-associated diseases. In an earlier study, we confirmed that 2 coding SNPs in the *LOXL1* gene were strongly associated with XFS.[31] The T allele of rs1048661 of the *LOXL1* SNP was highly associated with XFS in the Japanese population. However, the distribution of the rs1048661 allele was quite different in XFS and AMD in Japanese subjects. In subjects with exudative AMD, the T allele of the rs1048661 SNP of *LOXL1* was slightly higher than in control subjects, but the increase was not significant (Table 3). However, the genotype frequency of the rs1048661 SNP of *LOXL1* was significantly higher in the exudative AMD group than in the controls ($P = 6.8 \times 10^{-3}$; Table 4). In the exudative AMD subjects, only 1 subject had XFS, for a frequency of 2% (1/50). It is reported that the prevalence of XFS, including glaucoma resulting from XFS in Japan, is 1.0%.[32] Thus, this difference does not seem to be significant. However, whether the individuals with exudative AMD have a higher incidence of XFS needs to be investigated in more detail.

• BOTH MODIFYING GENES AND ENVIRONMENTAL FACTORS COULD INFLUENCE AGE-RELATED MACULAR DEGENERATION: Both modifying genes and environmental factors could influence the pathways that lead to either dry AMD or exudative AMD. The 2 major genetic variants *CFH*[9–11] and *HTRA1*[12,13] are independently associated with progression to the advanced stage of AMD. They are not necessary for the development of the disease, because some individuals with AMD who progress have the nonrisk genotype and some people without AMD progression carry the risk genotype. Nevertheless, we should remember that both genetic and environmental factors and their associations contribute to the progression of AMD,[2–6] which should help us to understand these mechanisms.

A systemic defect in elastic fiber deposition affects the integrity of the Bruch membrane and leads to more aggressive CNV growth. This may be mediated partially by abnormal signaling because of the accumulation of soluble elastin peptides. The elastic lamina of the Bruch membrane in the *LOXL1*-deficient mice was reported to be fragmented and led to more aggressive CNV growth after laser photocoagulation.[22] Thus, elastogenesis in the Bruch membrane should be related with CNV growth in eyes with AMD.

XFG is a relatively rare age-related disease characterized by a generalized fibrillar degeneration of elastin-containing tissues. So, we chose the *LOXL1* as a candidate for an AMD modifier gene. The genotype frequency of the SNPs of *LOXL1* was statistically higher in the exudative AMD group than in the normal people of older age ($P = 6.8 \times 10^{-3}$; Table 4). But, the polymorphisms in the *LOXL1* gene did not predispose the eye to dry AMD and PCV in Japanese patients.

Two-locus analyses were performed for the rs10490924 SNP of *ARMS2* and the rs1048661 SNP of *LOXL1*. Multivariate logistic regression analyses showed a significant interaction between the *ARMS2* and *LOXL1* SNPs in the case exudative AMD with T/T homozygote (Table 5).

The primary clinical sign of exudative AMD is new blood vessel formation and breaks beneath the retina to make the CNV. The *LOXL1* gene catalyzes oxidative deamination of lysine residues of tropoelastin and plays an important role in the elastogenesis of the elastic lamina in the Bruch membrane. This indicates the

existence of a synergic and permissive effect of the *ARMS2* and *LOXL1* polymorphisms on the growth of CNV and exudative AMD susceptibility.

In conclusion, the p.Ala69Ser polymorphism of the *ARMS2* gene is a common variant in Japanese populations and is strongly associated not only with exudative AMD, but also with PCV. The polymorphisms in the *LOXL1* gene did not predispose the subject to dry AMD or PCV in Japanese patients, but there is a possibility that there is a significant association between the prevalence of exudative AMD and the *ARMS2* and *LOXL1* genes. The *ARMS2* and *LOXL1* genes seem to be involved in a statistically significant fraction of exudative AMD cases in the Japanese population.

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REFERENCES

1. Klaver CC, Kliffen M, van Duijn CM, et al. Genetic association of apolipoprotein E with age-related macular degeneration. Am J Hum Genet 1998;63(1):200–206.
2. Seddon JM, Cote J, Page WF, Aggen SH, Neale MC. The US twin study of age-related macular degeneration: relative roles of genetic and environmental influences. Arch Ophthalmol 2005;123(3):321–327.
3. Seddon JM, George S, Rosner B, Klein ML. CFH gene variant, Y402H, and smoking, body mass index, environmental associations with advanced age-related macular degeneration. Hum Hered 2006;61(3):157–165.
4. Schmidt S, Hauser MA, Scott WK, et al. Cigarette smoking strongly modifies the association of LOC387715 and age-related macular degeneration. Am J Hum Genet 2006;78(5): 852–864.
5. Francis PJ, George S, Schultz DW, et al. The LOC387715 gene, smoking, body mass index, environmental associations with advanced age-related macular degeneration. Hum Hered 2007;63(3–4):212–218.
6. Schaumberg DA, Hankinson SE, Guo Q, Rimm E, Hunter DJ. A prospective study of 2 major age-related macular degeneration susceptibility alleles and interactions with modifiable risk factors. Arch Ophthalmol 2007;125(1): 55–62.
7. Seddon J. Epidemiology of age-related macular degeneration. 3rd ed. St. Louis: Mosby; 2001:1039–1050.
8. Oshima Y, Ishibashi T, Murata T, Tahara Y, Kiyohara Y, Kubota T. Prevalence of age related maculopathy in a representative Japanese population: the Hisayama study. Br J Ophthalmol 2001;85(10):1153–1157.
9. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. Science 2005;308(5720):385–389.
10. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. Science 2005;308(5720):419–421.
11. Edwards AO, Ritter R 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science 2005;308(5720): 421–424.
12. Dewan A, Liu M, Hartman S, et al. HTRA1 promoter polymorphism in wet age-related macular degeneration. Science 2006;314(5801):989–992.
13. Yang Z, Camp NJ, Sun H, et al. A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. Science 2006; 314(5801):992–993.
14. Kanda A, Chen W, Othman M, et al. A variant of mitochondrial protein LOC387715/ARMS2, not HTRA1, is strongly associated with age-related macular degeneration. Proc Natl Acad Sci U S A 2007;104(41):16227–16232.
15. Fritsche LG, Loenhardt T, Janssen A, et al. Age-related macular degeneration is associated with an unstable ARMS2 (LOC387715) mRNA. Nat Genet 2008;40(7):892–896.
16. Thorleifsson G, Magnusson KP, Sulem P, et al. Common sequence variants in the LOXL1 gene confer susceptibility to exfoliation glaucoma. Science 2007;317(5843):1397–1400.
17. Tarkkanen A, Kivela T, John G. Lindberg and the discovery of exfoliation syndrome. Acta Ophthalmol Scand 2002; 80(2):151–154.
18. Schlotzer-Schrehardt U, Naumann GO. Ocular and systemic pseudoexfoliation syndrome. Am J Ophthalmol 2006;141(5): 921–937.
19. Forsman E, Cantor RM, Lu A, et al. Exfoliation syndrome: prevalence and inheritance in a subisolate of the Finnish population. Acta Ophthalmol Scand 2007;85(5):500–507.
20. Thomassin L, Werneck CC, Broekelmann TJ, et al. The Pro-regions of lysyl oxidase and lysyl oxidase-like 1 are required for deposition onto elastic fibers. J Biol Chem 2005;280(52):42848–42855.
21. Liu X, Zhao Y, Gao J, et al. Elastic fiber homeostasis requires lysyl oxidase-like 1 protein. Nat Genet 2004;36(2):178–182.
22. Yu HG, Liu X, Kiss S, et al. Increased choroidal neovascularization following laser induction in mice lacking lysyl oxidase-like 1. Invest Ophthalmol Vis Sci 2008;49(6):2599–2605.
23. Kondo N, Honda S, Ishibashi K, Tsukahara Y, Negi A. Elastin gene polymorphisms in neovascular age-related macular degeneration and polypoidal choroidal vasculopathy. Invest Ophthalmol Vis Sci 2008;49(3):1101–1105.
24. Risk factors associated with age-related macular degeneration. A case-control study in the age-related eye disease study: Age-Related Eye Disease Study Report Number 3. Ophthalmology 2000;107(12):2224–2232.
25. Yannuzzi LA, Sorenson J, Spaide RF, Lipson B. Idiopathic polypoidal choroidal vasculopathy (IPCV). Retina 1990;10:1–8.
26. Gibbs D, Yang Z, Constantine R, et al. Further mapping of 10q26 supports strong association of HTRA1 polymorphisms with age-related macular degeneration. Vision Res 2008; 48(5):685–689.

27. Cameron DJ, Yang Z, Gibbs D, et al. HTRA1 variant confers similar risks to geographic atrophy and neovascular age-related macular degeneration. Cell Cycle 2007;6(9):1122–1125.
28. Gomi F, Ohji M, Sayanagi K, et al. One-year outcomes of photodynamic therapy in age-related macular degeneration and polypoidal choroidal vasculopathy in Japanese patients. Ophthalmology 2008;115(1):141–146.
29. Yannuzzi LA, Wong DW, Sforzolini BS, et al. Polypoidal choroidal vasculopathy and neovascularized age-related macular degeneration. Arch Ophthalmol 1999;117(11):1503–1510.
30. Forsius H. Exfoliation syndrome in various ethnic populations. Acta Ophthalmol Suppl 1988;184:71–85.
31. Fuse N, Miyazawa A, Nakazawa T, Mengkegale M, Otomo T, Nishida K. Evaluation of LOXL1 polymorphisms in eyes with exfoliation glaucoma in Japanese. Mol Vis 2008;14:1338–1343.
32. Yamamoto T, Iwase A, Araie M, et al. The Tajimi Study Report 2: Prevalence of primary angle closure and secondary glaucoma in a Japanese population. Ophthalmology 2005;112(10):1661–1669.

Biosketch

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