Table 3. Summary of the genotype association analysis.

SNP ID	Major/ Minor	Genotype frequency (%)			Genotype association results					
		Genotype (Major homo/Hetero/Minor homo)			PCV versus Control (dominant model)		tAMD versus Control (dominant model)		PCV versus tAMD (genotype model)	
		PCV	tAMD	Control	OR (95%CI)	Nominal p value	OR (95%CI)	Nominal p value	Nominal p value	Corrected p value*
rs10499862	T/C	75/22/3	81/17/2	67/29/4	0.58 (0.19–1.80)	0.34	0.52 (0.14–2.01)	0.34	0.5	0.52
rs3173798	T/C	28/51/21	45/44/11	36/40/24	0.8 (0.50–1.28)	0.36	0.41 (0.22–0.75)	0.0034	0.0031	0.0033
rs3211883	T/A	42/45/13	52/40/8	40/41/19	0.59 (0.35–1.02)	0.059	0.36 (0.18–0.74)	0.0038	0.13	0.14
rs3173800	A/T	52/44/4	37/50/13	54/36/10	0.35 (0.15–0.82)	0.012	1.32 (0.67–2.61)	0.42	0.00067	0.00069

Corrections were performed with permutation test. SNP: single nucleotide polymorphism, PCV: polypoidal choroidal vasculopathy, tAMD: typical neovascular AMD, OR: odds ratio, CI: coefficient interval.

TABLE 4. HAPLOTYPE-BASED ASSOCIATION STUDY.

Index	Haplotype	Frequency				Permutation P		
		Overall	PCV	tAMD	Control	PCV versus control	tAMD versus control	PCV versus tAMD
H1	T-T-T-T	0.32	0.26	0.38	0.28	0.45	0.01	0.001
H2	T-T-T-A	0.28	0.28	0.28	0.27	0.82	0.8	0.97
H3	T-C-A-A	0.19	0.21	0.17	0.21	0.85	0.27	0.2
H4	C-C-A-A	0.15	0.14	0.11	0.19	0.06	0.005	0.25
H5	T-C-T-A	0.05	0.11	0.06	0.05	0.001	0.63	0.018

Selected SNPs are rs10499862/rs3173798/rs3211883/rs3173800. SNP: single nucleotide polymorphism, PCV: polypoidal choroidal vasculopathy, tAMD: typical neovascular AMD.

site [41]. Thus, the SNPs in this region could have noncoding effects on gene expression and function. A recent study demonstrated that the C allele at rs3173798 tended to increase *CD36* expression while reducing high-density lipoprotein levels [41,42]. Since the C allele at rs3173798 was less frequent in the tAMD group than the PCV and control groups, the reduced expression of *CD36* might be correlated with tAMD but not PCV pathogenesis. Moreover, Picard et al. have recently demonstrated an accumulation of oxLDL in Bruch's membrane among aged *CD36* knockout mice [43]. The oxLDL accelerates an accumulation of deposits in Bruch's membrane and causes drusen. Interestingly, drusens are more frequently seen in tAMD than PCV [44]. However, exhaustive resequencing of this locus may elucidate potentially undiscovered and more important causative variants. In addition, it is essential to perform replication studies using other cohorts to verify and conclude the associations of *CD36* variants with tAMD and PCV.

The limitation of this study was a possible influence of lipid metabolism on AMD pathogenesis [45,46]. Since CD36 is known to associate with the metabolic syndrome and hyperlipidemia [41,47], *CD36* variants may indirectly contribute to AMD via abnormal lipid metabolism. However, the results of this study suggest a possible role of lipid metabolism in the different pathogeneses between PCV and tAMD.

In conclusion, the present study suggested some clinical possibilities for genetic association analysis that can be further investigated to determine the specific pathogenesis of PCV as distinct from that of tAMD.

ACKNOWLEDGMENTS

This study was supported by a Grant-in Aid (C) 23592567 from the Ministry of Education, Science and Culture, Tokyo, Japan (S.H.), and by a grant from the Takeda Science Foundation, Osaka, Japan (S.H.). The funding organization had no role in the design or conduct of this research. The authors have no proprietary or commercial interest in any of the materials discussed in this article.

REFERENCES

1. Congdon N, O'Colmain B, Klaver CC, Klein R, Muñoz B, Friedman DS, Kempen J, Taylor HR, Mitchell P, Eye Diseases Prevalence Research Group. Causes and prevalence of visual impairment among adults the United States. Arch Ophthalmol 2004; 122:477-85. [PMID: 15078664]
2. Rein DB, Wittenborn JS, Zhang X, Honeycutt AA, Lesesne SB, Saaddine J, Vision Health Cost-Effectiveness Study Group. Forecasting age-related macular degeneration through the year 2050: the potential impact of new treatments. Arch Ophthalmol 2009; 127:533-40. [PMID: 19365036]
3. Yannuzzi LA, Sorenson J, Spaide RF, Lipson B. Idiopathic polypoidal choroidal vasculopathy (IPCV). Retina 1990; 10:1-8. [PMID: 1693009]
4. Kleiner RC, Brucker AJ, Johnston RL. The posterior uveal bleeding syndrome. Retina 1990; 10:9-17. [PMID: 2343198]
5. Yannuzzi LA, Negrao S, Iida T, Carvalho C, Rodriguez-Coleman H, Slakter J, Freund KB, Sorenson J, Orlock D, Borodoker N. Retinal angiomatous proliferation in age-related macular degeneration. Retina 2001; 21:416-34. [PMID: 11642370]
6. Bessho H, Honda S, Imai H, Negi A. Natural course and funduscopic findings of polypoidal choroidal vasculopathy in a Japanese population over 1 year of follow-up. Retina 2011; 31:1598-602. [PMID: 21478804]
7. Honda S, Kurimoto Y, Kagotani Y, Yamamoto H, Takagi H, Uenishi M, Hyogo Macular Disease Study Group. Photodynamic therapy for typical age-related macular degeneration and polypoidal choroidal vasculopathy: a 30-month multicenter study in Hyogo, Japan. Jpn J Ophthalmol 2009; 53:593-7. [PMID: 20020237]
8. Honda S, Imai H, Yamashiro K, Kurimoto Y, Kanamori-Matsui N, Kagotani Y, Tamura Y, Yamamoto H, Ohoto S, Takagi H, Uenishi M, Negi A. Comparative assessment of photodynamic therapy for typical age-related macular degeneration and polypoidal choroidal vasculopathy: a multicenter study in Hyogo prefecture, Japan. Ophthalmologica 2009; 223:333-8. [PMID: 19478533]
9. Lim, JY.; Lee, SY.; Kim, JG.; Lee, JY.; Chung, H.; Yoon, YH. Intravitreal bevacizumab alone versus in combination with photodynamic therapy for the treatment of neovascular

maculopathy in patients aged 50 years or older: 1-year results of a prospective clinical study.. Acta Ophthalmol. 2010.

10. Matsumiya W, Honda S, Bessho H, Kusuhara S, Tsukahara Y, Negi A. Early responses to intravitreal ranibizumab in typical neovascular age-related macular degeneration and polypoidal choroidal vasculopathy. J Ophthalmol 2011; 2011:742020. [PMID: 21772985]
11. Bessho H, Honda S, Kondo N, Negi A. The association of *ARMS2* polymorphisms with phenotype in typical neovascular age-related macular degeneration and polypoidal choroidal vasculopathy. Mol Vis 2011; 17:977-82. [PMID: 21541271]
12. Bessho H, Honda S, Kondo N, Nishimura K, Negi A. Positive association of *complement factor H* gene variants with the effect of photodynamic therapy in polypoidal choroidal vasculopathy. J Clinic Experiment Ophthalmol. 2011; 2:122.
13. 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; 49:1101-5. [PMID: 18326737]
14. Chowers I, Meir T, Lederman M, Goldenberg-Cohen N, Cohen Y, Banin E, Averbukh E, Hemo I, Pollack A, Axer-Siegel R, Weinstein O, Hoh J, Zack DJ, Galbinur T. Sequence variants in HTRA1 and LOC387715/ARMS2 and phenotype and response to photodynamic therapy in neovascular age-related macular degeneration in populations from Israel. Mol Vis 2008; 14:2263-71. [PMID: 19065273]
15. Brantley MA Jr, Edelstein SL, King JM, Plotzke MR, Apte RS, Kymes SM, Shiels A. Association of complement factor H and LOC387715 genotypes with response of exudative age-related macular degeneration to photodynamic therapy. Eye (Lond) 2009; 23:626-31. [PMID: 18292785]
16. Gotoh N, Yamada R, Nakanishi H, Saito M, Iida T, Matsuda F, Yoshimura N. Correlation between CFH Y402H and HTRA1 rs11200638 genotype to typical exudative age-related macular degeneration and polypoidal choroidal vasculopathy phenotype in the Japanese population. Clin Experiment Ophthalmol 2008; 36:437-42. [PMID: 18939352]
17. Sakurada Y, Kubota T, Imasawa M, Tsumura T, Mabuchi F, Tanabe N, Iijima H. Angiographic lesion size associated with LOC387715 A69S genotype in subfoveal polypoidal choroidal vasculopathy. Retina 2009; 29:1522-6. [PMID: 19898184]
18. Mori K, Horie-Inoue K, Gehlbach PL, Takita H, Kabasawa S, Kawasaki I, Ohkubo T, Kurihara S, Iizuka H, Miyashita Y, Katayama S, Awata T, Yoneya S, Inoue S. Phenotype and genotype characteristics of age-related macular degeneration in a Japanese population. Ophthalmology 2010; 117:928-38. [PMID: 20132989]
19. Tsuchihashi T, Mori K, Horie-Inoue K, Gehlbach PL, Kabasawa S, Takita H, Ueyama K, Okazaki Y, Inoue S, Awata T, Katayama S, Yoneya S. Complement factor H and high-temperature requirement A-1 genotypes and treatment response of age-related macular degeneration. Ophthalmology 2011; 118:93-100. [PMID: 20678803]
20. Silverstein RL, Febbraio M. CD36 and atherosclerosis. Curr Opin Lipidol 2000; 11:483-91. [PMID: 11048891]
21. Febbraio M, Hajjar DP, Silverstein RL. CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. J Clin Invest 2001; 108:785-91. [PMID: 11560944]
22. Kuniyasu A, Ohgami N, Hayashi S, Miyazaki A, Horiuchi S, Nakayama H. CD36-mediated endocytic uptake of advanced glycation end products (AGE) in mouse 3T3–L1 and human subcutaneous adipocytes. FEBS Lett 2003; 537:85-90. [PMID: 12606036]
23. Kondo N, Honda S, Kuno S, Negi A. Positive Association of Common Variants in *CD36* with Neovascular Age-Related Macular Degeneration. Aging 2009; 1:266-74. [PMID: 20157514]
24. Yamashiro K, Mori K, Nakata I, Tsuchihashi T, Horie-Inoue K, Nakanishi H, Tsujikawa A, Saito M, Iida T, Yamada R, Matsuda F, Inoue S, Awata T, Yoneya S, Yoshimura N. Association of elastin gene polymorphism to age-related macular degeneration and polypoidal choroidal vasculopathy. Invest Ophthalmol Vis Sci 2011; 52:8780-4. [PMID: 22003121]
25. Tanaka K, Nakayama T, Yuzawa M, Wang Z, Kawamura A, Mori R, Nakashizuka H, Sato N, Mizutani Y. Analysis of candidate genes for age-related macular degeneration subtypes in the Japanese population. Mol Vis 2011; 17:2751-8. [PMID: 22065928]
26. Japanese Study Group of Polypoidal Choroidal Vasculopathy. Criteria for diagnosis of polypoidal choroidal vasculopathy. Nippon Ganka Gakkai Zasshi 2005; 109:417-27. [PMID: 16050460]
27. Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 2003; 164:1567-87. [PMID: 12930761]
28. Neale BM, Fagerness J, Reynolds R, Sobrin L, Parker M, Raychaudhuri S, Tan PL, Oh EC, Merriam JE, Souied E, Bernstein PS, Li B, Frederick JM, Zhang K, Brantley MA Jr, Lee AY, Zack DJ, Campochiaro B, Campochiaro P, Ripke S, Smith RT, Barile GR, Katsanis N, Allikmets R, Daly MJ, Seddon JM. Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (LIPC). Proc Natl Acad Sci USA 2010; 107:7395-400. [PMID: 20385826]
29. Chen W, Stambolian D, Edwards AO, Branham KE, Othman M, Jakobsdottir J, Tosakulwong N, Pericak-Vance MA, Campochiaro PA, Klein ML, Tan PL, Conley YP, Kanda A, Kopplin L, Li Y, Augustaitis KJ, Karoukis AJ, Scott WK, Agarwal A, Kovach JL, Schwartz SG, Postel EA, Brooks M, Baratz KH, Brown WL, Brucker AJ, Orlin A, Brown G, Ho A, Regillo C, Donoso L, Tian L, Kaderli B, Hadley D, Hagstrom SA, Peachey NS, Klein R, Klein BE, Gotoh N, Yamashiro K, Ferris Iii F, Fagerness JA, Reynolds R, Farrer LA, Kim IK, Miller JW, Cortón M, Carracedo A, Sanchez-Salorio M, Pugh EW, Doheny KF, Brion M, Deangelis MM, Weeks DE, Zack DJ, Chew EY, Heckenlively JR, Yoshimura N, Iyengar SK, Francis PJ, Katsanis N, Seddon JM, Haines JL, Gorin MB, Abecasis GR, Swaroop A. Genetic variants near TIMP3 and high-density lipoprotein-associated loci influence susceptibility to age-related macular degeneration. Proc Natl Acad Sci USA 2010; 107:7401-6. [PMID: 20385819]
30. Anderson CR, Hastings NE, Blackman BR, Price RJ. Capillary sprout endothelial cells exhibit a CD36low phenotype.

Regulation by shear stress and vascular endothelial growth factor-induced mechanism for attenuating anti-proliferative thrombospondin-1 signaling. Am J Pathol 2008; 173:1220-8. [PMID: 18772338]

31. Houssier M, Raoul W, Lavalette S, Keller N, Guillonneau X, Baragatti B, Jonet L, Jeanny JC, Behar-Cohen F, Coceani F, Scherman D, Lachapelle P, Ong H, Chemtob S, Sennlaub F. CD36 deficiency leads to choroidal involution via COX2 down-regulation in rodents. PLoS Med 2008; 5:e39. [PMID: 18288886]
32. Zarbin MA. Current concepts in the pathogenesis of age-related macular degeneration. Arch Ophthalmol 2004; 122:598-614. [PMID: 15078679]
33. Beatty S, Koh H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. Surv Ophthalmol 2000; 45:115-34. [PMID: 11033038]
34. van den Bergh H. Photodynamic therapy of age-related macular degeneration: History and principles. Semin Ophthalmol 2001; 16:181-200. [PMID: 15513440]
35. Yamada Y, Tian J, Yang Y, Cutler RG, Wu T, Telljohann RS, Mattson MP, Handa JT. Oxidized low density lipoproteins induce a pathologic response by retinal pigmented epithelial cells. J Neurochem 2008; 105:1187-97. [PMID: 18182060]
36. Kamei M, Yoneda K, Kume N, Suzuki M, Itabe H, Matsuda K, Shimaoka T, Minami M, Yonehara S, Kita T, Kinoshita S. Scavenger receptors for oxidized lipoprotein in age-related macular degeneration. Invest Ophthalmol Vis Sci 2007; 48:1801-7. [PMID: 17389514]
37. Scott LJ, Goa KL. Verteporfin. Drugs Aging 2000; 16:139-46. [PMID: 10755329]
38. Nakashizuka H, Mitsumata M, Okisaka S, Shimada H, Kawamura A, Mori R, Yuzawa M. Clinicopathologic findings in polypoidal choroidal vasculopathy. Invest Ophthalmol Vis Sci 2008; 49:4729-37. [PMID: 18586873]
39. Ueta T, Obata R, Inoue Y, Iriyama A, Takahashi H, Yamaguchi T, Tamaki Y, Yanagi Y. Background comparison of typical age-related macular degeneration and polypoidal choroidal vasculopathy in Japanese patients. Ophthalmology 2009; 116:2400-6. [PMID: 19815291]
40. Yuan HY, Chiou JJ, Tseng WH, Liu CH, Liu CK, Lin YJ, Wang HH, Yao A, Chen YT, Hsu CN. FASTSNP: an always up-to-date and extendable service for SNP function analysis and prioritization. Nucleic Acids Res 2006; 34:W635–41. [PMID: 16845089]
41. Love-Gregory L, Sherva R, Sun L, Wasson J, Schappe T, Doria A, Rao DC, Hunt SC, Klein S, Neuman RJ, Permutt MA, Abumrad NA. Variants in the CD36 gene associate with the metabolic syndrome and high-density lipoprotein cholesterol. Hum Mol Genet 2008; 17:1695-704. [PMID: 18305138]
42. Love-Gregory L, Sherva R, Schappe T, Qi JS, McCrea J, Klein S, Connelly MA, Abumrad NA. Common CD36 SNPs reduce protein expression and may contribute to a protective atherogenic profile. Hum Mol Genet 2011; 20:193-201. [PMID: 20935172]
43. Picard E, Houssier M, Bujold K, Qi JS, McCrea J, Klein S, Connelly MA, Abumrad NA. CD36 plays an important role in the clearance of oxLDL and associated age-dependent sub-retinal deposits. Aging (Albany NY) 2010; 2:981-9. [PMID: 21098885]
44. Laude A, Cackett PD, Vithana EN, Yeo IY, Wong D, Koh AH, Wong TY, Aung T. Polypoidal choroidal vasculopathy and neovascular age-related macular degeneration: same or different disease? Prog Retin Eye Res 2010; 29:19-29. [PMID: 19854291]
45. Kishan AU, Modjtahedi BS, Martins EN, Modjtahedi SP, Morse LS. Lipids and age-related macular degeneration. Surv Ophthalmol 2011; 56:195-213. [PMID: 21439604]
46. Stahl A, Krohne TU, Sapieha P, Chen J, Hellstrom A, Chew E, Holz FG, Smith LE. Lipid metabolites in the pathogenesis and treatment of neovascular eye disease. Br J Ophthalmol 2011; 95:1496-501. [PMID: 21421650]
47. Silverstein RL. Type 2 scavenger receptor CD36 in platelet activation: the role of hyperlipemia and oxidative stress. Clin Lipidol. 2009; 4:767. [PMID: 20161667]

Molecular Vision 2011; 17:3574-3582 <http://www.molvis.org/molvis/v17/a384>
Received 24 August 2011 | Accepted 28 December 2011 | Published 31 December 2011

Difference between age-related macular degeneration and polypoidal choroidal vasculopathy in the hereditary contribution of the A69S variant of the age-related maculopathy susceptibility 2 gene (*ARMS2*)

Suiho Yanagisawa, Naoshi Kondo, Akiko Miki, Wataru Matsumiya, Sentaro Kusuhara, Yasutomo Tsukahara, Shigeru Honda, Akira Negi

Department of Surgery, Division of Ophthalmology, Kobe University Graduate School of Medicine, Chuo-ku, Kobe, Japan

Purpose: To investigate whether the A69S variant of the age-related maculopathy susceptibility 2 gene (*ARMS2*) has a different hereditary contribution in neovascular age-related macular degeneration (AMD) and polypoidal choroidal vasculopathy (PCV).

Methods: We initially conducted a comparative genetic analysis of neovascular AMD and PCV, genotyping the *ARMS2* A69S variant in 181 subjects with neovascular AMD, 198 subjects with PCV, and 203 controls in a Japanese population. Genotyping was conducted using TaqMan technology. Results were then integrated into a meta-analysis of previous studies representing an assessment of the association between the *ARMS2* A69S variant and neovascular AMD and/or PCV, comprising a total of 3,828 subjects of Asian descent. The Q-statistic test was used to assess between-study heterogeneity. Summary odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using a fixed effects model.

Results: The genetic effect of the A69S variant was stronger in neovascular AMD (allelic summary OR=3.09 [95% CI, 2.71–3.51], fixed effects $p<0.001$) than in PCV (allelic summary OR=2.13 [95% CI, 1.91–2.38], fixed effects $p<0.001$). The pooled risk allele frequency was significantly higher in neovascular AMD (64.7%) than in PCV (55.6%). The population attributable risks for the variant allele were estimated to be 43.9% (95% CI, 39.0%–48.4%) and 29.7% (95% CI, 25.4%–34.0%) for neovascular AMD and PCV, respectively. No significant between-study heterogeneity was observed in any statistical analysis in this meta-analysis.

Conclusions: Our meta-analysis provides substantial evidence that the *ARMS2* A69S variant confers a significantly higher risk of neovascular AMD than PCV. Furthermore, there is compelling evidence that the risk attributable to the A69S variant differs between geographic atrophy and neovascular AMD. Together with defining the molecular basis of susceptibility, understanding the relationships between this genomic region and disease subtypes will yield important insights, elucidating the biologic architecture of this phenotypically heterogeneous disorder.

Age-related macular degeneration (AMD), a leading cause of irreversible blindness among older individuals in developed countries, is a common multifactorial disease with heterogeneous clinical manifestations [1]. An early hallmark lesion of AMD is large drusen and pigmentary abnormalities in the retinal pigment epithelium of the macula. The advanced form of the disease is classified into two main groups: "dry" and "wet" types; the former is characterized by geographic atrophy and the latter by the development of choroidal neovascularization (CNV) in the central macula (neovascular AMD). AMD has divergent clinical features between racial groups, and the ratio of neovascular AMD to dry AMD is higher in Asian than in European populations [2-5].

Correspondence to: Naoshi Kondo, Department of Surgery, Division of Ophthalmology, Kobe University Graduate School of Medicine, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan; Phone: +81-78-382-6048; FAX: +81-78-382-6059; email: nskondo@gmail.com

Remarkable progress has recently been made in understanding the genetic basis of AMD. Several AMD susceptibility loci have been established with convincing statistical evidence, including the complement factor H gene on chromosome 1q32 [6-8], two tightly linked genes on 10q26 (age-related maculopathy susceptibility 2 [*ARMS2*] [9] and high-temperature requirement factor H [*HTRA1*]) [10,11], the complement component 3 gene on 19p13 [12], two neighboring genes on 6p21 (complement factor B and complement component 2) [13], the complement factor I gene on 4q25 [14], the hepatic lipase gene on 15q22 [15,16], the cholesterylester transfer protein gene on 16q21 [15,16], and the tissue inhibitor of the metalloproteinase 3 gene on 22q12 [15,16]. Recent additions to the growing list of potential AMD risk loci include 6q21–q22.3 that encompass two genes—the collagen, type X, alpha 1 gene and the fyn-related kinase gene—and 6p12 harboring the vascular endothelial growth factor A gene, which were identified through a recent large-scale meta-analysis of genome-wide association study for advanced

Table 1. Characteristics of the Study Population

Groups	Neovascular AMD	PCV	Control
Number of subjects	181	198	203
Gender (male/female)	139/42	157/41	120/83
Mean age ± SD (years)	75±7.4	73±7.3	72±6.0
Age range (years)	55–94	54–93	56–95

Abbreviations: AMD, age-related macular degeneration; PCV, polypoidal choroidal vasculopathy; SD, standard deviation.

AMD [17]. The meta-analysis showed that the missense allele encoding A69S (rs10490924) in *ARMS2* confers the strongest disease risk, among others [17].

Polypoidal choroidal vasculopathy (PCV), characterized by inner choroidal vascular networks ending in polypoidal lesions [18], is now clinically classified as a specific type of AMD [19]. PCV is particularly prevalent in Asian populations, accounting for 54.7% of patients with the neovascular form of AMD in the Japanese population [20] and 24.5% in the Chinese population [21], but only 8% to 13% in Caucasians [22]. PCV shares many similarities with neovascular AMD, including demography [20], pathology [23,24], and manifestation [20]; however, important differences have been noted in histopathology [25], clinical behavior [22], and response to therapy [18,26]. These similarities and differences have been a subject of much interest and debate regarding whether the vascular abnormality in PCV represents neovascularization or a phenotype distinct from CNV [23-25,27].

We have previously shown that the *ARMS2* A69S variant is strongly associated with neovascular AMD and PCV, with a stronger association in neovascular AMD than in PCV [28]; however, the difference was not statistically significant, probably owing to a limitation in statistical power. Subsequent A69S association studies have consistently reported a trend toward stronger evidence for association in neovascular AMD than in PCV [29-31]. Interestingly, a significant difference in genetic susceptibility between geographic atrophy and neovascular AMD has been repeatedly observed at this locus [17,32]. Sub-phenotype associations are currently being actively researched in complex diseases, such as inflammatory bowel disease [33], rheumatoid arthritis [34], and various cancers [35-37]. Genotype–phenotype correlations between risk alleles and disease subtypes may provide an insight into the underlying etiologic pathways of complex diseases.

To date, some meta-analyses have been published regarding the association between AMD and the *ARMS2/HTRA1* region [38-40], but none of these studies focused on PCV. Here we conducted a comparative genetic analysis of neovascular AMD and PCV in our original sample set of Japanese ancestry, genotyping the *ARMS2* A69S variant in 181 subjects with neovascular AMD, 198 subjects with PCV, and 203 controls. Results were then integrated into a meta-analysis of previous studies representing an assessment of the association between the *ARMS2* A69S variant and neovascular AMD and/or PCV, comprising a total of 3,828 subjects of Asian descent, to more reliably compare the genetic effect of *ARMS2* A69S between neovascular AMD and PCV.

METHODS

New data set: Study participants: The study protocol was approved by the Institutional Review Board at Kobe University Graduate School of Medicine and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects before participation in this study. All cases and controls included in our original sample set were Japanese individuals recruited from the Department of Ophthalmology at Kobe University Hospital in Kobe, Japan. This cohort is an extension of one previously published for an association with the *ARMS2* A69S variant [28]. A portion of the subjects in the present study had participated in our previous studies in which phenotyping criteria were fully described [28,41,42]. In brief, all our subjects with neovascular AMD and PCV underwent a comprehensive ophthalmic examination including indocyanine green angiography, and were defined as having angiographically well defined lesions of CNV or PCV. The controls were not related to the cases and were defined as individuals without macular degeneration and changes such as drusen or pigment abnormalities, and were thus categorized as having clinical age-related maculopathy staging system stage 1 [43]. The demographic details of the study subjects are listed in Table 1.

Genotyping: Genomic DNA was extracted from peripheral blood using a standard methodology. Genotyping was performed using a pre-developed TaqMan SNP Genotyping Assay (Assay ID: C_29934973_20; Applied Biosystems, Foster City, CA) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems) in accordance with the manufacturer's recommendations.

Statistical analysis: Allelic associations were evaluated for the *ARMS2* A69S variant with chi-square tests on 2 × 2 contingency tables using the software package PLINK v1.07. Deviations from the Hardy–Weinberg equilibrium

(HWE) were tested using the exact test [44] implemented in PLINK. The odds ratio (OR) and corresponding 95% confidence interval (CI) were calculated relative to the major allele. Genotype-specific ORs were estimated for the heterozygous (GT) and risk homozygous (TT) genotypes, with the common homozygous (GG) genotype the baseline category with unconditional logistic regression using the JMP software (version 6.0.3; SAS Institute, Cary, NC). To test for heterogeneity between ORs for neovascular AMD and PCV, we conducted a logistic regression analysis of the cases (case-only analysis) using R project, where the subtypes were used as the outcome and the A69S genotype as the explanatory variable [45].

Meta-analysis: Identification and eligibility of relevant studies: We performed a systematic PubMed literature search (up to May 2011) using the following search terms in different combinations: "HtrA serine peptidase 1" or "*HTRA1*," "age-related maculopathy susceptibility 2," "*ARMS2,*" or "LOC387715," and "age-related macular degeneration" or "polypoidal choroidal vasculopathy." The literature search was performed in duplicate by two authors (S.Y. and N.K.).

Studies included in the meta-analysis had to fulfill the following criteria: (1) The study must be unrelated case-control or population-based representing an assessment of the association between the *ARMS2* A69S variant and neovascular AMD and/or PCV in East Asian populations. (2) The study must distinguish PCV from the neovascular form of AMD based on findings of indocyanine green angiography, and must look at PCV and/or neovascular AMD (CNV) as specific outcomes. (3) The study must present available data on allele and genotype distributions for cases and controls. (4) The study must be written in English and published in peer-reviewed journals. For duplicate publications, the largest data set was chosen for meta-analysis.

Data extraction: The following variables were extracted from each study: the name of the first author, the year of publication, ethnicity, and allele and genotype distributions in cases and controls.

Statistical analyses: For each study, deviations from the HWE in controls were tested using the exact test [44]. Pooled allele and genotype frequencies of the A69S variant were estimated with the fixed effects model [46] if heterogeneity among studies was absent, or with the random effects model [47] if heterogeneity was present. We estimated summary ORs and 95% CIs according to the Mantel–Haenszel fixed effects model [46] if heterogeneity among studies was absent or the DerSimonian–Laird random effects model [47] if there was evidence of between-study heterogeneity. The population attribute risk was calculated to demonstrate the number of cases in the total population that could be attributed to the risk genotype, as described previously [48].

Between-study heterogeneity was assessed using the Q-statistic test and I^2 statistic [49,50]. A p value of <0.1 was considered statistically significant for the Q-statistic test. I^2 ranges between 0% and 100% (where a value of 0% represents no heterogeneity), and larger values represent increasing heterogeneity.

All meta-analyses were conducted using the Stata software (version 11.0; Stata Corporation, College Station, TX). All tests were two tailed. A p value of <0.05 was considered statistically significant except for the test of between-study heterogeneity.

RESULTS

Comparative genetic analysis in our original sample set: We initially conducted a comparative genetic analysis of neovascular AMD and PCV, genotyping the *ARMS2* A69S variant (rs10490924) in our original sample set. Genotype distributions for this variant are given in Table 2, along with those of other studies included in the subsequent meta-analysis. No departure from the HWE was observed at this variant among the controls (p=0.88). As expected, the *ARMS2* A69S variant showed strong evidence of association with neovascular AMD and PCV. ORs for the risk allele T were 2.82 (95% CI, 2.10–3.78, $p=2.4\times10^{-12}$) and 2.39 (95% CI, 1.80–3.17, $p=1.3\times10^{-9}$) for neovascular AMD and PCV, respectively. For heterozygous and homozygous carriers of the risk allele, the genotype-specific OR was 2.62 (95% CI, 1.55–4.52) and 7.49 (95% CI, 4.11–14.07) for neovascular AMD and 1.56 (95% CI, 0.97–2.53) and 5.02 (95% CI, 2.89–8.90) for PCV, respectively. Similar to previous findings of *ARMS2* A69S association studies [29-31], the variant showed a trend toward stronger effect in neovascular AMD than in PCV. However, a case-only heterogeneity test with logistic regression analysis showed a nonsignificant value in our original sample set (heterogeneity p=0.31), possibly reflecting inadequate statistical power in this single study. Our own data were then combined with those from previously published studies in the subsequent meta-analysis.

Meta-analysis: Eligibility of studies: Our search identified five studies that met our inclusion criteria [29-31,51,52]. Data from these five studies and our original study were combined for the meta-analysis. Table 2 lists the studies included in the meta-analysis. The combined sample size for this meta-analysis was 3,828.

Allele and genotype frequency: None of the five previously published studies demonstrated significant deviation from the HWE among controls (Table 2). To estimate the pooled frequency of the A69S variant in Asian populations, we used allele data from controls. The pooled frequency for the risk allele T was 37.4% (95% CI, 35.9–38.8), and individuals carrying at least one copy of the risk allele (GT + TT) accounted for 60.8% (95% CI, 58.7–62.9) of the control populations. No evidence of heterogeneity in these frequencies was observed among controls across the six studies (allele frequency, Q=8.50, 5 degrees of freedom [d.f.],

Table 2. Allele and Genotype Distributions of the *ARMS2* A69S Variant of Case-Control Studies Contributing to the Meta-Analysis

			Genotype (GG/GT/TT)			Risk allele frequency			
Study	Year	Ethnicity	Neovascular AMD	PCV	Control	Neovascular AMD	PCV	Control	PHWE*
[51]	2008	Japanese	NA	15/49/45	39/32/14	NA	0.64	0.35	0.10
[52]	2008	Chinese	NA	17/30/25	33/48/12	NA	0.56	0.39	0.51
[29]	2009	Japanese	18/30/52	18/50/32	85/84/20	0.67	0.57	0.33	1.0
[30]	2010	Japanese	67/155/183	122/216/171	502/638/196	0.64	0.55	0.39	0.82
[31]	2011	Japanese	6/20/24	22/20/18	64/58/16	0.68	0.47	0.33	0.70
This study	2011	Japanese	26/81/74	42/77/79	79/94/30	0.63	0.59	0.38	0.88

Abbreviations: ARMS2, age-related maculopathy susceptibility 2; AMD, age-related macular degeneration; PCV, polypoidal choroidal vasculopathy; HWE, Hardy–Weinberg equilibrium; NA, not available. *p values generated by the exact test for Hardy–Weinberg equilibrium.

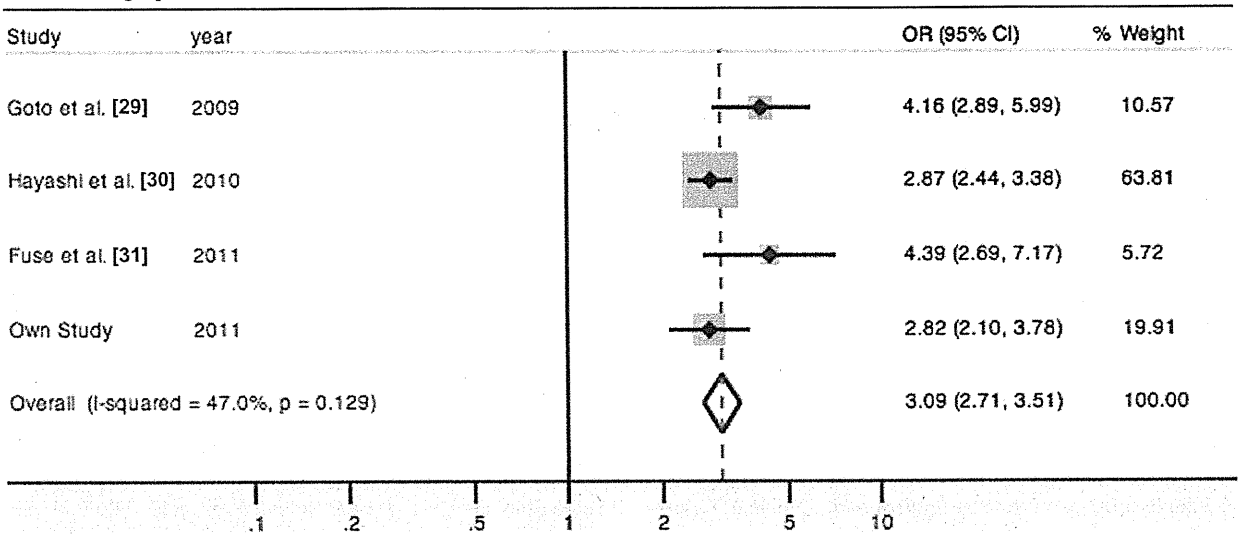

Figure 1. Forest plot showing the association between *ARMS2* A69S and neovascular age-related macular degeneration. Odds ratios (black squares) and 95% confidence intervals (bars) are given for each study. Also shown are the unshaded diamonds of the summary odds ratio based on the Mantel–Haenszel fixed effects model.

p=0.13, I^2=41.2%; frequency of GT/TT genotypes, Q=9.0, 5 d.f., p=0.11, I^2=44.5%).

Quantitative synthesis: We conducted a meta-analysis based on an allele contrast model. The A69S variant showed a significant summary OR of 3.09 ([95% CI, 2.71–3.51], fixed effects p<0.001; Figure 1) for neovascular AMD and 2.13 ([95% CI, 1.91–2.38], fixed effects p<0.001; Figure 2) for PCV. The Q-statistic test showed no significant between-study heterogeneity in association tests for neovascular AMD or PCV (p>0.1; Figure 1 and Figure 2). The population attribute risks for the risk allele were 43.9% (95% CI, 39.0%–48.4%) and 29.7% (95% CI, 25.4%–34.0%) for neovascular AMD and PCV, respectively. Next, we compared the allele frequencies of the variant between the two subtypes, combining data from four studies that included neovascular AMD and PCV subtypes in the case groups (Table 2). The pooled risk allele frequency was significantly higher in neovascular AMD than in PCV (64.7% versus 55.6%; p<0.001), without heterogeneity across studies (Q=5.38, 3 days.f., p=0.15, I^2=44.3%). This result, coupled with the finding that the 95% CIs for allelic summary ORs for neovascular AMD did not overlap with those for PCV, indicates that the genetic effect of the *ARMS2* A69S variant is significantly stronger in neovascular AMD than in PCV.

DISCUSSION

Several studies have reported that the *ARMS2* A69S variant is strongly associated with neovascular AMD and PCV, with a stronger association in neovascular AMD than in PCV [29-31]. However, the differences between the two were not statistically significant in most studies, probably owing to a limitation in the statistical power. Our meta-analysis has revealed that the *ARMS2* A69S variant confers a significantly greater risk of neovascular AMD than of PCV. The pooled

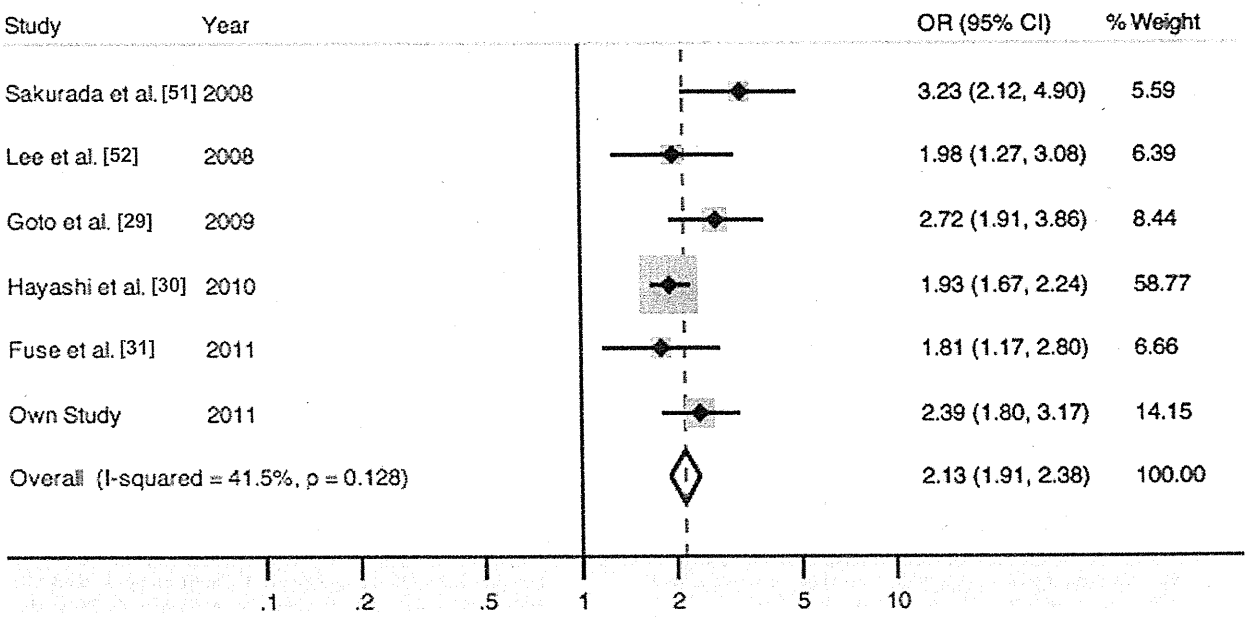

Figure 2. Forest plot showing the association between *ARMS2* A69S and polypoidal choroidal vasculopathy. Odds ratios (black squares) and 95% confidence intervals (bars) are given for each study. Also shown are the unshaded diamonds of the summary odds ratio based on the Mantel–Haenszel fixed effects model.

risk allele frequency was significantly higher in neovascular AMD (64.7%) compared with PCV (55.6%). The meta-analysis estimated the attributable risks for the variant allele were 43.9% and 29.7% for neovascular AMD and PCV, respectively. In the control populations, the pooled frequency for the risk allele T of the A69S variant was estimated to be 37.4%, and individuals carrying at least one copy of the risk allele accounted for 60.8%, indicating its population-wide epidemiological consequence in Asian populations owing to the high frequency of the risk allele. No significant between-study heterogeneity was observed in any statistical analysis in this meta-analysis of Asian populations.

There is increasing evidence that ethnicity influences disease via genetic background [53]. Risk allele frequencies of A69S diverge greatly between European and Asian populations from the HapMap sample, with almost 40% risk allele frequencies in Asian populations compared to 20% in individuals of European descent. The distributions of the neovascular subtype of AMD differ markedly between European and Asian populations, and parallel the risk allele frequencies of this variant, with Asians having a much higher rate of the neovascular subtype than Europeans [2-5], suggesting that this locus may contribute to ethnic heterogeneity in the manifestation of AMD subtypes.

Currently, how the *ARMS2/HTRA1* region on 10q26 is a source of genetic risk for AMD is unclear. Much effort has been made to localize variant(s) causally related to AMD in this region and to understand the molecular basis of the susceptibility [10,11,54-58]. However, there is high linkage disequilibrium (LD) across the *ARMS2/HTRA1* region, adding to the difficulty in identifying true causal variant(s) by association mapping alone [55]. The association signal at 10q26 converges on a region of an extensive LD block spanning *ARMS2* and *HTRA1* [54,55]. This LD block harbors multiple susceptibility alleles of which the *ARMS2* A69S variant has been reported to show the strongest evidence for association [54]. Two variants within this LD block that were correlated with A69S through strong LD—SNP rs11200638 in the promoter of *HTRA1* [10,11] and the insertion/deletion polymorphism (c.(*)372_815del443ins54) in the 3′-UTR region of *ARMS2* [55]—have recently been proposed as causal variants based on mechanistic functional evidence, but there is no agreement across studies [10,11,54-58]. Thus, the molecular basis of the susceptibility remains obscure.

In conclusion, our meta-analysis has identified a difference in the hereditary contribution of the *ARMS2* A69S variant between neovascular AMD and PCV. In addition, a significant difference has been reported between geographic atrophy and neovascular AMD with respect to genetic susceptibility at this locus [17,32]. This fact, coupled with our findings, indicates that the risk attributable to the A69S variant differs among AMD subtypes. Given the importance of the *ARMS2/HTRA1* region on 10q26 in AMD susceptibility, defining molecular mechanisms through which the genomic variants influence disease risk and understanding the relationships between this region and disease subtypes will yield important insights, elucidating the biologic architecture of this phenotypically heterogeneous disorder.

ACKNOWLEDGMENTS

This study was supported by a Grant-in Aid for (C) 23592567 from the Ministry of Education, Science, and Culture, Tokyo, Japan, and by a grant from the Takeda Science Foundation. None of the authors have any financial or conflicting interests to disclose.

REFERENCES

1. Jager RD, Mieler WF, Miller JW. Age-related macular degeneration. N Engl J Med 2008; 358:2606-17. [PMID: 18550876]
2. Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy. The Beaver Dam Eye Study. Ophthalmology 1992; 99:933-43. [PMID: 1630784]
3. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. Ophthalmology 1995; 102:1450-60. [PMID: 9097791]
4. 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; 85:1153-7. [PMID: 11567955]
5. Kawasaki R, Yasuda M, Song SJ, Chen SJ, Jonas JB, Wang JJ, Mitchell P, Wong TY. The prevalence of age-related macular degeneration in Asians: a systematic review and meta-analysis. Ophthalmology 2010; 117:921-7. [PMID: 20110127]
6. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J. Complement factor H polymorphism in age-related macular degeneration. Science 2005; 308:385-9. [PMID: 15761122]
7. Edwards AO, Ritter R 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science 2005; 308:421-4. [PMID: 15761121]
8. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Noureddine M, Gilbert JR, Schnetz-Boutaud N, Agarwal A, Postel EA, Pericak-Vance MA. Complement factor H variant increases the risk of age-related macular degeneration. Science 2005; 308:419-21. [PMID: 15761120]
9. Rivera A, Fisher SA, Fritsche LG, Keilhauer CN, Lichtner P, Meitinger T, Weber BH. Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. Hum Mol Genet 2005; 14:3227-36. [PMID: 16174643]
10. Dewan A, Liu M, Hartman S, Zhang SS, Liu DT, Zhao C, Tam PO, Chan WM, Lam DS, Snyder M, Barnstable C, Pang CP, Hoh J. *HTRA1* promoter polymorphism in wet age-related macular degeneration. Science 2006; 314:989-92. [PMID: 17053108]
11. Yang Z, Camp NJ, Sun H, Tong Z, Gibbs D, Cameron DJ, Chen H, Zhao Y, Pearson E, Li X, Chien J, Dewan A, Harmon J, Bernstein PS, Shridhar V, Zabriskie NA, Hoh J, Howes K, Zhang K. A variant of the *HTRA1* gene increases susceptibility to age-related macular degeneration. Science 2006; 314:992-3. [PMID: 17053109]
12. Yates JR, Sepp T, Matharu BK, Khan JC, Thurlby DA, Shahid H, Clayton DG, Hayward C, Morgan J, Wright AF, Armbrecht AM, Dhillon B, Deary IJ, Redmond E, Bird AC, Moore AT, Genetic Factors in AMD Study Group. Complement C3 variant and the risk of age-related macular degeneration. N Engl J Med 2007; 357:553-61. [PMID: 17634448]
13. Gold B, Merriam JE, Zernant J, Hancox LS, Taiber AJ, Gehrs K, Cramer K, Neel J, Bergeron J, Barile GR, Smith RT, AMD Genetics Clinical Study Group. Hageman GS, Dean M, Allikmets R. Variation in factor B (*BF*) and complement component 2 (*C2*) genes is associated with age-related macular degeneration. Nat Genet 2006; 38:458-62. [PMID: 16518403]
14. Fagerness JA, Maller JB, Neale BM, Reynolds RC, Daly MJ, Seddon JM. Variation near complement factor I is associated with risk of advanced AMD. Eur J Hum Genet 2009; 17:100-4. [PMID: 18685559]
15. Neale BM, Fagerness J, Reynolds R, Sobrin L, Parker M, Raychaudhuri S, Tan PL, Oh EC, Merriam JE, Souied E, Bernstein PS, Li B, Frederick JM, Zhang K, Brantley MA Jr, Lee AY, Zack DJ, Campochiaro B, Campochiaro P, Ripke S, Smith RT, Barile GR, Katsanis N, Allikmets R, Daly MJ, Seddon JM. Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (LIPC). Proc Natl Acad Sci USA 2010; 107:7395-400. [PMID: 20385826]
16. Chen W, Stambolian D, Edwards AO, Branham KE, Othman M, Jakobsdottir J, Tosakulwong N, Pericak-Vance MA, Campochiaro PA, Klein ML, Tan PL, Conley YP, Kanda A, Kopplin L, Li Y, Augustaitis KJ, Karoukis AJ, Scott WK, Agarwal A, Kovach JL, Schwartz SG, Postel EA, Brooks M, Baratz KH, Brown WL, Complications of Age-Related Macular Degeneration Prevention Trial Research Group. Brucker AJ, Orlin A, Brown G, Ho A, Regillo C, Donoso L, Tian L, Kaderli B, Hadley D, Hagstrom SA, Peachey NS, Klein R, Klein BE, Gotoh N, Yamashiro K, Ferris Iii F, Fagerness JA, Reynolds R, Farrer LA, Kim IK, Miller JW, Cortón M, Carracedo A, Sanchez-Salorio M, Pugh EW, Doheny KF, Brion M, Deangelis MM, Weeks DE, Zack DJ, Chew EY, Heckenlively JR, Yoshimura N, Iyengar SK, Francis PJ, Katsanis N, Seddon JM, Haines JL, Gorin MB, Abecasis GR, Swaroop A. Genetic variants near TIMP3 and high-density lipoprotein-associated loci influence susceptibility to age-related macular degeneration. Proc Natl Acad Sci USA 2010; 107:7401-6. [PMID: 20385819]
17. Yu Y, Bhangale TR, Fagerness J, Ripke S, Thorleifsson G, Tan PL, Souied EH, Richardson AJ, Merriam JE, Buitendijk GH, Reynolds R, Raychaudhuri S, Chin KA, Sobrin L, Evangelou E, Lee PH, Lee AY, Leveziel N, Zack DJ, Campochiaro B, Campochiaro P, Smith RT, Barile GR, Guymer RH, Hogg R, Chakravarthy U, Robman LD, Gustafsson O, Sigurdsson H, Ortmann W, Behrens TW, Stefansson K, Uitterlinden AG, van Duijn CM, Vingerling JR, Klaver CC, Allikmets R, Brantley MA Jr, Baird PN, Katsanis N, Thorsteinsdottir U, Ioannidis JP, Daly MJ, Graham RR, Seddon JM. Common Variants near FRK/COL10A1 and VEGFA are Associated with Advanced Age-related Macular Degeneration. Hum Mol Genet 2011; 20:3699-709. [PMID: 21665990]

18. Imamura Y, Engelbert M, Iida T, Freund KB, Yannuzzi LA. Polypoidal choroidal vasculopathy: a review. Surv Ophthalmol 2010; 55:501-15. [PMID: 20850857]
19. Takahashi K, Ishibashi T, Ogur Y, Yuzawa M, Working Group for Establishing Diagnostic Criteria for Age-Related Macular Degeneration. Classification and diagnostic criteria of age-related macular degeneration. Nippon Ganka Gakkai Zasshi 2008; 112:1076-84. [PMID: 19157028]
20. Maruko I, Iida T, Saito M, Nagayama D, Saito K. Clinical characteristics of exudative age-related macular degeneration in Japanese patients. Am J Ophthalmol 2007; 144:15-22. [PMID: 17509509]
21. Liu Y, Wen F, Huang S, Luo G, Yan H, Sun Z, Wu D. Subtype lesions of neovascular age-related macular degeneration in Chinese patients. Graefes Arch Clin Exp Ophthalmol 2007; 245:1441-5. [PMID: 17406882]
22. Ciardella AP, Donsoff IM, Huang SJ, Costa DL, Yannuzzi LA. Polypoidal choroidal vasculopathy. Surv Ophthalmol 2004; 49:25-37. [PMID: 14711438]
23. Kikuchi M, Nakamura M, Ishikawa K, Suzuki T, Nishihara H, Yamakoshi T, Nishio K, Taki K, Niwa T, Hamajima N, Terasaki H. Elevated C-reactive protein levels in patients with polypoidal choroidal vasculopathy and patients with neovascular age-related macular degeneration. Ophthalmology 2007; 114:1722-7. [PMID: 17400294]
24. Tong JP, Chan WM, Liu DT, Lai TY, Choy KW, Pang CP, Lam DS. Aqueous humor levels of vascular endothelial growth factor and pigment epithelium-derived factor in polypoidal choroidal vasculopathy and choroidal neovascularization. Am J Ophthalmol 2006; 141:456-62. [PMID: 16490490]
25. Nakashizuka H, Mitsumata M, Okisaka S, Shimada H, Kawamura A, Mori R, Yuzawa M. Clinicopathologic findings in polypoidal choroidal vasculopathy. Invest Ophthalmol Vis Sci 2008; 49:4729-37. [PMID: 18586873]
26. Honda S, Imai H, Yamashiro K, Kurimoto Y, Kanamori-Matsui N, Kagotani Y, Tamura Y, Yamamoto H, Ohoto S, Takagi H, Uenishi M, Negi A. Comparative assessment of photodynamic therapy for typical age-related macular degeneration and polypoidal choroidal vasculopathy: a multicenter study in Hyogo prefecture, Japan. Ophthalmologica 2009; 223:333-8. [PMID: 19478533]
27. Yuzawa M, Mori R, Kawamura A. The origins of polypoidal choroidal vasculopathy. Br J Ophthalmol 2005; 89:602-7. [PMID: 15834093]
28. Kondo N, Honda S, Ishibashi K, Tsukahara Y, Negi A. *LOC387715/HTRA1* variants in polypoidal choroidal vasculopathy and age-related macular degeneration in a Japanese population. Am J Ophthalmol 2007; 144:608-12. [PMID: 17692272]
29. Goto A, Akahori M, Okamoto H, Minami M, Terauchi N, Haruhata Y, Obazawa M, Noda T, Honda M, Mizota A, Tanaka M, Hayashi T, Tanito M, Ogata N, Iwata T. Genetic analysis of typical wet-type age-related macular degeneration and polypoidal choroidal vasculopathy in Japanese population. J Ocul Biol Dis Infor 2009; 2:164-75. [PMID: 20157352]
30. Hayashi H, Yamashiro K, Gotoh N, Nakanishi H, Nakata I, Tsujikawa A, Otani A, Saito M, Iida T, Matsuo K, Tajima K, Yamada R, Yoshimura N. *CFH* and *ARMS2* variations in age-related macular degeneration, polypoidal choroidal vasculopathy, and retinal angiomatous proliferation. Invest Ophthalmol Vis Sci 2010; 51:5914-9. [PMID: 20574013]
31. Fuse N, Mengkegale M, Miyazawa A, Abe T, Nakazawa T, Wakusawa R, Nishida K. Polymorphisms in *ARMS2* (*LOC387715*) and *LOXL1* genes in the Japanese with age-related macular degeneration. Am J Ophthalmol 2011; 151:550-6. [PMID: 21236409]
32. Sobrin L, Reynolds R, Yu Y, Fagerness J, Leveziel N, Bernstein PS, Souied EH, Daly MJ, Seddon JM. *ARMS2/HTRA1* locus can confer differential susceptibility to the advanced subtypes of age-related macular degeneration. Am J Ophthalmol 2011; 151:345-52. [PMID: 21122828]
33. Franke A, Balschun T, Karlsen TH, Sventoraityte J, Nikolaus S, Mayr G, Domingues FS, Albrecht M, Nothnagel M, Ellinghaus D, Sina C, Onnie CM, Weersma RK, Stokkers PC, Wijmenga C, Gazouli M, Strachan D, McArdle WL, Vermeire S, Rutgeerts P, Rosenstiel P, Krawczak M, Vatn MH, IBSEN Study Group. Mathew CG, Schreiber S. Sequence variants in *IL10, ARPC2* and multiple other loci contribute to ulcerative colitis susceptibility. Nat Genet 2008; 40:1319-23. [PMID: 18836448]
34. Thomson W, Barton A, Ke X, Eyre S, Hinks A, Bowes J, Donn R, Symmons D, Hider S, Bruce IN, Wellcome Trust Case Control Consortium. Wilson AG, Marinou I, Morgan A, Emery P. YEAR Consortium; Carter A, Steer S, Hocking L, Reid DM, Wordsworth P, Harrison P, Strachan D, Worthington J. Rheumatoid arthritis association at 6q23. Nat Genet 2007; 39:1431-3. [PMID: 17982455]
35. Stacey SN, Manolescu A, Sulem P, Thorlacius S, Gudjonsson SA, Jonsson GF, Jakobsdottir M, Bergthorsson JT, Gudmundsson J, Aben KK, Strobbe LJ, Swinkels DW, van Engelenburg KC, Henderson BE, Kolonel LN, Le Marchand L, Millastre E, Andres R, Saez B, Lambea J, Godino J, Polo E, Tres A, Picelli S, Rantala J, Margolin S, Jonsson T, Sigurdsson H, Jonsdottir T, Hrafnkelsson J, Johannsson J, Sveinsson T, Myrdal G, Grimsson HN, Sveinsdottir SG, Alexiusdottir K, Saemundsdottir J, Sigurdsson A, Kostic J, Gudmundsson L, Kristjansson K, Masson G, Fackenthal JD, Adebamowo C, Ogundiran T, Olopade OI, Haiman CA, Lindblom A, Mayordomo JI, Kiemeney LA, Gulcher JR, Rafnar T, Thorsteinsdottir U, Johannsson OT, Kong A, Stefansson K. Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. Nat Genet 2008; 40:703-6. [PMID: 18438407]
36. Goode EL, Chenevix-Trench G, Song H, Ramus SJ, Notaridou M, Lawrenson K, Widschwendter M, Vierkant RA, Larson MC, Kjaer SK, Birrer MJ, Berchuck A, Schildkraut J, Tomlinson I, Kiemeney LA, Cook LS, Gronwald J, Garcia-Closas M, Gore ME, Campbell I, Whittemore AS, Sutphen R, Phelan C, Anton-Culver H, Pearce CL, Lambrechts D, Rossing MA, Chang-Claude J, Moysich KB, Goodman MT, Dörk T, Nevanlinna H, Ness RB, Rafnar T, Hogdall C, Hogdall E, Fridley BL, Cunningham JM, Sieh W, McGuire V, Godwin AK, Cramer DW, Hernandez D, Levine D, Lu K, Iversen ES, Palmieri RT, Houlston R, van Altena AM, Aben KK, Massuger LF, Brooks-Wilson A, Kelemen LE, Le ND, Jakubowska A, Lubinski J, Medrek K, Stafford A, Easton DF, Tyrer J, Bolton KL, Harrington P, Eccles D, Chen A, Molina AN, Davila BN, Arango H, Tsai YY, Chen Z, Risch HA, McLaughlin J, Narod SA, Ziogas A, Brewster W, Gentry-

Maharaj A, Menon U, Wu AH, Stram DO, Pike MC, Wellcome Trust Case-Control Consortium; Beesley J, Webb PM, Australian Cancer Study (Ovarian Cancer); Australian Ovarian Cancer Study Group; Ovarian Cancer Association Consortium (OCAC); Chen X, Ekici AB, Thiel FC, Beckmann MW, Yang H, Wentzensen N, Lissowska J, Fasching PA, Despierre E, Amant F, Vergote I, Doherty J, Hein R, Wang-Gohrke S, Lurie G, Carney ME, Thompson PJ, Runnebaum I, Hillemanns P, Dürst M, Antonenkova N, Bogdanova N, Leminen A, Butzow R, Heikkinen T, Stefansson K, Sulem P, Besenbacher S, Sellers TA, Gayther SA, Pharoah PD, Ovarian Cancer Association Consortium (OCAC). A genome-wide association study identifies susceptibility loci for ovarian cancer at 2q31 and 8q24. Nat Genet 2010; 42:874-9. [PMID: 20852632]

37. Study Group of Millennium Genome Project for Cancer. Sakamoto H, Yoshimura K, Saeki N, Katai H, Shimoda T, Matsuno Y, Saito D, Sugimura H, Tanioka F, Kato S, Matsukura N, Matsuda N, Nakamura T, Hyodo I, Nishina T, Yasui W, Hirose H, Hayashi M, Toshiro E, Ohnami S, Sekine A, Sato Y, Totsuka H, Ando M, Takemura R, Takahashi Y, Ohdaira M, Aoki K, Honmyo I, Chiku S, Aoyagi K, Sasaki H, Ohnami S, Yanagihara K, Yoon KA, Kook MC, Lee YS, Park SR, Kim CG, Choi IJ, Yoshida T, Nakamura Y, Hirohashi S. Genetic variation in PSCA is associated with susceptibility to diffuse-type gastric cancer. Nat Genet 2008; 40:730-40. [PMID: 18488030]
38. Chen W, Xu W, Tao Q, Liu J, Li X, Gan X, Hu H, Lu Y. Meta-analysis of the association of the *HTRA1* polymorphisms with the risk of age-related macular degeneration. Exp Eye Res 2009; 89:292-300. [PMID: 19026638]
39. Tang NP, Zhou B, Wang B, Yu RB. *HTRA1* promoter polymorphism and risk of age-related macular degeneration: a meta-analysis. Ann Epidemiol 2009; 19:740-5. [PMID: 19375943]
40. Tong Y, Liao J, Zhang Y, Zhou J, Zhang H, Mao M. *LOC387715/HTRA1* gene polymorphisms and susceptibility to age-related macular degeneration: A HuGE review and meta-analysis. Mol Vis 2010; 16:1958-81. [PMID: 21031019]
41. Kondo N, Honda S, Kuno S, Negi A. Coding variant I62V in the complement factor H gene is strongly associated with polypoidal choroidal vasculopathy. Ophthalmology 2009; 116:304-10. [PMID: 19187823]
42. Kondo N, Honda S, Kuno S, Negi A. Role of *RDBP* and *SKIV2L* variants in the major histocompatibility complex class III region in polypoidal choroidal vasculopathy etiology. Ophthalmology 2009; 116:1502-9. [PMID: 19556007]
43. Seddon JM, Sharma S, Adelman RA. Evaluation of the clinical age-related maculopathy staging system. Ophthalmology 2006; 113:260-6. [PMID: 16458093]
44. Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of Hardy–Weinberg equilibrium. Am J Hum Genet 2005; 76:887-93. [PMID: 15789306]
45. Zhang XJ, Huang W, Yang S, Sun LD, Zhang FY, Zhu QX, Zhang FR, Zhang C, Du WH, Pu XM, Li H, Xiao FL, Wang ZX, Cui Y, Hao F, Zheng J, Yang XQ, Cheng H, He CD, Liu XM, Xu LM, Zheng HF, Zhang SM, Zhang JZ, Wang HY, Cheng YL, Ji BH, Fang QY, Li YZ, Zhou FS, Han JW, Quan C, Chen B, Liu JL, Lin D, Fan L, Zhang AP, Liu SX, Yang CJ, Wang PG, Zhou WM, Lin GS, Wu WD, Fan X, Gao M, Yang BQ, Lu WS, Zhang Z, Zhu KJ, Shen SK, Li M, Zhang XY, Cao TT, Ren W, Zhang X, He J, Tang XF, Lu S, Yang JQ, Zhang L, Wang DN, Yuan F, Yin XY, Huang HJ, Wang HF, Lin XY, Liu JJ. Psoriasis genome-wide association study identifies susceptibility variants within *LCE* gene cluster at 1q21. Nat Genet 2009; 41:205-10. [PMID: 19169255]
46. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959; 22:719-48. [PMID: 13655060]
47. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7:177-88. [PMID: 3802833]
48. Whittemore AS. Estimating attributable risk from case-control studies. Am J Epidemiol 1983; 117:76-85. [PMID: 6823955]
49. Ioannidis JP, Patsopoulos NA, Evangelou E. Heterogeneity in meta-analyses of genome-wide association investigations. PLoS ONE 2007; 2:e841. [PMID: 17786212]
50. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003; 327:557-60. [PMID: 12958120]
51. Sakurada Y, Kubota T, Mabuchi F, Imasawa M, Tanabe N, Iijima H. Association of *LOC387715* A69S with vitreous hemorrhage in polypoidal choroidal vasculopathy. Am J Ophthalmol 2008; 145:1058-62. [PMID: 18400199]
52. Lee KY, Vithana EN, Mathur R, Yong VH, Yeo IY, Thalamuthu A, Lee MW, Koh AH, Lim MC, How AC, Wong DW, Aung T. Association analysis of *CFH, C2, BF*, and *HTRA1* gene polymorphisms in Chinese patients with polypoidal choroidal vasculopathy. Invest Ophthalmol Vis Sci 2008; 49:2613-9. [PMID: 18515590]
53. Hardy J, Singleton A, Gwinn-Hardy K. Ethnic differences and disease phenotypes. Science 2003; 300:739-40. [PMID: 12730580]
54. Kanda A, Chen W, Othman M, Branham KE, Brooks M, Khanna R, He S, Lyons R, Abecasis GR, Swaroop A. A variant of mitochondrial protein *LOC387715/ARMS2*, not *HTRA1*, is strongly associated with age-related macular degeneration. Proc Natl Acad Sci USA 2007; 104:16227-32. [PMID: 17884985]
55. Fritsche LG, Loenhardt T, Janssen A, Fisher SA, Rivera A, Keilhauer CN, Weber BH. Age-related macular degeneration is associated with an unstable *ARMS2* (*LOC387715*) mRNA. Nat Genet 2008; 40:892-6. [PMID: 18511946]
56. Kanda A, Stambolian D, Chen W, Curcio CA, Abecasis GR, Swaroop A. Age-related macular degeneration-associated variants at chromosome 10q26 do not significantly alter *ARMS2* and *HTRA1* transcript levels in the human retina. Mol Vis 2010; 16:1317-23. [PMID: 20664794]
57. Yang Z, Tong Z, Chen Y, Zeng J, Lu F, Sun X, Zhao C, Wang K, Davey L, Chen H, London N, Muramatsu D, Salasar F, Carmona R, Kasuga D, Wang X, Bedell M, Dixie M, Zhao P, Yang R, Gibbs D, Liu X, Li Y, Li C, Li Y, Campochiaro B, Constantine R, Zack DJ, Campochiaro P, Fu Y, Li DY, Katsanis N, Zhang K. Genetic and functional dissection of HTRA1 and LOC387715 in age-related macular degeneration. PLoS Genet 2010; 6:e1000836. [PMID: 20140183]
58. Friedrich U, Myers CA, Fritsche LG, Milenkovich A, Wolf A, Corbo JC, Weber BH. Risk- and non-risk-associated variants at the 10q26 AMD locus influence *ARMS2* mRNA expression

but exclude pathogenic effects due to protein deficiency. Hum Mol Genet 2011; 20:1387-99. [PMID: 21252205]

OPEN ACCESS Freely available online

PLoS one

A Common Complement C3 Variant Is Associated with Protection against Wet Age-Related Macular Degeneration in a Japanese Population

Suiho Yanagisawa, Naoshi Kondo*, Akiko Miki, Wataru Matsumiya, Sentaro Kusuhara, Yasutomo Tsukahara, Shigeru Honda, Akira Negi

Department of Surgery, Division of Ophthalmology, Kobe University Graduate School of Medicine, Kobe, Japan

Abstract

Background: Genetic variants in the complement component 3 gene (*C3*) have been shown to be associated with age-related macular degeneration (AMD) in Caucasian populations of European descent. In particular, a nonsynonymous coding variant, rs2230199 (R102G), is presumed to be the most likely causal variant in the *C3* locus based on strong statistical evidence for disease association and mechanistic functional evidence. However, the risk allele is absent or rare (<1%) in Japanese and Chinese populations, and the association of R102G with AMD has not been reported in Asian populations. Genetic heterogeneity of disease-associated variants among different ethnicities is common in complex diseases. Here, we sought to examine whether other common variants in *C3* are associated with wet AMD, a common advanced-stage manifestation of AMD, in a Japanese population.

Methodology/Principal Findings: We genotyped 13 tag single nucleotide polymorphisms (SNPs) that capture the majority of common variations in the *C3* locus and tested for associations between these SNPs and wet AMD in a Japanese population comprising 420 case subjects and 197 controls. A noncoding variant in *C3* (rs2241394) exhibited statistically significant evidence of association (allelic $P = 8.32 \times 10^{-4}$; odds ratio = 0.48 [95% CI = 0.31–0.74] for the rs2241394 C allele). Multilocus logistic regression analysis confirmed that the effect of rs2241394 was independent of the previously described loci at *ARMS2* and *CFH*, and that the model including variants in *ARMS2* and *CFH* plus *C3* rs2241394 provided a better fit than the model without rs2241394. We found no evidence of epistasis between variants in *C3* and *CFH*, despite the fact that they are involved in the same biological pathway.

Conclusions: Our study provides evidence that *C3* is a common AMD-associated locus that transcends racial boundaries and provides an impetus for more detailed genetic characterization of the *C3* locus in Asian populations.

Citation: Yanagisawa S, Kondo N, Miki A, Matsumiya W, Kusuhara S, et al. (2011) A Common Complement C3 Variant Is Associated with Protection against Wet Age-Related Macular Degeneration in a Japanese Population. PLoS ONE 6(12): e28847. doi:10.1371/journal.pone.0028847

Editor: Ramani Ramchandran, Medical College of Wisconsin, United States of America

Received September 19, 2011; **Accepted** November 16, 2011; **Published** December 12, 2011

Funding: This study was supported by a Grant-in Aid for (C) 23592567 from the Ministry of Education, Science, and Culture, Tokyo, Japan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: nskondo@gmail.com

Introduction

Age-related macular degeneration (AMD) is a common multifactorial and heterogeneous disorder, characterized by progressive degeneration of the central region of the retina (macula) [1,2]. Pigmentary abnormalities of the retinal pigment epithelium (RPE) and extracellular deposits (drusen) under the retina are among the early-stage manifestations of AMD. As the condition progresses, extensive atrophy of the RPE and outer retina (geographic atrophy or dry AMD) or abnormal vessel growth underneath the macula (exudative or wet AMD) are common advanced-stage manifestations. AMD affects 30–50 million individuals worldwide and is a leading cause of legal blindness among older individuals in developed countries [1,2].

Although the precise etiology of AMD remains elusive, genetic studies have provided significant insights into the molecular basis of AMD. Several genes encoding proteins involved in the complement pathway have been shown to be associated with susceptibility to AMD, including the complement factor H gene (*CFH*) on chromosome 1q32 [3–5], two neighboring genes, complement component 2 (*C2*) and complement factor B (*CFB*) on 6p21 [6–8], the complement factor I gene (*CFI*) on 4q25 [9,10], and the complement component 3 gene (*C3*) on 19p13 [11–13]. These findings strongly implicate aberrant regulation and/or activation of the complement pathway in the mechanism of susceptibility to AMD. In addition to the association with complement pathway genes, AMD has been convincingly shown to be associated with two adjacent genes on 10q26 (age-related maculopathy susceptibility 2 [*ARMS2*] and high-temperature requirement factor H [*HTRA1*]) [14–16], which together account for nearly half of the heritability of AMD [7].

AMD susceptibility loci have been primarily discovered in populations of European descent, of which only the association of *CFH* [17–20] and the *ARMS2/HTRA1* loci [14,21,22] have been convincingly validated in Asian populations. We recently reported a significant association of wet AMD in a Japanese population with

the same susceptibility variant near *CFI* as that observed in individuals of European descent [23], indicating that, along with *CFH* and *ARMS2/HTRA1*, *CFI* is a susceptibility locus of AMD that transcends racial boundaries. However, studies have also revealed the existence of genetic heterogeneity in AMD susceptibility at the *C3* locus between populations of European and Asian descent. A nonsynonymous coding variant in *C3*, rs2230199 (R102G), was consistently found to be associated with AMD in Caucasian populations [11–13,24,25], but not in Asians [25–28]. Furthermore, the allelic frequency of the R102G variant is absent in Japanese and rare (<1%) in Chinese populations, according to the data from the International HapMap Project and published studies [25,27,28,29], while risk allele frequency is almost 20% in individuals of European descent [25]. It has been proposed that genetic effects of disease-associated variants are similar across racial boundaries regardless of their widely divergent allelic frequency between different populations [30]. However, it has also been documented that genetic heterogeneity of disease susceptibility between ethnic groups is common in complex diseases [31,32], and thus, disease-associated variants present in populations of European descent might not be applicable to Asian populations because of underlying genetic heterogeneity. Indeed, two recent studies have suggested a role for common intronic variants of the *C3* locus in susceptibility to wet AMD in Japanese and Chinese populations [26,27], implying that more common *C3* variants are associated with the disease in Asians. Here we genotyped 13 tag single nucleotide polymorphisms (SNPs) that capture the majority of common variations in the *C3* locus and tested for associations between these SNPs and wet AMD in a Japanese population comprising 420 case subjects and 197 controls.

Materials and Methods

Ethics Statement

The study protocol was approved by the Institutional Review Board at Kobe University Graduate School of Medicine and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects before participation in this study.

Study participants

All cases and controls included in this study were Japanese individuals recruited from the Department of Ophthalmology at Kobe University Hospital in Kobe, Japan. The demographic details of the study population are shown in Table 1. All cases and control subjects underwent comprehensive ophthalmic examination, including visual acuity measurement, slit-lamp examination, and dilated funduscopic examination. Fundus findings in each eye were classified according to the clinical age-related maculopathy staging system (CARMS) [33] as previously described [7,12]. All of our case subjects had wet AMD and associated manifestations such as nondrusenoid pigment epithelial detachment, serous or hemorrhagic retinal detachment, and subretinal or sub-RPE hemorrhages and fibrosis; they were categorized as having CARMS stage 5 [33]. The controls were individuals aged 56 years or older and were defined as cases without macular degeneration and changes, such as drusen or pigment abnormalities. Thus, controls were categorized as having CARMS stage 1 [33] on the basis of comprehensive ophthalmic examinations.

Table 1. Characteristics of the study population.

	Wet AMD	Controls
Number of subjects	420	197
Gender (male/female)	331/89	117/80
Mean age ± SD (years)	74±7.5	72±6.0
Age range (years)	54–94	56–95

AMD: age-related macular degeneration; SD: standard deviation.
doi:10.1371/journal.pone.0028847.t001

Genotyping

Genomic DNA was extracted from peripheral blood using standard methodology. Genotyping was performed using the TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems) in accordance with the manufacturer's recommendations.

SNP selection

To comprehensively yet efficiently screen *C3* sequences for common genetic variations, tag SNPs were selected from the HapMap Project database for the Japanese in Tokyo (JPT) population using the tag selection tool. Thirteen tag SNPs were selected for genotyping, which captured 29 of 34 SNPs in the *C3* locus exhibiting a minor frequency greater than 10% with a mean r^2 value of 0.986.

Statistical analysis

Allelic associations were evaluated for each SNP by chi-square tests on 2×2 contingency tables using the software package PLINK v1.00 (http://pngu.mgh.harvard.edu/purcell/plink/) [34]. The odds ratio (OR) and corresponding 95% confidence interval (CI) were calculated relative to the major allele. In addition to obtaining nominal *P* values, corrected empirical *P* values for multiple testing were generated by 10,000 permutation tests using the Max (T) permutation procedure implemented in PLINK [34]. We also applied a Bonferroni correction, where nominal *P*-values were multiplied by 13 (the number of SNPs tested for association). To adjust for age and gender differences between the case and control subjects, logistic regression analysis was performed using SNPStats (http://bioinfo.iconcologia.net/SNPStats), with age and gender controlled as covariates. Age and gender were included in this model as a continuous covariate measured in years and a categorical covariate, respectively. Deviations from the Hardy–Weinberg equilibrium were tested using the exact test implemented in PLINK [35]. Haploview software was used to assess linkage disequilibrium (LD) patterns and haplotype association statistics [36]. Haplotype blocks were determined using the solid spine of LD algorithm with a minimum D' of 0.8. To correct for multiple testing in the haplotype association analysis, 10,000 permutations were run using this software. An omnibus (or global) test of the haplotype association was performed with PLINK. To determine whether a single variant could explain an entire omnibus haplotype association, conditional haplotype-based likelihood ratio tests implemented in PLINK were conducted. The haplotype association was assessed further using sliding window analyses of four adjacent SNPs across the *C3* region. For this analysis, sliding windows of overlapping haplotypes were tested in sequence. For example, SNPs rs2250656, rs2230205, rs11569429, and rs11672613 were treated as a single haplotype, followed by SNPs rs2230205, rs11569429, rs11672613, and rs428453. The significance values were evaluated on the basis of omnibus test *P* values. The sliding window analyses were conducted using the PLINK software. The FASTSNP program (http://fastsnp.ibms.sinica.edu.tw/pages/input_CandidateGeneSearch.jsp) was used to predict the function of a SNP of interest [37].

Table 2. Results of single-marker association test.

SNP (location)	Position in NCBI build 36.3	Minor allele	Minor allele frequency		Association Results		
			Cases	Controls	Allelic *P*-value	Allelic OR (95% CI)	Corrected empirical *P*-value*
rs2250656 (intron 2)	6658534 bp	C	0.230	0.241	0.660	0.94 (0.71–1.24)	1
rs2230205 (exon 14; T612T)	6649704 bp	T	0.413	0.406	0.816	1.03 (0.81–1.31)	1
rs11569429 (intron 14)	6649074 bp	T	0.132	0.150	0.403	0.86 (0.61–1.22)	0.995
rs11672613 (intron 17)	6645246 bp	C	0.470	0.452	0.544	1.08 (0.85–1.37)	1
rs428453 (exon 19; V807V)	6642157 bp	C	0.096	0.140	0.0240	0.66 (0.46–0.95)	0.225
rs432001 (intron 24)	6633683 bp	G	0.152	0.155	0.912	0.98 (0.70–1.37)	1
rs7257062 (intron 29)	6625945 bp	C	0.241	0.211	0.247	1.19 (0.89–1.58)	0.929
rs2241393 (intron 29)	6625304 bp	G	0.329	0.305	0.40	1.12 (0.86–1.45)	0.995
rs2241394 (intron 29)	6625230 bp	C	0.052	0.104	8.32×10^{-4}	0.48 (0.31–0.74)	0.0102
rs1389623 (intron 33)	6624197 bp	A	0.082	0.102	0.263	0.79 (0.53–1.19)	0.942
rs7951 (exon 35; A1437A)	6621991 bp	A	0.082	0.102	0.263	0.79 (0.53–1.19)	0.942
rs344555 (intron 37)	6619360 bp	T	0.385	0.343	0.156	1.20 (0.93–1.54)	0.802
rs11569562 (intron 38)	6618753 bp	G	0.477	0.515	0.215	0.86 (0.68–1.09)	0.897

OR: odds ratio; CI: confidence intervals.
*Empirical *P*-values corrected for multiple testing (corrected empirical *P*-values) were generated by 10,000 permutation tests using Max (T) permutation procedure implemented in the PLINK software.
doi:10.1371/journal.pone.0028847.t002

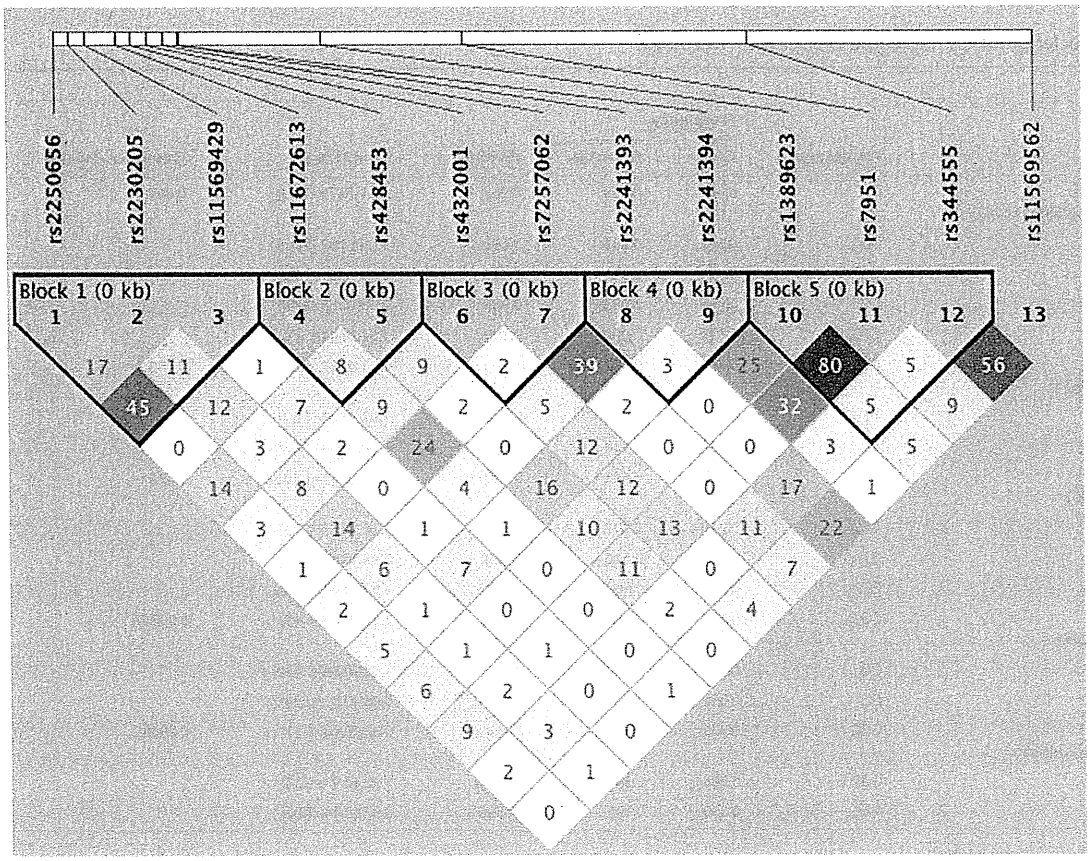

Figure 1. Linkage disequilibrium (LD) structure of the *C3* locus. LD was measured using data from all subjects in the present study. The haplotype blocks were determined by the solid spine of LD method implemented in the Haploview software. Each box provides estimated statistics of the coefficient of determination (r^2), with darker shades representing stronger LD.
doi:10.1371/journal.pone.0028847.g001

To examine a genetic effect detected here in the context of three validated AMD-risk loci for Asians (the A69S variant [rs10490924] in *ARMS2* [14,21,22] and the I62V variant [rs800292] and Y402H variant [rs1061170] in *CFH* [17–20]), we conducted logistic regression analyses with the R statistical analysis package (http://www.r-project.org/). For each locus, the genetic model of best fit was determined before genotypes were coded according to additive, dominant, and recessive models. Akaike Information Criterion (AIC) was used to select the model of best fit. The best models for each locus were then combined into multilocus models, and an effect of the *C3* variant after controlling for *ARMS2* A69S, *CFH* I62V, and *CFH* Y402H was estimated. Furthermore, we compared two logistic regression models (the full model including all four variants versus a reduced model in which the C3 variant was omitted) by using a likelihood ratio test and calculating AIC values. To determine epistatic effects between *C3* rs2241394 and *CFH* I62V or Y402H, pairwise interaction analysis was performed using the epistasis option in PLINK.

Results

None of the 13 SNPs reported in the present study showed significant deviation from the Hardy–Weinberg equilibrium in both the case and control subjects ($P>0.05$). Marker information, allelic frequencies, and summary statistics for all evaluated SNPs are shown in Table 2. In single-SNP analyses, two of the 13 SNPs showed nominally significant associations with wet AMD (rs2241394, nominal $P=8.32\times10^{-4}$; rs428453, nominal $P=0.0240$), of which only rs2241394 withstood multiple test corrections (corrected empirical $P=0.0102$; Bonferroni-corrected $P=0.0108$, Table 2). The minor allele C of rs2241394 was associated with protection against the disease, with a frequency of 0.052 in disease cases and 0.104 in controls (per allele OR = 0.48 [95% CI = 0.31–0.74]; Table 2). In a dominant genetic model, OR for individuals carrying at least one copy of the protective allele was 0.45 (95% CI = 0.28–0.72; $P=7.81\times10^{-4}$). Inclusion of age and gender as covariates in the logistic regression model did not substantially change the significance of the association (age- and gender-adjusted OR = 0.48 [95% CI = 0.30–0.75], $P=0.0016$, additive model; age- and gender-adjusted OR = 0.44 [95% CI = 0.27–0.72], $P=0.0012$, dominant model).

The pairwise LD structure was constructed with the 13 SNPs genotyped (Figure 1). Five haplotype blocks were defined, and association with the disease was restricted to block 4 where the disease-associated SNP rs2241394 resided as demonstrated by the significant omnibus result (omnibus $P=0.00367$ at 2 degrees of freedom, Table 3). Only one haplotype in block 4 was found to be significantly associated with the disease, with a haplotype frequency of 0.052 in affected individuals and 0.104 in controls ($P=8.0\times10^{-4}$; OR = 0.48 [95% CI = 0.31–0.74]; Table 3). This association remained statistically significant after correction for multiple testing (permutation $P=0.011$). The disease-associated

Table 3. Association of *C3* haplotype blocks with wet AMD.

		Frequency				
	Haplotype	**Cases**	**Controls**	***P*-value***	**OR (95% CI)**	**Omnibus *P*-value†**
Block 1 rs2250656|rs2230205|rs11569429	TTC	0.401	0.399	0.952	1.01 (0.79–1.29)	0.857
	TCC	0.362	0.351	0.699	1.05 (0.82–1.35)	
	CCT	0.125	0.141	0.442	0.86 (0.61–1.22)	
	CCC	0.092	0.093	0.967	0.99 (0.65–1.49)	
	CTC	0.012	0.0007	0.399	1.57 (0.43–5.74)	
Block 2 rs11672613|rs428453	CG	0.468	0.442	0.388	1.11 (0.87–1.41)	0.153
	TG	0.435	0.419	0.576	1.07 (0.84–1.37)	
	TC	0.094	0.130	0.0595	0.70 (0.48–1.02)	
Block 3 rs432001|rs7257062	AT	0.619	0.643	0.420	0.91 (0.71–1.16)	0.691
	AC	0.229	0.202	0.299	1.16 (0.87–1.56)	
	GT	0.140	0.146	0.779	0.95 (0.67–1.33)	
	GC	0.012	0.008	0.574	1.57 (0.43–5.74)	
Block 4 rs2241393|rs2241394	CG	0.619	0.591	0.353	1.12 (0.88–1.43)	0.00367
	GG	0.329	0.305	0.40	1.12 (0.86–1.45)	
	CC	0.052	0.104	8.0×10^{-4}	0.48 (0.31–0.74)	
Block 5 rs1389623|rs7951|rs344555	GGC	0.521	0.556	0.260	0.87 (0.68–1.11)	0.0846
	GGT	0.385	0.343	0.156	1.20 (0.93–1.54)	
	AAC	0.070	0.101	0.0596	0.67 (0.44–1.02)	

OR: odds ratio; CI: confidence intervals.
The association of haplotype CC in block 4 remained statistically significant after correction for multiple testing (permutation $P=0.011$).
*The *P*-values were calculated by the chi-square test on haplotype counts (1 degree of freedom).
†The omnibus *P*-values were calculated by the PLINK software (4 degrees of freedom for block 1; 2 degrees of freedom for block 2, 4, and 5; 3 degrees of freedom for block 3).
doi:10.1371/journal.pone.0028847.t003

haplotype was completely described by the protective allele C of rs2241394, and a conditional haplotype-based likelihood ratio test revealed that the significant omnibus haplotype association detected in haplotype block 4 disappeared when it was estimated to be conditional on rs2241394 (omnibus $P=0.85$), confirming that rs2241394 is responsible for the haplotype association detected in this LD block. To further assess haplotype associations, we conducted a sliding window analysis of four adjacent SNPs across the *C3* region. Significant associations were observed only around rs2241394 (Table 4), and the strongest association was found when four variants—rs2241393, rs2241394, rs1389623, and rs7951—were included together (omnibus $P=9.81\times10^{-4}$, Table 4).

To examine the possibility that the disease-associated SNP rs2241394 might be correlated with untyped SNPs, we investigated the LD structure across the genomic region extending approximately 200 kb upstream and downstream of the *C3* locus. Genotype data were retrieved from the 1000 Genome Project (August 2010 release) [38] and International HapMap (release 24) JPT+CHB datasets [39], and correlations (as defined by r^2 values) were examined. In this genomic region, we found 594 SNPs but did not identify any SNPs that are highly correlated with rs2241394 (all pairwise $r^2<0.45$).

Next, we examined the genetic effect of rs2241394 in the context of three validated AMD-risk loci for Asians (*ARMS2* A69S [14,21,22], *CFH* I62V [17–19], and *CFH* Y402H [20]). Using unconditional logistic regression, the genetic model of best fit for *C3* rs2241394, *ARMS2* A69S, *CFH* I62V, and *CFH* Y402H was determined and genotypes were coded according to additive, dominant, and recessive models. On the basis of AIC values, *ARMS2* A69S, and *CFH* I62V had the best fit under an additive model, and *C3* rs2241394 and *CFH* Y402H had the best fit under a dominant model. The best models were then combined into multilocus logistic regression models. After including the effects of *CFH* I62V, *CFH* Y402H, and *ARMS2* A69S, *C3* rs2241394 retained significant association (model 1; Table 5). Furthermore, we found that the model including all four variants–*C3* rs2241394, *ARMS2* A69S, *CFH* I62V, and *CFH* Y402H–fit significantly better than the model without *C3* rs2241394 (likelihood ratio test $\chi^2=10.32$, $P=0.00132$, model 1 vs. model 2; AIC = 692.0 and 700.3 for model 1 and 2, respectively; Table 5).

Finally, we conducted pairwise interaction analysis to evaluate potential epistatic effects between *C3* rs2241394 and *CFH* I62V or *CFH* Y402H, because the proteins encoded by these loci biologically interact in the complement pathway [40]. However, we did not find any evidence of epistasis between rs2241394 and *CFH* variants (all $P>0.05$).

Table 4. Four-marker sliding window haplotype analysis over the entire *C3* locus.

	Omnibus *P* Value*
SNP	**4-Marker**
rs2250656	0.959
rs2230205	0.706
rs11569429	0.476
rs11672613	0.503
rs428453	0.269
rs432001	0.0515
rs7257062	0.0555
rs2241393	9.81×10^{-4}
rs2241394	0.00103
rs1389623	0.0738
rs7951	-
rs344555	-
rs11569562	-

SNP: single nucleotide polymorphism.
*Omnibus *P* value corresponding to the haplotype with the listed SNP as the first SNP in the haplotype.
doi:10.1371/journal.pone.0028847.t004

Discussion

We genotyped 13 tag SNPs that capture the majority of common genetic variations in the *C3* locus and found statistically significant evidence for association between an intronic *C3* variant (rs2241394) and wet AMD in a Japanese population ($P=8.32\times10^{-4}$). Haplotype analyses identified the LD block where rs2241394 resides as being the only significant locus, and haplotype association was completely explained by rs2241394. Logistic regression analysis showed that the effect of rs2241394 is independent of the established associations of *ARMS2* A69S, *CFH* I62V, and *CFH* Y402H, and that the model including these three established loci plus *C3* rs2241394 provides a better fit than the model without rs2241394. Although the proteins encoded by *C3* and *CFH* are involved in the same biological pathway, we found no evidence of epistasis between rs2241394 and the two *CFH* variants.

Complement has emerged as an important element in AMD pathology [41,42], because of the identification of various complement-related molecules in drusen and nearby RPE [42]. In addition, recent successes in the identification of genetic susceptibility loci for AMD have revealed several molecules involved in the complement pathway, including CFH [3–5], CFB [6–8], C2 [6–8], CFI [9,10,23], and C3 [11–13]. Furthermore, systemic complement activation was observed in AMD patients [43–45] and nutritional supplementation with zinc was shown to delay the progression of AMD [46], an effect likely mediated by an inhibitory effect of zinc on complement activity [47]. C3 is a central component of all three pathways of complement activation: the alternative, classical, and mannose-binding lectin pathways, all of which lead to the cleavage of C3 into biologically active C3a and C3b fragments [40]. Notably, an animal study has shown that C3 deficiency prevented the formation of choroidal neovascularization induced by the rupture of Bruch's membrane with laser photocoagulation in eyes of

Table 5. Multilocus logistic regression analysis of *C3* rs2241394, *ARMS2* A69S, *CFH* I62V, and *CFH* Y402H.

Model	Effect	*P*-value	OR (95% CI)	AIC
1	*C3* rs2241394	0.00125	0.43 (0.26–0.72)	692.0
	ARMS2 A69S	2.30×10^{-9}	2.15 (1.67–2.77)	
	CFH I62V	3.24×10^{-5}	1.76 (1.35–2.30)	
	CFH Y402H	0.00302	2.34 (1.33–4.10)	
2	*ARMS2* A69S	1.66×10^{-9}	2.15 (1.68–2.77)	700.3
	CFH I62V	1.87×10^{-5}	1.78 (1.37–2.32)	
	CFH Y402H	0.00523	2.20 (1.26–3.82)	

AIC: Akaike information criterion.
doi:10.1371/journal.pone.0028847.t005

$C3^{-/-}$mice [48], indicating that C3 is a key factor in the development of choroidal neovascularization.

A nonsynonymous coding *C3* variant, rs2230199 (R102G), is strongly associated with AMD in populations of European descent, and this variant is presumed to be the most likely causal variant responsible for the disease association based on mechanistic functional evidence [11–13,49,50]. However, the association of R102G has not been reported in Asian populations [25–28], and allele frequencies of R102G vary widely among different ethnicities. For example, the risk allele is absent in Japanese [29] and rare (<1%) in Chinese populations [25,27,28], while the corresponding rate in Caucasians is 20% [25]. In the present study, we have found that a more common SNP of *C3*, rs2241394, is associated with AMD in a Japanese population. This association has not been documented by any previous genetic studies of AMD in European populations. These findings suggest that the susceptibility conferred by the R102G variant does not transcend ethnic lines and that there may be a significant difference in disease susceptibility loci in the *C3* region of populations of European and Asian descent. Notably, rs2241394 has previously been reported in a Japanese population to be associated with polypoidal choroidal vasculopathy [26], a major subphenotype of wet AMD in East Asian populations [51–53], and the direction of association was consistent with our findings. However, suggestive evidence for association of rs2250656 with wet AMD previously reported in a Chinese cohort [27] was not detected in the present study. We sought further evidence from a recent genome-wide association study of wet AMD in Japanese populations [29]; however, the arrays used in this study (Illumina HumanHap610-Quad BeadChip and Illumina HumanHap550v3 Beadchip) did not suit rs2241394.

The *C3* variant rs2241394 is an intronic SNP, and there is currently no evidence supporting its functional relevance. Using the FASTSNP program [37], we investigated potential functions of rs2241394. According to the analysis, this SNP was identified as lying in an intronic enhancer region created by a "C→G" change at rs2241394 that may lead to the creation of a binding site for the transcriptional factor *GATA-1*. Therefore, this SNP may have a functional relevance to disease risk for Japanese populations in the absence of surrounding 1000 Genome Project and HapMap SNPs that are highly correlated with rs2241394; however, fine-mapping and resequencing efforts are required to identify any potential as yet unidentified variants of more functional relevance.

In conclusion, we report a significant association between wet AMD and a common noncoding *C3* variant in a Japanese population. Our study provides evidence that *C3* is a common AMD-associated locus that transcends racial boundaries and provides an impetus for more detailed genetic characterization of the *C3* locus in Asian populations.

Author Contributions

Conceived and designed the experiments: SY NK AM WM SK SH YT AN. Performed the experiments: SY NK AM WM SK. Analyzed the data: SY NK AM WM SK SH YT AN. Contributed reagents/materials/analysis tools: SY NK AM WM SK SH YT AN. Wrote the paper: SY NK. Critical revision of the article: AM WM SK SH YT AN.

References

1. Gehrs KM, Anderson DH, Johnson LV, Hageman GS (2006) Age-related macular degeneration–emerging pathogenetic and therapeutic concepts. Ann Med 38: 450–471.
2. Coleman HR, Chan CC, Ferris FL, III, Chew EY (2008) Age-related macular degeneration. Lancet 372: 1835–1845.
3. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, et al. (2005) Complement factor H polymorphism in age-related macular degeneration. Science 308: 385–389.
4. Edwards AO, Ritter R, III, Abel KJ, Manning A, Panhuysen C, et al. (2005) Complement factor H polymorphism and age-related macular degeneration. Science 308: 421–424.
5. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, et al. (2005) Complement factor H variant increases the risk of age-related macular degeneration. Science 308: 419–421.
6. Gold B, Merriam JE, Zernant J, Hancox LS, Taiber AJ, et al. (2006) Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. Nat Genet 38: 458–462.
7. Maller J, George S, Purcell S, Fagerness J, Altshuler D, et al. (2006) Common variation in three genes, including a noncoding variant in CFH, strongly influences risk of age-related macular degeneration. Nat Genet 38: 1055–1059.
8. Spencer KL, Hauser MA, Olson LM, Schmidt S, Scott WK, et al. (2007) Protective effect of complement factor B and complement component 2 variants in age-related macular degeneration. Hum Mol Genet 16: 1986–1992.
9. Fagerness JA, Maller JB, Neale BM, Reynolds RC, Daly MJ, et al. (2009) Variation near complement factor I is associated with risk of advanced AMD. Eur J Hum Genet 17: 100–104.
10. Ennis S, Gibson J, Cree AJ, Collins A, Lotery AJ (2010) Support for the involvement of complement factor I in age-related macular degeneration. Eur J Hum Genet 18: 15–16.
11. Yates JR, Sepp T, Matharu BK, Khan JC, Thurlby DA, et al. (2007) Complement C3 variant and the risk of age-related macular degeneration. N Engl J Med 357: 553–561.
12. Maller JB, Fagerness JA, Reynolds RC, Neale BM, Daly MJ, et al. (2007) Variation in complement factor 3 is associated with risk of age-related macular degeneration. Nat Genet 39: 1200–1201.
13. Spencer KL, Olson LM, Anderson BM, Schnetz-Boutaud N, Scott WK, et al. (2008) C3 R102G polymorphism increases risk of age-related macular degeneration. Hum Mol Genet 17: 1821–1824.
14. Dewan A, Liu M, Hartman S, Zhang SS, Liu DT, et al. (2006) HTRA1 promoter polymorphism in wet age-related macular degeneration. Science 314: 989–992.
15. Yang Z, Camp NJ, Sun H, Tong Z, Gibbs D, et al. (2006) A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. Science 314: 992–993.
16. Fritsche LG, Loenhardt T, Janssen A, Fisher SA, Rivera A, et al. (2008) Age-related macular degeneration is associated with an unstable ARMS2 (LOC387715) mRNA. Nat Genet 40: 892–896.
17. Mori K, Gehlbach PL, Kabasawa S, Kawasaki I, Oosaki M, et al. (2007) Coding and noncoding variants in the CFH gene and cigarette smoking influence the risk of age-related macular degeneration in a Japanese population. Invest Ophthalmol Vis Sci 48: 5315–5319.
18. Kim NR, Kang JH, Kwon OW, Lee SJ, Oh JH, et al. (2008) Association between complement factor H gene polymorphisms and neovascular age-related macular degeneration in Koreans. Invest Ophthalmol Vis Sci 49: 2071–2076.
19. Ng TK, Chen LJ, Liu DT, Tam PO, Chan WM, et al. (2008) Multiple gene polymorphisms in the complement factor H gene are associated with exudative age-related macular degeneration in Chinese. Invest Ophthalmol Vis Sci 49: 3312–3317.
20. Kondo N, Bessho H, Honda S, Negi A (2011) Complement factor H Y402H variant and risk of age-related macular degeneration in Asians: a systematic review and meta-analysis. Ophthalmology 118: 339–344.
21. Kondo N, Honda S, Ishibashi K, Tsukahara Y, Negi A (2007) LOC387715/HTRA1 variants in polypoidal choroidal vasculopathy and age-related macular degeneration in a Japanese population. Am J Ophthalmol 144: 608–612.
22. Tam PO, Ng TK, Liu DT, Chan WM, Chiang SW, et al. (2008) HTRA1 variants in exudative age-related macular degeneration and interactions with smoking and CFH. Invest Ophthalmol Vis Sci 49: 2357–2365.
23. Kondo N, Bessho H, Honda S, Negi A (2010) Additional evidence to support the role of a common variant near the complement factor I gene in susceptibility to age-related macular degeneration. Eur J Hum Genet 18: 634–635.
24. Zerbib J, Richard F, Puche N, Leveziel N, Cohen SY, et al. (2010) R102G polymorphism of the C3 gene associated with exudative age-related macular degeneration in a French population. Mol Vis 16: 1324–1330.
25. Thakkinstian A, McKay GJ, McEvoy M, Chakravarthy U, Chakrabarti S, et al. (2011) Systematic review and meta-analysis of the association between complement component 3 and age-related macular degeneration: a HuGE review and meta-analysis. Am J Epidemiol 173: 1365–1379.
26. Goto A, Akahori M, Okamoto H, Minami M, Terauchi N, et al. (2009) Genetic analysis of typical wet-type age-related macular degeneration and polypoidal choroidal vasculopathy in Japanese population. J Ocul Biol Dis Infor 2: 164–175.
27. Pei XT, Li XX, Bao YZ, Yu WZ, Yan Z, et al. (2009) Association of C3 gene polymorphisms with neovascular age-related macular degeneration in a chinese population. Curr Eye Res 34: 615–622.
28. Liu X, Zhao P, Tang S, Lu F, Hu J, et al. (2010) Association study of complement factor H, C2, CFB, and C3 and age-related macular degeneration in a Han Chinese population. Retina 30: 1177–1184.

29. Arakawa S, Takahashi A, Ashikawa K, Hosono N, Aoi T, et al. (2011) Genome-wide association study identifies two susceptibility loci for exudative age-related macular degeneration in the Japanese population. Nat Genet (in press).
30. Ioannidis JP, Ntzani EE, Trikalinos TA (2004) 'Racial' differences in genetic effects for complex diseases. Nat Genet 236: 1312–1318.
31. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG (2001) Replication validity of genetic association studies. Nat Genet 2001 29: 306–309.
32. Sun LD, Xiao FL, Li Y, Zhou WM, Tang HY, et al. (2011) Genome-wide association study identifies two new susceptibility loci for atopic dermatitis in the Chinese Han population. Nat Genet 43: 690–694.
33. Seddon JM, Sharma S, Adelman RA (2006) Evaluation of the clinical age-related maculopathy staging system. Ophthalmology 113: 260–266.
34. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559–575.
35. Wigginton JE, Cutler DJ, Abecasis GR (2005) A note on exact tests of Hardy-Weinberg equilibrium. Am J Hum Genet 76: 887–893.
36. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21: 263–265.
37. Yuan HY, Chiou JJ, Tseng WH, Liu CH, Liu CK, et al. (2006) FASTSNP: an always up-to-date and extendable service for SNP function analysis and prioritization. Nucleic Acids Res 34(Web Server issue): W635–641.
38. Genomes Project Consortium (2010) A map of human genome variation from population-scale sequencing. Nature 467: 1061–1073.
39. International HapMap Consortium (2005) A haplotype map of the human genome. Nature 437: 1299–1320.
40. Ricklin D, Hajishengallis G, Yang K, Lambris JD (2010) Complement: a key system for immune surveillance and homeostasis. Nat Immunol 11: 785–797.
41. Anderson DH, Radeke MJ, Gallo NB, Chapin EA, Johnson PT, et al. (2010) The pivotal role of the complement system in aging and age-related macular degeneration: hypothesis re-visited. Prog Retin Eye Res 29: 95–112.
42. Ding X, Patel M, Chan CC (2009) Molecular pathology of age-related macular degeneration. Prog Retin Eye Res 28: 1–18.
43. Scholl HP, Charbel Issa P, Walier M, Janzer S, Pollok-Kopp B, et al. (2008) Systemic complement activation in age-related macular degeneration. PLoS One 3: e2593.
44. Reynolds R, Hartnett ME, Atkinson JP, Giclas PC, Rosner B, et al. (2009) Plasma complement components and activation fragments: associations with age-related macular degeneration genotypes and phenotypes. Invest Ophthalmol Vis Sci 50: 5818–5827.
45. Hecker LA, Edwards AO, Ryu E, Tosakulwong N, Baratz KH, et al. (2010) Genetic control of the alternative pathway of complement in humans and age-related macular degeneration. Hum Mol Genet 19: 209–215.
46. Age-Related Eye Disease Study Research Group (2001) A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol 119: 1417–1436.
47. Blom AM, Kask L, Ramesh B, Hillarp A (2003) Effects of zinc on factor I cofactor activity of C4b-binding protein and factor H. Arch Biochem Biophys 418: 108–118.
48. Bora PS, Sohn JH, Cruz JM, Jha P, Nishihori H, et al. (2005) Role of complement and complement membrane attack complex in laser-induced choroidal neovascularization. J Immunol 174: 491–497.
49. Arvilommi H (1974) Capacity of complement C3 phenotypes to bind on to mononuclear cells in man. Nature 251: 740–741.
50. Welch TR, Beischel L, Kleesattel A (1990) Functional consequences of the genetic polymorphism of the third component of complement. J Pediatr 116: S92–S97.
51. Maruko I, Iida T, Saito M, Nagayama D, Saito K (2007) Clinical characteristics of exudative age-related macular degeneration in Japanese patients. Am J Ophthalmol 144: 15–22.
52. Liu Y, Wen F, Huang S, Luo G, Yan H, et al. (2007) Subtype lesions of neovascular age-related macular degeneration in Chinese patients. Graefes Arch Clin Exp Ophthalmol 245: 1441–1445.
53. Imamura Y, Engelbert M, Iida T, Freund KB, Yannuzzi LA (2010) Polypoidal choroidal vasculopathy: a review. Surv Ophthalmol 55: 501–515.