Vascular Endothelial Growth Factor Gene Polymorphisms and Choroidal Neovascularization in Highly Myopic Eyes

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Purpose. To investigate a potential association between VEGF gene polymorphisms and the occurrence and/or the size of choroidal neovascularization (CNV) in highly myopic eyes.

Methods. In the case-control study for CNV occurrence, 327 highly myopic Japanese patients were enrolled. One hundred and eighty-four patients had CNV in at least one eye, and 143 did not have CNV in either eye. Of the 184 patients with CNV, 83 patients were used to evaluate an association with CNV size, and an additional 76 patients with CNV were used to confirm the association. We genotyped four tag single nucleotide polymorphisms (SNPs) and four functional SNPs previously reported to be correlated with VEGF gene expression to evaluate the associations of these eight SNPs with CNV occurrence and size. To confirm the association between CNV size and VEGF gene polymorphism, the associated SNP was genotyped in 76 additional patients with myopic CNV.

Results. There was no significant association between the occurrence of myopic CNV and the SNPs in the VEGF gene ($P > 0.16$). Of the eight SNPs evaluated, however, rs2010963 showed significant association with CNV area ($P = 0.0047$). This association was successfully replicated in the additional 76 eyes with myopic CNV, and pooled analysis revealed significant association of rs2010963 with CNV size ($P = 0.00078$).

Conclusions. VEGF gene polymorphisms were not associated with CNV occurrence in highly myopic eyes but were significantly associated with the size of CNV, suggesting roles in the growth rather than the emergence of CNV. (*Invest Ophthalmol Vis Sci.* 2012;53:2349–2353) DOI:10.1167/iovs.11-9405

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Supported in part by Grants-in-Aid for Scientific Research (Nos. 21249084 and 200791294) from the Japan Society for the Promotion of Science, Tokyo, Japan, and the Japan National Society for the Prevention of Blindness, Tokyo, Japan.

Submitted for publication December 27, 2011; revised February 14, 2012; accepted February 17, 2012.

Disclosure: **Y. Akagi-Kurashige**, None; **K. Kumagai**, None; **K. Yamashiro**, None; **H. Nakanishi**, None; **I. Nakata**, None; **M. Miyake**, None; **A. Tsujikawa**, None; **M. Moriyama**, None; **K. Ohno-Matsui**, None; **M. Mochizuki**, None; **R. Yamada**, None; **F. Matsuda**, None; **N. Yoshimura**, None

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Myopia is one of the most common ocular disorders worldwide. The prevalence of myopia is much higher in Asian populations, with a reported incidence of roughly 40% in the Japanese and Chinese population and 25% in Caucasians.[1–3] Pathological myopia, also called high myopia, is defined as a spherical equivalent refractive error of at least −6 diopters or an axial length ≥26.5 mm. Myopic axial length elongation can lead to chorioretinal atrophy and choroidal neovascularization (CNV), which is the most vision-threatening complication in highly myopic eyes.[4] Since the long-term visual outcomes of myopic CNV are extremely poor,[5] it is critical to determine in which highly myopic patients CNV will occur. CNV usually occurs in young adults with high myopia in the fourth and fifth decades of life. However, many eyes with high myopia do not have CNV even after 60 years of age. Furthermore, the size of the CNV seriously affects the visual prognosis because it determines the size of the scotoma, and some smaller CNVs can regress without treatment.[6] Since it is difficult to prevent the development of myopia, it is important to investigate the mechanisms underlying CNV occurrence and growth in myopic eyes; this may lead to the prevention of CNV development and the subsequent visual disturbance.

Genetic backgrounds may affect the development of high myopia; recently, we have determined a susceptible locus for pathological myopia using a genome-wide association study (GWAS).[7] Furthermore, recent GWASs reveal that myopia susceptibility loci exist in chromosome 15.[8–10] The occurrence of CNV in highly myopic eyes might also depend on genetic variations. Thus far, however, few studies have investigated the genetic background of patients with CNV in highly myopic eyes.

Since anti-VEGF treatment has been developed for neovascular AMD, it has become a popular treatment for ocular neovascularization. Anti-VEGF drugs have been shown to be effective in treating CNV secondary to high myopia.[11–13] In contrast to neovascular AMD, myopic CNV is easily inactivated with anti-VEGF treatment. In this study, we evaluated the associations between VEGF gene polymorphisms and CNV development in highly myopic eyes in Japanese patients.

Methods

This study was performed in accordance with the tenets of the Declaration of Helsinki. The Institutional Review Board/Ethics Committee of each institution approved the study protocols. All patients were fully informed of the study purpose and procedures, and written consent was obtained from each patient. For the case-control study of CNV occurrence, 327 highly myopic, unrelated Japanese patients with axial lengths of >26.0 mm in both eyes and who were ≥60 years of age were recruited from Kyoto University Hospital and Tokyo Medical and

Table 1. Characteristics of the Study Population

	With CNV	Without CNV	*P* Value
Number	184	143	
Mean age ± SD (years)	69.97 ± 6.35	69.23 ± 6.74	0.52*
Axial length ± SD (mm)			
Right	28.97 ± 1.72	29.11 ± 1.72	0.49*
Left	28.75 ± 1.72	28.84 ± 1.86	0.68*
Sex (male/female)	32/152	58/85	3.27×10^{-6}†

* Unpaired *t*-test.
† χ^2 test.

Dental University Hospital. The number of patients with macular CNV in at least one eye was 184, and the number of patients without macular CNV in either eye was 143 (Table 1). All patients underwent detailed ophthalmologic examinations, including dilated indirect and contact lens slit-lamp biomicroscopy, automatic objective refraction, measurement of the axial length by A-scan ultrasound (UD-6000; Tomey, Nagoya, Japan) or partial coherence interferometry (IOLMaster; Carl Zeiss Meditec, Dublin, CA), color fundus photography, optical coherence tomography, and fluorescein angiography. Individuals with a history of ocular surgery, with the exception of cataract surgery, were excluded from the study. Patients with secondary choroidal neovascular diseases, such as angioid streaks, presumed ocular histoplasmosis syndrome, and ocular trauma, were also excluded.

Of the 184 patients with myopic CNV, 83 patients underwent angiography with HRA2 (Heidelberg Engineering, Heidelberg, Germany) in Kyoto University Hospital. To evaluate the association between VEGF gene polymorphisms and CNV size, the area of CNV (mm^2) in these 83 patients was measured with the HRA-2 software. An additional 76 patients with myopic CNV were enrolled from Kyoto University Hospital to confirm the aforementioned associations. The average age of these patients was 63.8 ± 12.6 years, and the average axial length was 30.1 ± 1.1 mm.

For selecting tag single nucleotide polymorphisms (SNPs), we used the public dbSNP database build 126 (NCBI build 36.1) and HapMap database phase 2, release 22,[35] to extract the relevant sequencing information for the *VEGFA* gene and the genotyping information for the SNPs. A set of four tagging VEGF SNPs were selected for investigation: two SNPs on the promoter region, named rs699946 and rs699947, and two intronic SNPs, rs3025033 and rs3025035. This set of four tagging SNPs provided 100% coverage for all 14 common HapMap SNPs within a 26.3 kb region (16.3 kb gene length; 10 kb upstream) spanning the VEGF gene on chromosome 6 (r^2 threshold of 0.95). Furthermore, we evaluated four functional SNPs (rs1570360, rs2010963, rs833061, and rs3025039). Since these SNPs have been shown to affect VEGF expression,[14–17] many studies have evaluated the association of these SNPs with various diseases such as AMD, diabetic retinopathy, Behçet's disease, Alzheimer's disease, and diabetes.[18–26]

Genomic DNA was prepared from peripheral blood by a DNA extraction kit (QuickGene-610L; Fujifilm, Minato, Tokyo, Japan). VEGF-tagged SNPs (rs699946, rs699947, rs3025033, and rs3025035) and functional SNPs (rs1570360, rs2010963, rs833061, and rs3025039) were genotyped by a Taqman SNP assay with the ABI PRISM 7700 system (Applied Biosystems, Foster, CA). Deviations in genotype distributions from the Hardy-Weinberg equilibrium (HWE) were assessed with the HWE exact test. A χ^2 test for trend or its exact counterpart was used to compare the genotype distributions of the two groups. To adjust for age and sex, we performed logistic regression analysis. Mean age and axial length were compared using unpaired *t*-test or ANOVA, and sex ratio was compared with the χ^2 test. The associations between genotype and CNV size were evaluated using the Jonckheere-Terpstra trend test. *P* values of less than 0.05 were considered statistically significant.

Results

The demographics of the study population are shown in Table 1; there was no significant difference between patients with CNV and patients without CNV with respect to either age or axial length. The mean age of each group was 70.0 ± 6.4 years and 69.2 ± 6.7 years, respectively ($P = 0.52$). However, CNV is more predominant in women compared with men ($P = 3.27 \times 10^{-6}$) with an odds ratio (OR) of 3.24 (95% confidence interval [CI] = 2.27–4.64).

The genotype counts, associations, and ORs for the eight SNPs are shown in Table 2. The genotype distributions were not significantly different between patients with CNV and patients without CNV (nominal $P > 0.16$). Evaluation of the associations in a recessive model and a dominant model also showed no associations ($P > 0.10$). Even when adjusted for age and sex, the genotype distributions were not significantly different ($P > 0.10$).

In addition, we performed subset analysis for patients aged 70 years or older. In our cohort, 86 patients with CNV and 63 patients without CNV were ≥70 years of age. Associations between the eight SNPs with the occurrence of CNV were not statistically significant ($P > 0.17$).

Of the 184 patients with myopic CNV, the area of CNV was measured in 83 patients who underwent angiography with HRA2 in Kyoto University Hospital. The genotype distribution of rs2010963 was significantly correlated with CNV area ($P = 0.0047$), while the other seven SNPs did not show significant associations with CNV area (Fig. 1). The size of CNV was largest (1.71 ± 1.29 mm^2) in patients with a CC genotype of rs2010963, intermediate (0.98 ± 0.84 mm^2) with a CG genotype, and smallest (0.78 ± 0.78 mm^2) with a GG genotype. There was no significant difference in axial length, age of patients, or male/female ratio among the three

Table 2. Genotype Counts, Associations, and Odds Ratios for VEGF SNPs

		CNV (+)			CNV (−)				Age- and Sex-Adjusted	
SNP	Genotype	Genotype Count	MAF	HWE *P*	Genotype Count	MAF	HWE *P*	Nominal *P*	*P*	OR (95% CI)
rs699946	AA/AG/GG	64/82/33	G, 0.41	0.399	40/73/23	G, 0.44	0.250	0.543	0.10	0.80 (0.62–1.04)
rs699947	AA/AC/CC	22/77/85	A, 0.33	0.477	17/60/63	A, 0.34	0.626	0.856	0.68	0.93 (0.66–1.31)
rs3025033	AA/AG/GG	125/53/4	G, 0.17	0.286	90/44/8	G, 0.21	0.151	0.160	0.60	0.94 (0.73–1.20)
rs3025035	CC/CT/TT	90/71/17	T, 0.29	0.391	79/49/12	T, 0.26	0.200	0.355	0.34	1.13 (0.88–1.44)
rs1570360	AA/AG/GG	11/42/130	A, 0.17	0.005	8/32/102	A, 0.17	0.020	0.858	0.79	0.94 (0.60–1.47)
rs2010963	CC/GC/GG	34/84/62	C, 0.42	0.547	23/73/42	C, 0.43	0.348	0.820	0.42	0.88 (0.65–1.20)
rs833061	CC/CT/TT	22/75/82	C, 0.33	0.451	17/60/66	C, 0.33	0.554	0.922	0.69	0.93 (0.66–1.31)
rs3025039	CC/CT/TT	116/56/5	T, 0.19	0.402	87/45/8	T, 0.22	0.298	0.328	0.81	0.97 (0.76–1.24)

MAF, minor allele frequency.

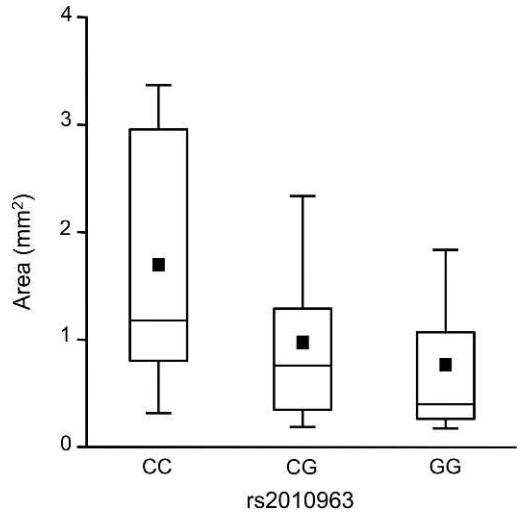

Figure 1. The area (mm²) of choroidal neovascularization among the three genotypes of rs2010963 in 83 patients. The area was significantly associated with the genotype ($P = 0.0047$).

genotypes of rs2010963 ($P = 0.54$, 0.98, and 0.69, respectively). To confirm the aforementioned association between rs2010963 and CNV size, we genotyped rs2010963 in an additional 76 patients with myopic CNV (20 male and 56 female). The genotype distribution of rs2010963 was significantly correlated with the CNV area ($P = 0.032$), while there was no significant difference in the axial length, age of patients, or male/female ratio among the three genotypes of rs2010963 ($P = 0.91$, 0.15, and 0.20, respectively). When these two cohorts were pooled for further evaluation of this association, the genotype distribution of rs2010963 was significantly correlated with the CNV area (Fig. 2, $P = 0.00078$).

Discussion

In the present study, we found no association between VEGF gene polymorphisms and the occurrence of CNV in highly myopic eyes in Japanese patients, although rs2010963 was significantly associated with the size of CNV. To evaluate factors associated with CNV occurrence in highly myopic eyes, the age of the cohort is of critical importance. Therefore, when a younger cohort is used, some patients assigned to the group without CNV may eventually develop CNV, which can obscure potential differences between the two groups. Fernandez-Robredo et al. have evaluated the association of CFH Y402H and ARMS2 A69S polymorphisms with myopic CNV using 196 myopic patients who were aged $\geq$30 years.[27] We have previously evaluated the same association using 353 myopic patients who were $\geq$50 years of age,[28] and the present study consisted of 327 myopic patients who were aged $\geq$60 years. However, the association of VEGF gene polymorphism with CNV occurrence was not statistically significant. Furthermore, we evaluated the association using a cohort of patients older than 70 years, but statistical significance was still not found.

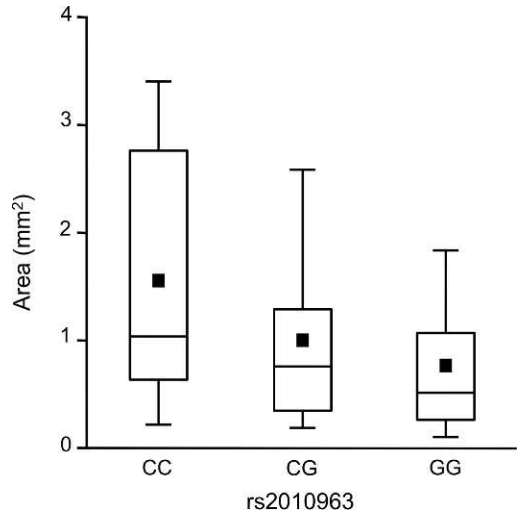

Figure 2. The area (mm²) of choroidal neovascularization among the three genotypes of rs2010963 in 159 patients. The area was significantly associated with the genotype ($P = 0.00078$).

Genetic associations with myopia have been investigated for several decades. Linkage studies have identified 18 possible loci for myopia (MYP1-18). Numerous candidate genes have been evaluated, and we have recently completed a GWAS study.[7] Furthermore, recent GWAS studies have revealed myopia susceptibility loci on chromosome 15, and we have successfully reproduced the association of these susceptibility loci with high myopia.[8–10] However, susceptibility genes for myopia have not been revealed; this makes it difficult to determine how to prevent myopia. Compared with the prevention of myopia, prevention and/or control of CNV occurrence and growth in highly myopic eyes might be a more practical approach. Since CNV is one of the most vision-threatening complications in highly myopic eyes, it is of great value to investigate the mechanism underlying CNV development in these eyes.

Although anti-VEGF treatments have been developed for the management of neovascular AMD, they are also substantially effective in treating myopic CNV.[11–13] Considering the effectiveness of these anti-VEGF treatments, we had hypothesized that VEGF is associated with the occurrence of CNV in highly myopic eyes. The present study, however, suggests that VEGF gene variations do not affect the occurrence of CNV in these eyes. In contrast with CNV occurrence, VEGF gene polymorphism rs2010963 was significantly associated with CNV size. Thus, it appears that VEGF contributes to CNV growth rather than CNV occurrence in highly myopic eyes. Experimental studies have shown that inhibition of VEGF leads to smaller CNV in laser-induced CNV models.[29–31] However, inhibition of VEGF does not always completely suppress CNV occurrence after laser photocoagulation to disrupt Bruch's membrane. This evidence suggests that VEGF only affects CNV size/growth, and other factors are responsible for triggering CNV occurrence, partly by interacting with Bruch's membrane.

The size of CNV is critical for visual prognosis in highly myopic eyes. Smaller CNVs can lead to smaller scotomas and spare the visual functions of the surrounding retina. Furthermore, very small CNVs can disappear completely after treatment.[6] Our findings suggest that development of larger CNVs in highly myopic eyes can be prevented by targeting VEGF, while prevention of CNV occurrence might be accomplished by targeting other factors.

Watson et al. reported that the amount of lipopolysaccharide-induced VEGF production from peripheral blood mononuclear cells (PBMNCs) is highest in individuals with a GG genotype of rs2010963, intermediate with a CG genotype, and lowest with a CC genotype.[17] In contrast to the findings of this study, we discovered that the size of CNV was largest in patients with a CC genotype, intermediate with a CG genotype, and smallest with a GG genotype. Considering that VEGF is a pro-angiogenic factor, these two findings seem contradictory. However, an evaluation of PBMNC function in in-vitro studies

does not always reflect their function in in-vivo situations. Furthermore, PBMNCs include several cell types such as lymphocytes, monocytes, and macrophages, and we have performed in vivo experiments that show that PBMNCs induce endothelium apoptosis[32] and that lymphocytes are negative regulators of pathological neovascularization, while monocytes are positive regulators in an ischemic retinopathy model.[33] Further studies are required to evaluate the roles of VEGF produced individually by monocytes or lymphocytes during myopic CNV development. In addition to VEGF produced from PBMNCs, VEGF produced from the RPE could also affect the growth of CNV in highly myopic eyes. Although we cannot evaluate the VEGF-producing ability of the RPE in an in-vivo situation, elucidation of the roles of the RPE in myopic CNV development might lead to better control of CNV size. It is also important to consider that VEGF can have several isoforms with different properties; we have demonstrated that VEGF165 is associated with pathological neovascularization, while VEGF121 is associated with physiological neovascularization.[33] Furthermore, recent studies have shown that some isoforms of VEGF are anti-angiogenic.[34] Additional studies on the role of different VEGF isoforms in myopic CNV development may lead to prevention of larger CNV secondary to high myopia.

Limitations of the present study include the age of the cohort and the small sample size. Although we used a cohort older than 60 years of age and performed a subanalysis using samples with patients older than 70 years, some participants included in the group without CNV might develop CNV in the future. Furthermore, our study is retrospective in nature, and the associations discovered herein need to be evaluated in prospective studies.

In conclusion, we have shown that VEGF gene polymorphisms have no association with the occurrence of CNV in highly myopic eyes in Japanese individuals; however, VEGF rs2010963 affects the size of CNV. Treatments that target VEGF may prevent large CNV formation in highly myopic eyes and help achieve better visual prognosis. To prevent CNV occurrence, further studies are needed to clarify the mechanism and/or background causes of CNV occurrence in highly myopic eyes.

References

1. Sawada A, Tomidokoro A, Araie M, Iwase A, Yamamoto T. Refractive errors in an elderly Japanese population: the Tajimi study. *Ophthalmology*. 2008;115:363–370, e363. http://www.ophsource.org/periodicals/ophtha/article/S0161-6420%2807%2900379. Accessed March 29, 2012.
2. Wong TY, Foster PJ, Hee J, et al. Prevalence and risk factors for refractive errors in adult Chinese in Singapore. *Invest Ophthalmol Vis Sci*. 2000;41:2486–2494.
3. Kempen JH, Mitchell P, Lee KE, et al. The prevalence of refractive errors among adults in the United States, Western Europe, and Australia. *Arch Ophthalmol*. 2004;122:495–505.
4. Hayashi K, Ohno-Matsui K, Shimada N, et al. Long-term pattern of progression of myopic maculopathy a natural history study. *Ophthalmology*. 2010; 117:1595–1611.
5. Yoshida T, Ohno-Matsui K, Yasuzumi K, et al. Myopic choroidal neovascularization: a 10-year follow-up. *Ophthalmology*. 2003; 110:1297–1305.
6. Hayashi K, Ohno-Matsui K, Yoshida T, et al. Characteristics of patients with a favorable natural course of myopic choroidal neovascularization. *Graefes Arch Clin Exp Ophthalmol*. 2005; 243:13–19.
7. Nakanishi H, Yamada R, Gotoh N, et al. A genome-wide association analysis identified a novel susceptible locus for pathological myopia at 11q24.1. *PLoS Genet*. 2009;5:e1000660. http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.100. Accessed March 29, 2012.
8. Solouki AM, Verhoeven VJ, van Duijn CM, et al. A genome-wide association study identifies a susceptibility locus for refractive errors and myopia at 15q14. *Nat Genet*. 2010;42:897–901.
9. Hysi PG, Young TL, Mackey DA, et al. A genome-wide association study for myopia and refractive error identifies a susceptibility locus at 15q25. *Nat Genet*. 2010;42:902–905.
10. Hayashi H, Yamashiro K, Nakanishi H, et al. Association of 15q14 and 15q25 with high myopia in Japanese. *Invest Ophthalmol Vis Sci*. 2011;52:4853–4858.
11. Chan WM, Lai TY, Liu DT, Lam DS. Intravitreal bevacizumab (Avastin) for myopic choroidal neovascularization: six-month results of a prospective pilot study. *Ophthalmology*. 2007;114:2190–2196.
12. Gharbiya M, Allievi F, Mazzeo L, Gabrieli CB. Intravitreal bevacizumab treatment for choroidal neovascularization in pathologic myopia: 12-month results. *Am J Ophthalmol*. 2009; 147:84–93, e81. http://www.ajo.com/. Accessed March 29, 2012.
13. Wu PC, Chen YJ. Intravitreal injection of bevacizumab for myopic choroidal neovascularization: 1-year follow-up. *Eye (Lond)*. 2009;23:2042–2045.
14. Renner W, Kotschan S, Hoffmann C, Obermayer-Pietsch B, Pilger E. A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. *J Vasc Res*. 2000;37:443–448.
15. Shahbazi M, Fryer AA, Pravica V, et al. Vascular endothelial growth factor gene polymorphisms are associated with acute renal allograft rejection. *J Am Soc Nephrol*. 2002;13:260–264.
16. Stevens A, Soden J, Brenchley PE, Ralph S, Ray DW. Haplotype analysis of the polymorphic human vascular endothelial growth factor gene promoter. *Cancer Res*. 2003;63:812–816.
17. Watson CJ, Webb NJ, Bottomley MJ, Brenchley PE. Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production, Cytokine. 2000;12:1232–1235.
18. Mori K, Horie-Inoue K, Gehlbach PL, et al. Phenotype and genotype characteristics of age-related macular degeneration in a Japanese population. *Ophthalmology*. 2010;117:928–938.
19. Lin JM, Wan L, Tsai YY, et al. Vascular endothelial growth factor gene polymorphisms in age-related macular degeneration. *Am J Ophthalmol*. 2008;145:1045–1051.
20. Fang AM, Lee AY, Kulkarni M, Osborn MP, Brantley MA Jr. Polymorphisms in the VEGFA and VEGFR-2 genes and neovascular age-related macular degeneration. *Mol Vis*. 2009; 15:2710–2719.
21. Janik-Papis K, Zaras M, Krzyzanowska A, et al. Association between vascular endothelial growth factor gene polymorphisms and age-related macular degeneration in a Polish population. *Exp Mol Pathol*. 2009;87:234–238.
22. Kangas-Kontio T, Vavuli S, Kakko SJ, et al. Polymorphism of the manganese superoxide dismutase gene but not of vascular endothelial growth factor gene is a risk factor for diabetic retinopathy. *Br J Ophthalmol*. 2009;93:1401–1406.
23. Chun MY, Hwang HS, Cho HY, et al. Association of vascular endothelial growth factor polymorphisms with nonproliferative and proliferative diabetic retinopathy. *J Clin Endocrinol Metab*. 2010;95:3547–3551.
24. Salvarani C, Boiardi L, Casali B, et al. Vascular endothelial growth factor gene polymorphisms in Behcet's disease. *J Rheumatol*. 2004;31:1785–1789.
25. Landgren S, Palmer MS, Skoog I, et al. No association of VEGF polymorphisms with Alzheimer's disease. *Neuromolecular Med*. 2010; 12:224–228.

26. Freathy RM, Weedon MN, Shields B, et al. Functional variation in VEGF is not associated with type 2 diabetes in a United Kingdom Caucasian population. *JOP*. 2006;7:295–302.
27. Fernandez-Robredo P, Maestre SR, Zarranz-Ventura J, Mulero HH, Salinas-Alaman A, Garcia-Layana A. Myopic choroidal neovascularization genetics. *Ophthalmology*. 2008;115:1632, 1632. e1. http://www.ophsource.org/periodicals/ophtha/article/S0161-6420(08)00226-1/. Accessed March 29, 2012.
28. Nakanishi H, Gotoh N, Yamada R, et al. ARMS2/HTRA1 and CFH polymorphisms are not associated with choroidal neovascularization in highly myopic eyes of the elderly Japanese population. *Eye (Lond)*. 2010;24:1078–1084.
29. Seo MS, Kwak N, Ozaki H, et al. Dramatic inhibition of retinal and choroidal neovascularization by oral administration of a kinase inhibitor. *Am J Pathol*. 1999;154:1743–1753.
30. Kwak N, Okamoto N, Wood JM, Campochiaro PA. VEGF is major stimulator in model of choroidal neovascularization. *Invest Ophthalmol Vis Sci*. 2000;41:3158–3164.
31. Krzystolik MG, Afshari MA, Adamis AP, et al. Prevention of experimental choroidal neovascularization with intravitreal anti-vascular endothelial growth factor antibody fragment. *Arch Ophthalmol*. 2002;120:338–346.
32. Ishida S, Yamashiro K, Usui T, et al. Leukocytes mediate retinal vascular remodeling during development and vaso-obliteration in disease. *Nat Med*. 2003;9:781–788.
33. Ishida S, Usui T, Yamashiro K, et al. VEGF164-mediated inflammation is required for pathological, but not physiological, ischemia-induced retinal neovascularization. *J Exp Med*. 2003;198:483–489.
34. Bates DO, Cui TG, Doughty JM, et al. VEGF165b, an inhibitory splice variant of vascular endothelial growth factor, is down-regulated in renal cell carcinoma. *Cancer Res*. 2002;62:4123–4131.
35. The International HapMap Project. International HapMap Consortium. *Nature*. 2003;426:789–796.