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Three Major Loci Involved in Age-Related Macular Degeneration are also Associated with Polypoidal Choroidal Vasculopathy

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Abstract

Purpose—To investigate the frequency of variants in three major age-related macular degeneration (AMD)-associated loci in patients with polypoidal choroidal vasculopathy (PCV) of European-American descent.

Design—Cross-sectional case-control association study.

Participants—Fifty-five patients with PCV, 368 patients with advanced AMD and 368 age- and ethnically-matched unaffected controls of European-American descent.

Methods—Association analysis of allele and genotype frequencies, determined by TaqMan assays, was performed for the following haplotype-tagging single nucleotide polymorphisms (htSNPs): risk alleles in the complement factor H (*CFH*) gene (Y402H and IVS14) in the *ARMS2/HTRA1* locus on 10q26 (A69S) and protective alleles in *CFH* (IVS1 and IVS6) and in the complement factor B/complement component C2 (*CFB/C2*) locus (IVS10 and H9L).

Main Outcome Measures—Allele and genotype frequencies of the htSNPs in the *CFH*, *CFB/C2*, and *ARMS2/HTRA1* loci.

Results—Four AMD-associated haplotype-tagging alleles (rs547154, rs1061170, rs1410996, rs10490924) in the three major loci, *CFH*, *CFB/C2* and *ARMS2/HTRA1*, were statistically significantly associated also with the PCV phenotype ($P < 0.05$). Three other alleles from the same loci (rs4151667, rs529825, rs3766404) showed a trend towards association ($P < 0.2$), but did not reach statistical significance possibly because of the combined effects of a relatively small sample size and low minor allele frequency in the screened populations.

Conclusion—The PCV phenotype in Caucasian patients is associated with the major alleles/genotypes in the AMD-associated loci, suggesting that PCV and AMD are genetically similar in the tested loci.

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Polypoidal choroidal vasculopathy (PCV) is characterized by aneurysmal dilations with interconnecting vessels that are often best demonstrated by indocyanine green (ICG) angiography.¹ There is still controversy about PCV pathogenesis; whether this condition represents inner choroidal vascular abnormalities or a particular variety of choroidal neovascularization (CNV) remains undetermined.^{2,3} The ophthalmoscopic appearance of PCV shares some similarities with that of the CNV caused by age-related macular degeneration (AMD);^{4–6} both cause hemorrhages and other signs of exudation and also scarring and fibrosis. However there are significant differences, typical AMD has other preceding stigmata such as soft drusen and focal hyperpigmentation, and the clinical course and visual outcomes differ as well.^{7–9} In addition the response to treatment, particularly the value of photodynamic therapy (PDT), is dissimilar. While PCV often has angiographic characteristics of occult disease during fluorescein angiography, several reports have shown that PDT is effective in managing PCV.^{10–12}

Heredity has been established as the main contributor to the susceptibility of AMD.^{13,14} Common variants in the *ARMS2/HTRA1* locus, in complement factor H (*CFH*) and in the *CFB/C2* locus have been strongly associated with AMD.^{15–21} *ARMS2/HTRA1* polymorphisms have also been associated with PCV in Asian (Japanese and Chinese) population.^{22–26} Association of genetic variants in AMD-associated loci with PCV has not been investigated in the Caucasian population, therefore, the purpose of this study was to analyze the major AMD-associated alleles in individuals of European-American descent diagnosed with PCV.

Methods

The PCV cohort corresponded to 55 consecutive patients of Caucasian descent referred to two ophthalmologic centers (Edward Harkness Eye Institute, Columbia University and The Vitreous, Retina, Macula Consultants of New York) during a 2-year interval (from November 5, 2006, to December 27, 2008) enrolled after giving informed consent. The principles outlined in the Declaration of Helsinki were followed and an institutional review board approval was obtained specifically for this study. All patients had a comprehensive ophthalmic examination and their medical records were reviewed retrospectively.

All enrolled patients undertook clinical ophthalmic examination, including best visual acuity measurement, ocular tonometry, biomicroscopy, and indirect ophthalmoscopy. Color fundus retinography, fluorescein angiography, and ICG were performed in all patients. The diagnosis of PCV was based on ICG results, which showed a branching vascular network that terminated in aneurysmal enlargements which have been called polypoidal lesions. Eyes with other causes of CNV, for example, pathologic myopia, idiopathic CNV, presumed ocular histoplasmosis, angioid streaks, and other secondary CNV, were excluded.

The AMD cohort included 368 subjects of European-American descent. The control cohort consisted of 368 disease-free individuals (no AMD or PCV) matched by ethnicity and age with the AMD group. Both groups were the same as reported in previous studies, where the ascertainment procedures and clinical characterization are described in detail.^{15,16} Genomic DNA was produced from peripheral blood leukocytes collected from the enrolled subjects by means of kits (QIAamp DNA Blood Maxi; Qiagen, Valencia, California, USA).

The htSNPs for complement factor H (*CFH*) (OMIM 134370) and complement factor B (*CFB*) (OMIM 138470) genes were described in prior studies.^{15,16} The *LOC387715/HTRA1* gene high-risk AMD-associated A69S variant has been described in several previous studies.^{19,21} The genotyped htSNPs included the following: in the *CFH* locus, (1) rs1061170 (Y402H), (2) rs1410996 (IVS14T>C), (3) rs529825 (IVS1C>T), and (4) rs3766404

(IVS6C>T); in the *CFB/C2* locus, rs4151667 (H9L), rs547154 (IVS10); and in the *ARMS2* gene, rs10490924 (A69S).

Genotyping was performed by polymerase chain reaction–restriction fragment length polymorphism and/or by *TaqMan* assays (Applied Biosystems, Foster City, California). The technique used was identical to that previously described.¹⁵ Briefly, 5 ng of DNA was subjected to 50 cycles on a 384-well thermocycler (ABI 9700, Applied Biosystems), and plates were read in a sequence detection system (7900 HT, Applied Biosystems). Further genotyping details are available on request. Statistical analyses were performed by standard 2×2 table and Fisher exact tests. Multiple-comparison tests (e.g., Bonferroni correction) were not applied because each htSNP was analyzed independently.

Results

All study subjects (55) with PCV were not related to each other and of European-American descent with an approximate 2:1 male to female ratio (32/55 (60%) were male). The average (SD) age at the time of enrollment in the study was 73 (+/−8.2) years.

Genotype and allele frequencies of the 7 haplotype-tagging SNPs from *CFH* (4), *ARMS2* (1) and *CFB/C2* (2) loci in 55 patients with PCV were analyzed and compared with the same data acquired previously on a cohort of 368 subjects with advanced AMD and a control cohort of 368 individuals matched by age and ethnicity. All the results are summarized in Table 1. Four htSNPs (IVS1, IVS6, Y402H and IVS14) were analyzed in the *CFH* locus, two in *CFB/C2* locus (H9L and IVS10) and one in *ARMS2* (A69S). As in AMD, the Y402H allele (rs1061170) was highly elevated in the PCV cohort; the frequency of this allele in the PCV cohort (49.1%) was approximately the same of the AMD cohort (53.8%), whereas it was much lower (32.4%) in the control cohort ($p=0.0002$; odds ratio [OR], 2.16; 95% confidence interval [CI], 1.44;3.24). The frequency of IVS14 (rs1410996) SNP was also nearly identical in the PCV and AMD groups (65.5% vs 72.8%), which is significantly higher than in the control group (52.8%; $p=0.01$; OR, 21.16; 95% CI, 1.44; 3.24). The same was true for the A69S variant and for one of the tested protective haplotypes (C2 IVS10). The frequency of the A69S variant (rs10490924) in the *ARMS2* gene in the PCV cohort (31.8%) was lower than in the AMD cohort (43.3%), however it was still significantly higher than in the control group (22.3%; $p=0.03$; OR, 1.63; 95% CI, 1.05;2.52). The frequency of the IVS10 allele was 3.6% in the PCV cohort, 4.9% in the AMD group (difference not statistically significant), and 11.8% in the control cohort (a significant difference with both AMD and PCV groups). The other three tested SNPs, i.e *CFB* H9L (rs4151667), *CFH* IVS1 (rs529825) and *CFH* IVS6 (rs3766404) showed a trend towards association ($P<0.2$).

Discussion

The current study showed that the allele frequencies in Caucasian PCV patients are in good correlation with the data in AMD patients in the three loci. Four AMD-associated alleles (rs547154, rs1061170, rs1410996, rs10490924) in the three major loci, *CFH*, *CFB/C2* and *ARMS2/HTRA1*, were significantly associated also with the PCV phenotype ($P<0.05$) and the other three tested SNPs (rs4151667, rs529825, rs3766404) demonstrated a tendency towards correlation ($P<0.2$). Therefore, common genetic variation in all three loci was associated with PCV similarly to that seen in AMD; i.e., SNPs conferring higher risk or protection from the disease in AMD were associated with the same in PCV.

Currently, there is no agreement about etiology for polypoidal choroidal vasculopathy (PCV) which can be considered as a distinctive macular disorder or a subset of AMD. PCV

is as common as exudative AMD in Asian populations.^{27,28} and is thought to occur more frequently in Asian population than in Caucasian individuals.²⁹ The rs10490924 single-nucleotide polymorphism (SNP) in the *ARMS2* gene on chromosome 10q26 has been demonstrated to have a strong association with advanced AMD in both Caucasian and Asian populations.³⁰ Also, association of the *ARMS2* rs10490924 SNP with PCV has been reported in both Japanese and Chinese populations.^{22–26} This study investigated the possible association of the PCV phenotypes in European-American population with previously confirmed AMD-associated htSNPs from three loci, *CFH*, *ARMS2* and *CFB/C2*. In Caucasians, PCV and AMD occur in the same age group and based on its angiographic features PCV would be classified as an occult (type 1) CNV in the angiographic AMD classification. Also, a strong association between elevated serum C-reactive protein levels and PCV or advanced AMD has been described implying that inflammation is a common component for both AMD and PCV.³¹ Therefore, it is possible that PCV in Caucasians may be a subset of AMD with similar demographic and genetic risk factors, clinical features, angiographic characteristics, natural course, and visual prognosis.

Direct comparison of our data (allele and genotype frequencies) with that obtained from the cohorts of Asian origin is complex due to the documented significant differences of allele frequencies of the htSNPs in the three loci between the general populations of Caucasian and Asian descent.³² For example, the minor allele frequency of the *ARMS2* rs10490924 SNP in the general population of Caucasian origin is ~0.20, while it is 0.35–0.40 in the general population in Asia (Table 2). However, relative differences of allele frequencies between cases and controls in all studies in the *ARMS2/HTRA1* locus suggest that the AMD-associated variants in at least this locus confer a higher risk for both PCV and AMD in the two populations (Table 2). The relatively small size of our PCV cohort may explain some deviation from the AMD data. The present study suggests that PCV and AMD are genetically similar in the three tested loci, and that differences in these phenotypes may represent the effects of currently unknown alleles in these or other loci.

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References

1. Spaide RF, Yannuzzi LA, Slakter JS, et al. Indocyanine green videoangiography of idiopathic polypoidal choroidal vasculopathy. *Retina* 1995;15:100–110. [PubMed: 7542796]
2. Yannuzzi LA, Sorenson J, Spaide RF, Lipson B. Idiopathic polypoidal choroidal vasculopathy (PCV). *Retina* 1990;10:1–8. [PubMed: 1693009]
3. Terasaki H, Miyake Y, Suzuki T, et al. Polypoidal choroidal vasculopathy treated with macular translocation: clinical pathological correlation. *Br J Ophthalmol* 2002;86:321–327. [PubMed: 11864892]
4. Yuzawa M, Mori R, Kawamura A. The origins of polypoidal choroidal vasculopathy. *Br J Ophthalmol* 2005;89:602–607. [PubMed: 15834093]
5. Terasaki H, Ishikawa K, Suzuki T, et al. Morphologic and angiographic assessment of the macula after macular translocation surgery with 360 degrees retinotomy. *Ophthalmology* 2003;110:2403–2408. [PubMed: 14644725]
6. Yannuzzi LA, Wong DW, Sforzolini BS, et al. Polypoidal choroidal vasculopathy and neovascularized age-related macular degeneration. *Arch Ophthalmol* 1999;117:1503–1510. [PubMed: 10565519]

7. Gomi F, Ohji M, Sayanagi K, et al. One-year outcomes of photodynamic therapy in age-related macular degeneration and polypoidal choroidal vasculopathy in Japanese patients. *Ophthalmology* 2008;115:141–146. [PubMed: 17582498]
8. Moorthy RS, Lyon AT, Rabb MF, et al. Idiopathic polypoidal choroidal vasculopathy of the macula. *Ophthalmology* 1998;105:1380–1385. [PubMed: 9709746]
9. Yannuzzi LA, Ciardella A, Spaide RF, et al. The expanding clinical spectrum of idiopathic polypoidal choroidal vasculopathy. *Arch Ophthalmol* 1997;115:478–485. [PubMed: 9109756]
10. Spaide RF, Donsoff I, Lam DL, et al. Treatment of polypoidal choroidal vasculopathy with photodynamic therapy. *Retina* 2002;22:529–535. [PubMed: 12441716]
11. Lee SC, Seong YS, Kim SS, et al. Photodynamic therapy with verteporfin for polypoidal choroidal vasculopathy of the macula. *Ophthalmologica* 2004;218:193–201. [PubMed: 15103216]
12. Chan WM, Lam DS, Lai TY, et al. Photodynamic therapy with verteporfin for symptomatic polypoidal choroidal vasculopathy : one-year results of a prospective case series. *Ophthalmology* 2004;111:1576–1584. [PubMed: 15288991]
13. Swaroop A, Branham KE, Chen W, Abecasis G. Genetic susceptibility to age-related macular degeneration: a paradigm for dissecting complex disease traits. *Hum Mol Genet* 2007;16:R174–R182. [PubMed: 17911160]
14. Gorin MB. A clinician's view of the molecular genetics of age-related maculopathy. *Arch Ophthalmol* 2007;125:21–29. [PubMed: 17210848]
15. Hageman GS, Anderson DH, Johnson LV, et al. A common haplotype in the complement regulatory gene factor H (*HF1/CFH*) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci U S A* 2005;102:7227–7232. [PubMed: 15870199]
16. Gold B, Merriam JE, Zernant J, et al. Variation in factor B (*BF*) and complement component 2 (*C2*) genes is associated with age-related macular degeneration. *Nat Gen* 2006;38:458–462.
17. Edwards AO, Ritter R III, Abel KJ, et al. Complement factor H polymorphism and age-related macular degeneration. *Science* 2005;308:421–424. [PubMed: 15761121]
18. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science* 2005;308:419–421. [PubMed: 15761120]
19. Jakobsdottir J, Conley YP, Weeks DE, et al. Susceptibility genes for age-related maculopathy on chromosome 10q26. *Am J Hum Genet* 2005;77:389–407. [PubMed: 16080115]
20. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science* 2005;308:385–389. [PubMed: 15761122]
21. Rivera A, Fisher SA, Fritsche LG, et al. 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;14:3227–3236. [PubMed: 16174643]
22. Kondo N, Honda S, Ishibashi K, et al. *LOC387715/HTRA1* variants in polypoidal choroidal vasculopathy and age-related macular degeneration in a Japanese population. *Am J Ophthalmol* 2007;144:608–612. [PubMed: 17692272]
23. Sakurada Y, Kubota T, Mabuchi F, et al. Association of *LOC387715 A69S* with vitreous hemorrhage in polypoidal choroidal vasculopathy. *Am J Ophthalmol* 2008;145:1058–1062. [PubMed: 18400199]
24. Gotoh N, Nakanishi H, Hayashi H, et al. *ARMS2 (LOC387715)* variants in Japanese patients with exudative age-related macular degeneration and polypoidal choroidal vasculopathy. *Am J Ophthalmol* 2009;147:1037–1041. [PubMed: 19268887]
25. Lee KY, Vithana EN, Mathur R, et al. Association analysis of *CFH*, *C2*, *BF*, and *HTRA1* gene polymorphisms in Chinese patients with polypoidal choroidal vasculopathy. *Invest Ophthalmol Vis Sci* 2008;49:2613–2619. [PubMed: 18515590]
26. 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;116:304–310. [PubMed: 19187823]
27. Byeon SH, Lee SC, Oh HS, et al. Incidence and clinical patterns of polypoidal choroidal vasculopathy in Korean patients. *Jpn J Ophthalmol* 2008;52:57–62. [PubMed: 18369702]
28. Maruko I, Iida T, Saito M, et al. Clinical characteristics of exudative age-related macular degeneration in Japanese patients. *Am J Ophthalmol* 2007;144:15–22. [PubMed: 17509509]

29. Sho K, Takahashi K, Yamada H, et al. Polypoidal choroidal vasculopathy: incidence, demographic features, and clinical characteristics. *Arch Ophthalmol* 2003;121:1392–1396. [PubMed: 14557174]
30. Yoshida T, DeWan A, Zhang H, et al. *HRTA1* promoter polymorphism predisposes Japanese to age-related macular degeneration. *Mol Vis* [serial online]. 2007 13:545–548. Available at <http://www.molvis.org/molvis/v13/a58/>.
31. Kikuchi M, Nakamura M, Ishikawa K, et al. Elevated C-reactive protein levels in patients with polypoidal choroidal vasculopathy and patients with neovascular age-related macular degeneration. *Ophthalmology* 2007;114:1722–1727. [PubMed: 17400294]
32. Hageman GS, Hancox LS, Taiber AJ, et al. Extended haplotypes in the complement factor H (*CFH*) and *CFH*-related (*CFHR*) family of genes that protect against age-related macular degeneration: characterization, ethnic distribution and evolutionary implications. *Ann Med* 2006;38:592–604. [PubMed: 17438673]

Table 1
Association of seven Age-related Macular Degeneration-Associated hSNPs with Polypoidal Choroidal Vasculopathy

Haplotype tagging SNP	Minor Allele Frequency		Comparisons			
	PCV n=55)	Controls (n=368)	AMD (n=368)	PCV – Controls	PCV – AMD	AMD – Controls
CFB H9L (rs4151667)	0.027	0.044	0.02	odds ratio = 0.62 95% confidence interval [0.18;2.04] <i>p</i> =.11	odds ratio = 0.35 95% confidence interval [0.41;4.42] <i>p</i> =.5	odds ratio = 0.46 95% confidence interval [0.28;0.73] <i>p</i> =.002
C2 IVS10 (rs547154)	0.036	0.118	0.049	odds ratio = 0.28 95% confidence interval [0.10;0.79] <i>p</i> =.009	odds ratio = 0.74 95% confidence interval [0.27;2.05] <i>p</i> =.82	odds ratio = 0.38 95% confidence interval [0.27;0.53] <i>p</i> <.0000001
CFH IVS1 (rs529825)	0.2	0.261	0.145	odds ratio = 0.71 95% confidence interval [0.43;1.17] <i>p</i> =.19	odds ratio = 1.46 95% confidence interval [0.90;2.37] <i>p</i> =.13	odds ratio = 0.48 95% confidence interval [0.38;0.60] <i>p</i> <.0000001
CFH IVS6 (rs3766404)	0.118	0.172	0.092	odds ratio = 0.65 95% confidence interval [0.35;1.19] <i>p</i> =.17	odds ratio = 1.32 95% confidence interval [0.73;2.40] <i>p</i> =.4	odds ratio = 0.49 95% confidence interval [0.38;0.63] <i>p</i> <.0000001
CFH Y402H (rs1061170)	0.491	0.324	0.538	odds ratio = 2.16 95% confidence interval [1.44;3.24] <i>p</i> =.0002	odds ratio = 1.12 95% confidence interval [0.77;1.65] <i>p</i> =.56	odds ratio = 2.43 95% confidence interval [2.03;2.91] <i>p</i> <.000001
CFH IVS14 (rs1410996)	0.345	0.472	0.272	odds ratio = 0.59 95% confidence interval [0.39;0.90] <i>p</i> =.01	odds ratio = 1.41 95% confidence interval [0.94;2.12] <i>p</i> =.1	odds ratio = 0.42 95% confidence interval [0.35;0.50] <i>p</i> <.000001
ARMS2 A69S (rs10490924)	0.318	0.223	0.433	odds ratio = 1.63 95% confidence interval [1.05;2.52] <i>p</i> =.03	odds ratio = 0.61 95% confidence interval [0.41;0.92] <i>p</i> =.02	odds ratio = 2.66 95% confidence interval [2.19;3.23] <i>p</i> <.000001

PCV = polypoidal choroidal vasculopathy

AMD = age-related macular degeneration

Table 2
Association Between Polypoidal Choroidal Vasculopathy and *ARMS2/HTRA1* Locus in Five Studies

No of participants	Current Study		Kondo et al., 2007 ²²		Sakurada et al., 2008 ²³		Gotoh et al., 2009 ²⁴		Lee et al., 2008 ²⁵	
	PCV	Controls	PCV	Controls	PCV	Controls	PCV	Controls	PCV	Controls
	(55)	(368)	(76)	(94)	(109)	(85)	(55)	(77)	(72)	(93)
Allele count (%)										
G	38 (68.2)	286 (77.7)	57 (37.5)	117 (62.2)	79 (36.2)	110 (64.7)	49 (44.5)	95 (61.7)	64 (44.4)	114 (61.3)
T	17 (31.8)	82 (22.3)	95 (62.5)	71 (37.8)	139 (63.8)	60 (35.3)	61 (55.5)	59 (38.3)	80 (55.6)	72 (38.7)
Genotype count (%)										
GG	26 (47.3)	218 (59.2)	12 (15.8)	38 (40.4)	15 (13.8)	39 (45.9)	12 (21.8)	26 (35.1)	17 (23.6)	33 (35.5)
GT	23 (41.8)	136 (37.0)	33 (43.4)	41 (43.6)	49 (44.9)	32 (37.6)	25 (45.5)	36 (48.6)	30 (41.7)	48 (51.6)
TT	6 (10.9)	14 (3.8)	31 (40.8)	15 (16.0)	45 (41.3)	14 (16.5)	18 (32.7)	12 (16.2)	25 (34.7)	12 (12.9)
Allele association										
<i>p</i> values	0.03		<0.0001		<0.0001		0.0058		0.0027	

PCV = polypoidal choroidal vasculopathy