

C3 R102G polymorphism increases risk of age-related macular degeneration

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Received January 24, 2008; Revised and Accepted March 4, 2008

Inflammation has long been suspected to play a role in the pathogenesis of age-related macular degeneration (AMD). Association of variants in the complement factor H (CFH) and complement factor B (CFB) genes has targeted the search for additional loci to the alternative complement cascade, of which C3 is a major component. Two non-synonymous coding polymorphisms within C3, R102G and L314P, have previously been strongly associated with increased risk. These variants are in strong linkage disequilibrium (LD), making the contribution of this locus to AMD even more difficult to ascertain. We sought to determine whether the C3 association resulted primarily from only one of these two variants or from a combined effect of both in 223 families and an independent dataset of 701 cases and 286 unrelated controls. The C3 polymorphisms were in strong LD ($r^2 = 0.85$), and both were associated in the family-based and case-control datasets (R102G genoPDT $P = 0.02$, case-control genotypic $P = 0.004$; L314P genoPDT $P = 0.001$, case-control genotypic $P = 0.04$). In conditional analyses in the case-control dataset, R102G remained associated with disease in the L314P risk allele carriers ($P = 0.01$), but there was no effect of L314P in the R102G risk allele carriers ($P = 0.2$). After adjusting for age, smoking, CFH Y402H, LOC387715 A69S, and CFB R32Q, the effect of R102G remained strong [$P = 0.015$, odds ratio = 1.55, 95% confidence interval 1.09 to 2.21, adjusted PAR(population attributable risk) = 0.17]. Therefore, while the strong LD between R102G and L314P makes it difficult to disentangle their individual effects on disease risk, the R102G polymorphism acting alone provides the best model for disease in our data.

INTRODUCTION

Age-related macular degeneration (AMD, MIM 603075) is a progressive retinal disorder and the primary cause of blindness in the elderly in developed nations. Inflammation has been suggested to contribute to the pathogenesis of AMD through encouragement of drusen development, retinal pigment epithelium/photoreceptor degeneration, and disruption of Bruch's membrane (1). Association of variants in the complement factor H (CFH) and complement factor B (CFB) genes with AMD has targeted the alternative complement cascade, of which C3 is a major component, for the

search for additional susceptibility loci. R102G and L314P, two non-synonymous coding polymorphisms within C3, have previously been strongly associated with increased AMD risk (2,3).

C3 R102G, the polymorphism responsible for the 'fast' and 'slow' electrophoretic allotypes of C3 (C3F and C3S) (4), has known functional consequences on the C3 protein. C3 allotype affects binding to monocyte-complement receptors in humans (5), and C3F (the glycine allele) has been associated with IgA nephropathy (6), systemic vasculitis (7), partial lipodystrophy, and membranoproliferative glomerulonephritis type II (MPGNII) (8,9). Variants in CFH are associated with

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both MPGNII and AMD, further tying these diseases and the alternative complement cascade together.

To our knowledge, no data supporting a functional role for C3 L314P have been discovered. However, this polymorphism does cause a non-synonymous coding change in the protein, so the possibility for functional differences exists. The fact that C3 R102G and L314P are in strong linkage disequilibrium (LD) has long been recognized (4), and makes determining the contribution of this locus to AMD even more difficult. Therefore, we sought to determine whether the C3 association resulted primarily from only one of these two variants or from a combined haplotype effect.

RESULTS

Both C3 polymorphisms were associated with AMD in both the family-based and case-control datasets (C3 R102G genoPDT $P = 0.02$, case-control genotypic $P = 0.004$; C3 L314P genoPDT $P = 0.001$, case-control genotypic $P = 0.04$, Tables 1 and 2). The association remained significant for both single nucleotide polymorphisms (SNPs) when examining only neovascular AMD cases in the case-control dataset (C3 R102G case-control genotypic $P = 0.002$; C3 L314P case-control genotypic $P = 0.02$), but was no longer significant in the smaller number of families with neovascular AMD (C3 R102G genoPDT $P = 0.68$; C3 L314P genoPDT $P = 0.26$, Tables 1 and 2). There was no strong evidence of linkage for either SNP (max LOD = 0.13 under a recessive model for L314P).

The strong LD between these two SNPs ($D' > 0.94$, $r^2 = 0.85$ for both datasets) makes it difficult to determine using only statistical methods which of the two polymorphisms is the functional variant at this locus or whether the association is due to a combined haplotype effect. Nevertheless, the association between the haplotype carrying risk alleles at both SNPs was either equal to or weaker than the association at each individual SNP (case-control dataset haplotype association $P = 0.004$ compared with C3 R102G allelic $P = 0.001$, genotypic $P = 0.004$ and C3 L314P allelic $P = 0.014$, genotypic $P = 0.004$; family-based HBAT $P = 0.35$ compared with C3 R102G genoPDT $P = 0.02$ and C3 L314P genoPDT $P = 0.001$, Tables 1 and 2). Furthermore, in conditional analyses in the case-control dataset, C3 R102G remained associated with disease in the C3 L314P risk allele carriers ($P = 0.014$), but there was no effect of C3 L314P in the C3 R102G risk allele carriers, implying that the best model for disease in our dataset includes only the C3 R102G variant.

Several genetic variants have consistently been associated with AMD susceptibility, especially polymorphisms in the CFH, LOC387715/ARMS2, and CFB loci (reviewed in 10), in addition to environmental risk factors such as age and smoking. It is important to consider the effect of any novel risk factor in the context of these known associations. Though some controversy surrounds whether polymorphisms in LOC387715/ARMS2 or the adjacent gene HTRA1 represent the functional variant at the 10q26 locus (11,12), the associated SNPs are in very strong LD, and either SNP can be used as a proxy for the other (13). After adjusting for

Table 1. Family-based dataset association analysis results

	Analysis ^a	MAF ^b	PDTave <i>P</i> -value	PDTgeno <i>P</i> -value
C3 L314P	All AMD	0.30	0.97	0.001
	Neovascular AMD	0.30	0.69	0.26
C3 R102G	All AMD	0.30	0.66	0.02
	Neovascular AMD	0.29	0.45	0.68

^aThe 'All AMD' analysis compares AMD grade 3, 4 and 5 cases with grade 1 and 2 controls. The 'Neovascular AMD' analysis compares only grade 5 cases with grade 1 controls.

^bMAF, minor allele frequency.

age, smoking, CFH Y402H, LOC387715 A69S, and CFB R32Q in the case-control dataset, the effect of C3 R102G remained significant ($P = 0.015$, odds ratio = 1.55, 95% confidence interval 1.09–2.21). The population attributable risk (PAR) for C3 R102G was ~17% after controlling for age, smoking, CFH Y402H, and LOC387715 (Table 3), similar to the PAR = 22% estimated in another study (2).

Because C3, CFH, and CFB all operate within the same biological pathway, the alternative complement cascade, we tested for interactions between C3 and CFH or CFB. We did not detect evidence of epistasis between these loci (C3 R102G-CFH Y402 LRT $P = 0.59$, C3 R102G-CFB R32Q $P = 0.83$ in the case-control dataset).

DISCUSSION

Variation in C3 is associated with increased risk for AMD, with the R102G polymorphism showing the strongest evidence for the functional variant in our dataset. Distinguishing between the possible genetic etiologies for an association at this locus (single SNP effects of C3 R102G or L314P or a haplotype effect) may prove valuable as predictive algorithms and novel therapeutics for AMD are developed.

Rather than undertaking a more comprehensive screening of the C3 gene, we chose specifically to test the R102G and L314P polymorphisms because of their potential for functional consequences on the C3 protein and prior reports of association, leaving open the possibility that other variants in this region may also affect susceptibility. However, both previous studies took a tagging approach for selecting SNPs within this candidate gene and did not observe any other strong associations; no other SNPs in C3 were significantly associated with AMD in these studies after Bonferroni correction for the number of SNPs tested within the gene (2,3). This fact, coupled with the evidence for a true functional effect of the polymorphism [differential capacity to bind monocyte-complement receptors (5)] and our conditional association analyses, strongly suggests that R102G is biologically related to disease.

In comparison with smoking, CFH Y402H, and LOC387715/ARMS2 A69S, C3 R102G explains a somewhat smaller, though still substantial, portion of the cases of AMD in our study population (PAR = 0.17, Table 3). The wide confidence interval on the PAR estimate (confidence interval 0.01–0.30) indicates that future studies are needed to refine this measurement. Notably, even though C3, CFH, and CFB all belong to the

Table 2. Case–control dataset association analysis results

	Analysis	Case MAF ^a	Control MAF	Allelic <i>P</i> -value	Genotypic <i>P</i> -value
C3 L314P	All AMD	0.27	0.22	0.014	0.041
	Neovascular AMD	0.27	0.21	0.024	0.023
C3 R102G	All AMD	0.29	0.21	0.001	0.004
	Neovascular AMD	0.28	0.19	<0.001	0.002

^aMAF, minor allele frequency.

Table 3. Population attributable risk (PAR) for known AMD risk factors

	PAR ^a	95% Confidence interval	
Smoking	0.29	0.12	0.42
CFH Y402H	0.49	0.29	0.63
LOC387715 A69S	0.34	0.20	0.46
C3 R102G	0.17	0.01	0.30
Total	0.76	0.61	0.86

^aModel includes adjustment for age.

same biological pathway, we did not observe interactive effects between C3 and these other genes.

Perhaps most encouraging, the total PAR for the model with all known AMD risk factors was 0.76, showing that great strides have been made in identifying susceptibility factors for this complex disease. As the field continues to tease out the genetic etiologies of AMD, we are optimistic that these and future genetic associations will provide a starting place for a fruitful avenue of inquiry into the development of preventative and therapeutic treatments.

MATERIALS AND METHODS

Multiplex and singleton families totaling 223 pedigrees in all and an independent dataset of 701 cases and 286 unrelated controls, all of Caucasian, non-Hispanic descent, were ascertained at Vanderbilt University Medical Center (VUMC) and Duke University Medical Center (DUMC) (Table 4). All patients and controls received an eye examination and had stereoscopic fundus photographs graded according to a modified version of the age-related eye disease study (AREDS) grading system as described elsewhere (14,15). Briefly, grades 1 and 2 represent controls. Grade 1 controls have no evidence of drusen or small non-extensive drusen without pigmentary abnormalities, while grade 2 controls may show signs of extensive small drusen, non-extensive intermediate drusen and/or pigmentary abnormalities. Grade 3 AMD cases have extensive intermediate drusen or large, soft drusen with or without drusenoid retinal pigment epithelial detachment. Grade 4 AMD cases exhibit geographic atrophy and grade 5 individuals have exudative AMD, which includes non-drusenoid retinal pigment epithelial detachment, choroidal neovascularization, and subretinal hemorrhage or disciform scarring. Individuals were classified according to status in the more severely affected eye. Approval for the study was obtained from the appropriate institutional review boards at

VUMC and DUMC; all study participants gave informed consent, and this research adhered to the tenets of the Declaration of Helsinki.

C3 R102G (rs2230199) and C3 L314P (rs1047286) were genotyped using Taqman Assays on Demand from Applied Biosystems. Quality control samples were duplicated within and between plates, and we required that 95% of individuals assayed received a genotype for SNPs to be used in further analyses.

We verified that all SNPs were in Hardy–Weinberg equilibrium (HWE) and examined the LD between SNPs in both the family-based and case–control datasets using Haploview software (16). HWE and LD in the case–control dataset were examined both in the overall dataset and separately in cases and controls. The results were similar in each analysis (data not shown). We used only founders to estimate allele frequencies in the family-based dataset, except when a family did not have any founders genotyped. For those families, one individual was selected at random to contribute the allele frequency calculation. We calculated two-point parametric and non-parametric LOD scores for both SNPs in the families. We tested SNPs for allelic and genotypic association in the family-based dataset using the pedigree disequilibrium test (PDT) (17,18), and for haplotype association using the haplotype family-based association test (haplotype FBAT) (19). In the case–control dataset we assessed the association of each SNP with AMD using a χ^2 test for allelic association and a 2×3 contingency table likelihood ratio test for genotypic association. We used conditional analyses in the case–control dataset to test for an effect of C3 R102G in C3 L314P carriers and vice versa. The effect of C3 R102G after controlling for age, smoking status, the Y402H variant in complement factor H, the A69S variant in LOC387715, and the R32Q variant in CFB was estimated using logistic regression, assuming an additive genetic model for each locus. Smokers (those who had smoked at least 100 cigarettes) were coded as ‘1’ and non-smokers (those who had smoked fewer than 100 cigarettes over their lifetime) were coded as ‘0’. We tested for interactions with C3 R102G and CFH Y402H or CFB R32Q by comparing full and reduced logistic regression models with a likelihood ratio statistics (LRT, twice the difference in the deviance of the full compared with reduced logistic regression models) and determined significance by comparing the LRT with a χ^2 distribution with 1 degree of freedom. All case–control analyses, including PAR calculations, were performed using either Intercooled Stata 9.1 (StataCorp LP, College Station, TX, USA) or PowerMarker (20).

Table 4. Clinical features of the family-based and independent case-control datasets

	Family dataset	Independent case-control dataset Cases	Controls
Total individuals	559 Phenotyped (144 Mx, 79 Singleton families)	701 Cases (grades 3,4,5)	286 Controls (grades 1,2)
Grade	1: 60.8%, 2: 39.2%, 3: 29.6%, 4: 14.3%, 5: 56.1%	3: 26.7%, 4: 12.6%, 5: 60.8%	1: 72.6%, 2: 27.4%
Mean age, SD	67.3, 9.9 (Unaffected); 74.7, 9.2 (Affected)	76.5 (7.7)	66.9 (8.3)
% Female	66.5	63.6	55.6
% Ever smoked	46.9 (Unaffected); 56.2 (Affected)	60.9	48.7

ACKNOWLEDGEMENTS

Special thanks to the patients, their families, and the controls who participated in the study.

Conflict of Interest statement. The authors have no conflicts of interest to declare.

FUNDING

National Institutes of Health (EY12118 to M.A.P.-V. and J.L.H., M01 RR-00095 to Vanderbilt University); Funding Agency (National Eye Institute, National Center for Research Resources).

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