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Genetic variation in complement regulators and susceptibility to age-related macular degeneration

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ABSTRACT

Objectives: Age-related macular degeneration (AMD) is the commonest cause of blindness in Western populations. Risk is influenced by age, genetic and environmental factors. Complement activation appears to be important in the pathogenesis and associations have been found between AMD and genetic variations in complement regulators such as complement factor H. We therefore investigated other complement regulators for association with AMD.
Methods: We carried out a case–control study to test for association between AMD and single nucleotide polymorphisms (SNPs) spanning the genes encoding complement factor P (CFP, properdin), CD46 (membrane cofactor protein, MCP), CD55 (decay accelerating factor, DAF) and CD59 (protectin). All cases and controls were examined by an ophthalmologist and had independent grading of fundus photographs to confirm their disease status.
Results: 20 SNPs were genotyped in 446 cases and 262 controls. For two SNPs with *p*-values approaching significance additional subjects were genotyped to increase the numbers to 622 cases and 359 controls. There was no evidence of association between AMD and any of the SNPs typed in CFP, CD46, CD55 or CD59.
Conclusions: In a case–control sample that has shown the well established associations between AMD and variants in CFH, CFB and C3 there was absence of association with SNPs in CFP, CD46, CD55 and CD59. This suggests that these are not important susceptibility genes for AMD.

Introduction

Age-related macular degeneration (AMD) is a major cause of visual impairment in people over 50 years of age and the commonest cause of blindness in Western populations (Jager et al. 2008). It affects the macula, the region of the retina rich in photoreceptors which provides detailed central vision. In the early stages of the disease (age-related maculopathy, ARM), deposits called drusen form between the retinal pigment epithelium (RPE) and underlying choroid. Later in the disease there is atrophy of the RPE and overlying photoreceptor cells (geographic atrophy, GA) and/or aberrant choroidal neovascularisation (CNV, also called wet AMD) (Jager et al. 2008). Susceptibility to AMD is influenced by age, ethnic background, genetic and environmental factors, particularly smoking (Jager et al. 2008; Swaroop et al. 2009).

Common variants in a number of genes have been shown to influence the risk of developing AMD (Swaroop et al. 2009), including complement factor H (CFH) (Klein et al. 2005; Haines et al. 2005; Edwards et al. 2005; Hughes et al. 2006), complement factor B (CFB) and/or complement 2 (C2) (Gold et al. 2006), complement 3 (C3) (Yates et al. 2007) and complement factor I (CFI) (Fagerness et al. 2009). The involvement of genes in the complement pathway, particularly complement regulators, together with the finding that drusen contain proteins associated with inflammation and

Abbreviations: AMD, age-related macular degeneration; ARM, age-related maculopathy; CFB, complement factor B; CFH, complement factor H; CI, confidence interval; CPI, complement factor I; CFP, complement factor P; CNV, choroidal neovascularisation; DAF, decay accelerating factor; DNA, deoxyribonucleic acid; GA, geographic atrophy; HWE, Hardy–Weinberg equilibrium; MAC, membrane attack complex; MAF, minor allele frequency; MCP, membrane cofactor protein; OR, odds ratio; RPE, retinal pigment epithelium; SNP, single nucleotide polymorphism.

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Table 1
Disease status, sex, age and smoking history of subjects.[a]

	Core sample		Enlarged sample	
	Controls	Cases	Controls	Cases
Number of subjects	262	446	359	622
Disease status				
Age-related maculopathy (ARM)		19		26
Geographic atrophy (GA)		88		126
Choroidal neovascularisation (CNV)		267		373
Both GA and CNV		72		97
Sex				
Male	105 (40%)	207 (46%)	146 (41%)	283 (46%)
Female	157 (60%)	239 (54%)	213 (59%)	339 (54%)
Age				
Mean age, years (SD)	**75.7 (7.8)**	**80.4 (6.8)**	**75.1 (7.9)**	**79.4 (7.1)**
Pack years of cigarette smoking[b]				
0	**106 (41%)**	**169 (38%)**	**150 (42%)**	**230 (37%)**
0.1–20	**103 (39%)**	**127 (29%)**	**133 (37%)**	**175 (28%)**
20.1–40	**39 (15%)**	**99 (22%)**	**56 (15%)**	**137 (22%)**
>40	**14 (5%)**	**51 (11%)**	**20 (6%)**	**79 (13%)**

[a] Significant differences (at p-value ≤ 0.05) between cases and controls are reported in bold.
[b] Information on smoking was missing for 1 case in the enlarged sample.

immune-mediated processes (Mullins et al. 2000; Anderson et al. 2010) supports the hypothesis that complement activation is important in the pathogenesis of AMD. We have investigated variants in the major genes encoding proteins in the alternative complement pathway for evidence of association with AMD and the results for CFH, CFB, CFI, C3 and C5 have been reported elsewhere (Sepp et al. 2006; Yates et al. 2007; Cipriani et al. 2011). Because complement activation appears to be central to the pathogenesis of AMD, we have studied other regulators of the alternative complement pathway and the results are presented here.

Materials and methods

Patients and controls

The sample comprised cases with predominantly advanced AMD (GA or CNV) and spouse controls recruited from hospital ophthalmic clinics in London and the South East of England. All subjects described themselves as "white" on a recruitment questionnaire. The study had Research Ethics Committee approval and written consent was obtained from all participants. Subjects were examined by an ophthalmologist and health, lifestyle and smoking data were collected. All subjects had colour, stereoscopic fundus photography of the macular region. These images were independently graded at the Reading Centre, Moorfields Eye Hospital, London using the International Classification of Age-related Maculopathy and Macular Degeneration (Bird et al. 1995).

SNP selection and genotyping

Genomic DNA was extracted from peripheral blood leucocytes and typed for variants spanning the genes encoding complement factor P (CFP, properdin), CD46 (membrane cofactor protein, MCP), CD55 (decay accelerating factor, DAF) and CD59 (protectin). SNPs were selected from the International HapMap Project (The International HapMap Consortium 2003) database (release 19) for the CEPH population (Utah residents with ancestry from northern and western Europe). In an initial round of genotyping, SNPs with a minor allele frequency of at least 10% were selected to cover the main blocks of linkage disequilibrium. Subsequently, genetic coverage of the four genes of interest was formally calculated using the Tagger tag SNP selection algorithm (de Bakker et al. 2005) implemented in Haploview v4.1 (Barrett et al. 2006) and reported as percentage number of CEPH HapMap SNPs (genotype rate $\geq$ 90%; minor allele frequency (MAF) $\geq$ 1%; Hardy–Weinberg equilibrium (HWE) p-value $\geq 10^{-4}$; maximum number of Mendelian errors = 1) captured by (at least) one genotyped SNP (at $r^2 \geq 0.80$). On the basis of this analysis, additional genotyping was carried out and the final coverage achieved was 83% for CFP, 71% for CD46, 92% for CD55 and 71% for CD59. SNPs were genotyped using the ABI PRISM SNaPshot ddNTP Primer Extension Kit and a 3100 Genetic Analyser (Applied Biosystems) with the exception of rs7060246 which was typed using Taqman (Applied Biosystems). Manufacturers' protocols were followed.

Statistical analysis

Differences in the demographic characteristics of cases and controls were assessed using the Fisher's Exact test for categorical variables and the two sample Mann–Whitney test for continuous variables as implemented in STATA (Version 11.1, StataCorp LP, College Station, TX). A difference was considered significant if the p-value was found to be less than 0.05. Genetic association analysis was conducted using PLINK v1.07 (Purcell et al. 2007). Departure from HWE was assessed in controls. SNP association analysis was performed using the Cochran–Armitage trend test and corresponding p-values are reported. Odds ratios (ORs) were calculated using referent minor allele and are presented with 95% confidence intervals (CIs). We performed logistic regression analyses adjusting for age and pack years of cigarette smoking to address the possibility that SNP genotypes were confounded by these demographic factors.

Results

20 SNPs spanning the four genes of interest were genotyped in 446 cases and 262 controls. For two SNPs with p-values approaching statistical significance, additional subjects were genotyped to increase the numbers to 622 cases and 359 controls. Data on disease status, sex, age and smoking history of subjects are given in Table 1. The SNPs that were typed in each gene are listed in Table 2 together with the results of genotyping and tests for association. For all SNPs no departure from HWE was observed in control samples. There was no evidence of association between AMD and any of the SNPs in CFP, CD46, CD55 and CD59. Adjusting the analysis for age and pack years of cigarette smoking, excluding cases with ARM or confining the analysis to cases with either CNV or GA did not significantly alter the estimates (results not shown).