EXTENDED REPORT

Polymorphism p.402Y>H in the complement factor H protein is a risk factor for age related macular degeneration in an Italian population

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Br J Ophthalmol 2006;90:1142–1145. doi: 10.1136/bjo.2006.096487

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Accepted for publication
1 June 2006

Aims: To evaluate the complement factor H (CFH) p.402Y>H polymorphism as a risk factor in age related macular degeneration (AMD) in an Italian population.

Methods: 104 unrelated Italian AMD patients and 131 unrelated controls were screened for the CFH polymorphism p.402Y>H (c.1277 T>C), which has been associated with AMD. Retinography was obtained for patients and controls; the AMD diagnosis was confirmed by fluorescein angiograms. The c.1277 T>C polymorphism was genotyped with the TaqMan real time polymerase chain reaction single nucleotide polymorphism assay.

Results: The frequency of c.1277C allele was higher in AMD patients than in controls (57.2% v 39.3%; $p < 0.001$). The odds ratio (OR; logistic regression analysis) for AMD was 3.9 (95% confidence interval (CI): 1.9 to 8.2) for CC homozygotes. The CC genotype conferred a higher risk for sporadic (OR 4.6; CI: 2.0 to 10.5) than for familial AMD (OR 2.9; CI: 1.0 to 8.4). Genotypes were not related to either age at AMD diagnosis or to AMD phenotype. However, geographic atrophy and choroidal neovascularisation were more frequent in sporadic than in familial AMD ($p = 0.027$). Overall, the percentage of population attributable risk for the CC genotype was 28% (95% CI: 18% to 33%).

Conclusion: The association between the p.402Y>H (c.1277T>C) polymorphism and AMD applies to the Italian population and the CC genotype is more frequent in sporadic than in familial AMD cases.

Age related macular degeneration (AMD) is a complex disorder that leads to irreversible vision loss.^{1–3} The aetiology is unknown, but smoking, hypertension, age, obesity, and diet have been implicated in its insurgence.^{4–5} A genetic predisposition has been inferred from reports of a twofold to fourfold greater risk of AMD in first degree relatives of AMD patients versus first degree relatives of unaffected subjects,⁶ and from high levels of concordance in monozygotic twins.⁷ Thus far, the ABCA4, Apo E, SOD, ELOVL-4, hemicentin-1, and fibulin-5 genes^{8–14} have been associated with AMD. In addition, a major AMD locus has been linked to chromosomal region 1q.¹⁵ Very recently, the complement factor H gene (CFH) has been identified in this region and its c.1277T>C polymorphism, which produces the p.402Y>H amino acid change, is significantly associated with AMD.^{12–20} It has been suggested that the CFH gene protects the host arterial wall from excess complement activation, and that the p.402H variant, by altering this ability, could provoke vessel damage thereby triggering the neovascularisation that is sometimes present in AMD. This theory is supported by the finding that "drusen," the deposits often present in the macula of AMD patients, contain factors of the complement cascade, which are products typical of atherosclerotic lesions, apo E lipoproteins, and inflammatory molecules.^{19–21–23} These findings prompted us to investigate if the CFH p.402Y>H polymorphism was also involved in macular degeneration in our European white AMD patients from Italy.

MATERIAL AND METHODS

Between 2000 and 2004, 104 unrelated Italian patients with clinically documented AMD and 131 unrelated healthy controls were consecutively recruited from the department of ophthalmology (Second University of Naples), from the department of ophthalmology (San Raffaele Hospital, University of Milan), and from the Maugeri Institute

(Telese). All subjects belonged to the same geographic area and had a similar lifestyle including light exposure, smoking, alcohol use, and physical exercise. The study, which was conducted according to the Helsinki declaration, was approved by the ethics committees of the three institutions, and all participants provided informed consent. Patients were examined by trained ophthalmologists. Ophthalmic records, stereofundus photographs, and fluorescein angiograms were obtained for all patients. Family and medical histories were collected from all subjects, and familial AMD was recorded when a patient had at least one first or second degree relative with AMD. In all these cases the diagnosis in relatives was confirmed by fluorescein angiography.

AMD patients (mean age 73.3 (SD 9.7); 58 females and 46 males) were diagnosed according to the International Classification System and subdivided into three phenotype categories: early AMD (stages 1a, 1b, 2a, 2b, and 3; $n = 31$), geographic atrophy (stage 4ga; $n = 19$), and exudative AMD (choroidal neovascularisation; stage 4cnv; $n = 54$).^{24–25} The AMD stage assigned was based on the most severe eye at the time of recruitment. About 19% of our AMD patients had a familial history of the disease. Control subjects (mean age 71.6 (6.7); 66 females and 65 males) did not have a family history of AMD or ocular diseases that might simulate AMD or preclude its diagnosis—namely, previous laser photocoagulation, cryopexy, media opacity, or inflammatory diseases. The general characteristics of our AMD patients and controls are reported in table 1.

A blood sample was collected from all subjects for genotyping analysis. DNA was extracted with a commercial kit (Nucleon, Amersham Biosciences, UK). We genotyped the single nucleotide polymorphism (SNP) c.1277T>C

Abbreviations: AMD, age related macular degeneration; CFH, complement factor H

Table 1 General characteristics of the AMD patients and controls

	AMD patients		Controls	
	No	%	No	%
Sample size	104		131	
Females	58	56	66	50.4
Mean age at diagnosis (SD)	73.3 (9.7)			
Mean age at most recent examination (SD)			71.6 (6.7)	
Familial AMD	20	19		
AMD phenotypic categories:				
Early	31	30		
Geographic atrophy	19	18		
Choroidal neovascularisation	54	52		

(p.402Y>H) located in exon 9 of the CFH gene using the TaqMan SNP Genotyping Assay (Applied BioSystem, Foster City, CA, USA). Custom TaqMan genotyping probes for each allele were obtained from Applera (Applied BioSystem). Briefly, we amplified 20 ng of DNA on an Applied Biosystem 9700 96 well thermocycler, and read the plates with an Applied Biosystem 7900HT Sequence Detection System. Three samples, representative of the three CFH c.1277T>C genotypes—namely, CC, CT, and TT (amino acid sequences: HH, HY, YY), previously typed by sequence analysis on the ABI Prism 3700 analyser (Applied BioSystem), were analysed as positive controls in each TaqMan run, to verify allele identification. We tested the paraoxonase 1 (PON 1) 55 M>L polymorphism, as unassociated marker, in the AMD and control groups to look for a possible substructure in our population. Hardy-Weinberg equilibrium, based on allele frequencies, was verified with the formula: $p^2 + 2pq + q^2 = 1$. Student's *t* test or Fisher's exact test and the χ^2 test were performed to compare sex, age, alleles, and genotype frequencies between AMD cases and controls. Odds ratios (OR) and 95% confidence intervals (CI) were calculated by logistic regression analysis to assess the relative association between disease and age, sex, and genotypes. The percentage of population attributable risk of carrying the CC genotype was calculated with the formula $f(R - 1)/R$, where *f* is the fraction of cases with the CC genotype and *R* is the measure of OR.¹⁶ We used the SPSS software (Release 12.0) for statistical analysis.

RESULTS

The AMD cases and controls did not differ with regard to age ($p = 0.09$) or sex ($p = 0.33$). To determine whether there was a population substructure in the two groups, we evaluated Hardy-Weinberg equilibrium for the CFH c.1277T>C (p.402Y>H) polymorphism. We found that CFH c.1277T>C genotype frequencies were in Hardy-Weinberg equilibrium in controls ($p = 0.08$), slightly skewed in the whole AMD group ($p = 0.047$) and heavily skewed in the familial AMD subset ($p = 0.0004$). Consequently, we verified Hardy-Weinberg

equilibrium in our AMD and control groups for the PON 1 55 M>L polymorphism, which is an unassociated marker. PON 1 55 genotype frequencies were in Hardy-Weinberg equilibrium ($p = 0.51$) in the AMD and control groups, so we excluded bias in our population recruitment. The c.1277T>C (p.402Y>H) allele and genotype frequencies in AMD patients and controls are reported in table 2.

The CFH c.1277C allele was significantly more frequent in AMD patients ($p < 0.001$) than in controls (57.2% *v* 39.3%). The CC genotype was more frequent in patients than in controls ($p = 0.001$; univariate analysis). To account for age and sex effects, we did a logistic regression analysis with age, sex, and genotypes as independent variables. The results confirmed the significant association ($p < 0.001$) between the CC genotype and AMD (OR = 3.9; 95% CI: 1.9 to 8.2). As shown in table 3, the CC genotype conferred a higher risk ($p = 0.008$) in sporadic AMD than in familial AMD (OR 4.6 and 2.9, respectively), whereas the CT genotype conferred a moderate, not significant, increased risk (OR; CI: 2.0; 0.9 to 4.0) in sporadic AMD patients; no trend was identified for the familial AMD group.

Overall, the attributable risk of AMD based on the frequencies of CC in our patients indicated that histidine homozygous at position 402 of the CFH protein accounts for 28% (95% CI: 18% to 33%) of disease risk and for 27% (95% CI: 17% to 31%) when familial cases were excluded from the analysis. Geographic atrophy and choroidal neovascularisation AMD forms were more frequent ($p = 0.027$) in sporadic than in familial AMD cases (74% and 55%, respectively; data not shown). The mean age at AMD diagnosis did not vary significantly ($p = 0.3$) among patients in relation to genotypes CC (72.3 (9.9) years), CT (73.9 (9.7) years) or TT (73.3 (11.2) years). The distribution of the CC genotype did not differ from that of the CT + TT genotypes in relation to AMD phenotype (table 4).

DISCUSSION

We have screened a large Italian sample of AMD patients and controls for the CFH p.402Y>H polymorphism. Genotype

Table 2 Allele and genotype distributions of the CFH c.1277T>C polymorphism in Italian AMD patients and controls. The genotype risk for AMD (OR and CI, by logistic regression analysis) in CC or CT *v* TT genotypes is also reported

Allele	Allele distribution				Genotype	Genotype distribution				OR (CI)
	AMD patients		Controls			AMD patients		Controls		
	No	%	No	%		No	%	No	%	
C	119	57.2	103	39.3	CC	39	37.7	21	16.0	3.9 (1.9 to 8.2)
T	89	42.8	159	60.7	CT	41	39.7	61	46.6	1.4 (0.7 to 2.6)
					TT	24	22.6	49	37.4	
	p<0.001*					p<0.001				

*Fisher's exact test.

Table 3 Genotype distributions of the CFH c.1277T>C polymorphism in Italian familial and sporadic AMD patients. The genotype risk for AMD (OR and CI, by logistic regression analysis) in CC or CT v TT genotypes is also reported

Genotypes	Familial AMD*			Sporadic AMD†		
	No	%	OR (CI)	No	%	OR (CI)
CC	10	50.0	2.9 (1.0 to 8.4)	29	34.6	4.6 (2.0 to 10.5)
CT	2	10.0	0.2 (0.0 to 0.9)	39	46.4	2.0 (0.9 to 4.0)
TT	8	40.0		16	19.0	
	p=0.05			p=0.002		

*9 early, 6 geographic atrophy, and 5 choroidal neovascularisation.

†22 early, 13 geographic atrophy, and 49 choroidal neovascularisation.

c.1277T>C departed from Hardy-Weinberg equilibrium in the familial AMD subgroup. This observation was not related to a population substructure or genotyping error. Consequently, it probably reflects linkage disequilibrium between the CFH polymorphism and disease susceptibility loci.²⁶ Other instances of linkage disequilibrium have been reported in the CFH gene region.^{15–17}

We show that p.402Y>H (c.1277T>C) is also associated with AMD in an Italian population. Our finding that CC homozygotes were more frequent among patients than controls (37.7% v 16.0%) coincides with earlier data (31.4–34.6% v 12.1–13.7%).^{16–27} Moreover, the CC genotype conferred an OR for AMD of 3.9, which is similar to another report (3.33),¹⁷ and lower than reported in French (6.93)²⁷ and US (7.4 or 5.93) white AMD patients.^{18–20} The CC genotype accounts for 28% of disease risk in our cases, which is lower versus other white AMD patients—that is, 43% and 50%.^{16–17}

In our patients, the p.402Y>H polymorphism was not prognostic for the AMD phenotype. This supports the concept that it is involved in both exudative and atrophic AMD.¹²

Should this be the case, it follows that endothelial cells are not adequately protected during an inflammatory insult, thereby predisposing to thrombotic microangiopathy, which would explain the initial stages of exudative and atrophic AMD. In contrast, Haines *et al*¹⁷ found that the OR for AMD in carriers of two C alleles increased from 3.33 to 5.57 when considering only neovascular AMD. They suggested that the p.402Y>H polymorphism alters the ability of CFH to suppress complement related damage to arterial walls and might lead to vessel injury and subsequent neovascular/exudative changes like those seen in neovascular AMD. Very recently, CFH was found to be a major risk factor for soft drusen, and it was suggested that additional genetic and/or environmental factors may be required for progression to advanced AMD.²⁸

The genotype frequencies of c.1277T>C (p.402Y>H) were unrelated to age at AMD diagnosis in our patients, which concurs with another study.¹⁶ Our data suggest that p.402Y>H is a predisposing factor for both sporadic and familial AMD, even though the CC genotype confers a higher risk in sporadic cases, and geographic and neovascularisation

AMD are more frequent in sporadic than in familial AMD cases. Our results support the concept that the CFH polymorphism confers a risk of AMD irrespective of family history.¹⁶ However, a trend, albeit not significant, has recently been associated with familial AMD.²⁷

In conclusion, our data show that the p.402Y>H polymorphism is associated with both sporadic and familial AMD in an Italian population. The CC genotype association with AMD was not related to the disease phenotype or to age at diagnosis, and accounts for 28% of AMD risk.

ACKNOWLEDGEMENTS

This work was supported by grants from Ceinge-Regione Campania (Convenzione-Del GR 27/12/2002 N 6276) and MIUR (PRIN 2004). We are grateful to Jean Ann Gilder for revising and editing the text.

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Competing interests: none.

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Table 4 Genotype c.1277T>C distributions (CC v CT + TT) in the three phenotype categories in Italian AMD patients

AMD phenotypic categories	c.1277T>C genotypes	
	CC (%)	CT+TT (%)
Early (1a, 1b, 2a, 2b, 3)	15 (38.5)	16 (24.6)
Geographic atrophy (ga 4)	9 (23.0)	10 (15.4)
Choroidal neovascularisation (cnv 4)	15 (38.5)	39 (60.0)
	p=0.1*	

* χ^2 test

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