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Variation in complement component C1 inhibitor in age-related macular degeneration

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ABSTRACT

This study assessed variation in plasma levels of the complement regulatorC1 inhibitor (C1inh) in patients with age related macular degeneration (AMD) and controls. Plasma from391 AMD cases and 370 controls was assayed by rate nephelometry to determine C1inh protein levels. Protein levels were analysed for relationships with age, gender, smoking, AMD disease status and genetic variation in the *SERPING1* gene, which encodes C1inh, using a multivariate analysis. *t*-Tests show a significant difference in C1inh levels in AMD cases compared with controls ($p = 2.340E\text{-}6$), smokers compared to non-smokers ($p = 1.022E\text{-}4$) and females compared to males ($p = 1.661E\text{-}7$). Multivariate analysis shows that after accounting for gender and smoking AMD status remained significant. Age was included in the model but was not significant. Including genetic variation in the model shows that one significant SNP (rs2649663) 5′ of the *SERPING1* gene is associated with C1inh levels though this SNP is not associated with AMD. This suggests that genetic variation in the promoter region of the *SERPING1* gene may influence expression of the gene.

Introduction

Age-related Macular degeneration (AMD) is a major cause of blindness affecting up to 64% of the population over the age of 80 years (de Jong 2006; Friedman et al. 2004). Genetic susceptibility to this disease has been investigated and many genes have now been implicated (Conley et al. 2006; Francis et al. 2008; Klein et al. 2005; Kanda et al. 2007; Yang et al. 2006). Several complement component and regulator genes have been identified highlighting the importance of the complement pathway in AMD pathogenesis. The most studied of these is the complement regulator factor H (CFH) gene (Klein et al. 2005) but others include the complement components C3, C2 and factor B (CFB) (Francis et al. 2008). Protein levels of complement components and activation fragments have been measured in AMD cases and controls and support systemic activation of the complement system (Hecker et al. 2009; Scholl et al. 2008; Reynolds et al. 2009; Sivaprasad et al. 2007).

Among the loci implicated in AMD susceptibility is the *SERPING1* gene which was identified in a candidate gene study in a UK AMD sample, and replicated in an independent US sample (Ennis et al. 2008). Recently there have been conflicting reports over the association of this gene with AMD. Park et al. (2009), Allikmets et al. (2009) and Carter and Churchill (2011) failed to find an association but Ramsden et al. (2009) did find an association and Lu et al. (2010) show a haplotype across the *SERPING1* gene is significantly associated with soft drusen in a Han Chinese sample. One study in a small number of AMD donor eyes demonstrated that the C1inh protein, encoded by *SERPING1*, is more highly expressed in AMD donor eyes than control eyes (Mullins et al. 2009).

To further define the role of C1inh in AMD pathogenesis this study assessed the levels of C1inh in the plasma of AMD patients and controls. C1inh plasma levels were also compared with genetic variability in and around the *SERPING1* gene.

Materials and methods

Samples

761 plasma samples were analysed in this study, 391 AMD cases and 370 controls were selected from the collection of samples previously described by Ennis et al. (2008). Recruitment to this

Abbreviations: AMD, age-related macular degeneration; C1inh, complement component C1 inhibitor; *SERPING1*, serpin peptidase inhibitor clade G (C1 inhibitor), member 1 gene; SNP, single nucleotide polymorphism; HWE, Hardy–Weinberg equilibrium.

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Table 1
Descriptive statistics for the study population.

	Southampton UK		
	AMD cases	Controls	*p*-Value
No. of individuals	*N* = 391	*N* = 370	
Diagnosis			
No AMD	0	370 (100%)	
Early AMD (AREDS 2–3)	152 (39%)	0	
GA (AREDS 4)	45 (11%)	0	
CNV (AREDS 4)	86 (22%)	0	
Mixed (AREDS 4)	108 (28%)	0	
AREDS grade			
0	0	370 (100%)	
2	60 (15%)	0	
3	92 (24%)	0	
4	239 (61%)	0	
Age in years (all >50 years)			4.03E-27[a]
Mean	78.1	71.0	
Standard Deviation	7.8	9.4	
Gender			3.31E-4[b]
Female	249	188	
Male	142	182	
Smoking			0.30[b]
Current smoker	53	41	
Not current smoker	337	327	

[a] Independent samples *t*-test.
[b] Pearson's chi square.

collection is ongoing and for this collaborative study we chose to analyse plasma samples for 761 individuals selected on the basis of available plasma and irrespective of age, gender, smoking and other risk factors. A ratio of approximately 1:1 cases and controls was chosen. AMD can be categorised in order of severity with a grading system, such as that used in the Age Related Eye Disease Study (AREDS) (Age-Related Eye Disease Study Research and Group 2001). Under this scheme our cases belong to AREDS classes 2–4. Our cases have also been stratified by disease subtypes; early AMD cases (AREDS2 and 3), late AMD cases (AREDS 4) characterised by Geographic Atrophy (GA), choroidal neovascularisation (CNV), and mixed late AMD cases (with both CNV and GA). The mean age of our case samples was 78.1 years and the mean age of our control samples was 71.0 years. Other sample descriptive statistics are given in Table 1. Recruitment was approved by the Southampton and South West Hants local research ethics committee and followed the tenets of the Declaration of Helsinki. All participants provided informed written consent and underwent a detailed ophthalmic examination.

Plasma samples and assay

Plasma was obtained from 10mls of fresh peripheral blood collected in lithium heparin tubes. Within four hours, samples were centrifuged at 2600 rpm for 10 min. Plasma (supernatant) was collected, aliquoted and stored at −80 °C minimising the number of freeze/thaw cycles prior to analysis. Plasma samples were stored frozen until assayed for C1inh levels in a clinically validated and externally quality controlled (UK NEQUAS) rate nephelometric assay (Clinical Biochemistry and Immunology, UHW, Cardiff; Dade Behring, Milton Keynes). The normal range in the Cardiff laboratory is 0.15–0.40 g/l.

Genotypes

The genotype data used in this study consists of 11 single nucleotide polymorphisms (SNPs) close to the *SERPING1* gene on chromosome 11q12.1, which were previously reported in Ennis et al. (Ennis et al. 2008). Genotyping was carried out using KASPar chemistry (http://www.kbioscience.co.uk/genotyping/genotyping_chemistry.html). All SNPs included in the analysis passed standard quality control procedures ensuring all had sample minor allele frequencies greater than 5%. In addition the genotype distribution conformed to Hardy–Weinberg equilibrium (HWE) ($p > 0.05$) when tested in the controls, deviation may be indicative of genotyping error. The SNPs tested and results of genetic association with AMD in this sample are presented in Supplementary Table 1. Allele frequencies in the cases and controls and association results are comparable to those previously published (Ennis et al. 2008). Genetic association testing was carried out using PLINK (Purcell et al. 2007).

Statistical analysis

Independent samples *t*-tests were used to test the relationship between C1inh levels and gender, smoking, AMD status (Table 2). To determine the contribution of these significant variables as well as genetic variation at *SERPING1* to variation in C1inh levels a multivariate analysis was performed. The independent variables included were, gender, smoking, AMD status, age, and genotypes

Table 2
t-Tests comparing C1inh levels between groups (*n* = number of individuals in each group).

Groups	*n*	Mean C1inh (mg/l)	Standard deviation	*p*-Value
No AMD	370	0.33	0.077	
AMD	391	0.36	0.081	2.340E-6
Smoker	94	0.37	0.075	
Non-smoker	664	0.34	0.080	1.022E-4
Male	324	0.33	0.081	
Female	437	0.36	0.077	1.661E-7