Complement factor I and age-related macular degeneration

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Purpose: The complement system has been implicated in the pathogenesis of age-related macular degeneration (AMD). Complement factor I (CFI) is a serum protease that inhibits all complement pathways. A previous multicenter study identified a single missense *CFI* mutation (p.Gly119Arg) in 20/3,567 (0.56%) of AMD cases versus 1/3,937 (0.025%) of controls, thus suggesting that this mutation confers a high risk of AMD. A second *CFI* mutation, p.Gly188Ala, was identified in one patient with AMD.
Methods: We screened 521 unrelated AMD cases and 627 controls for the p.Gly119Arg and p.Gly188Ala variants. All participants were Caucasian and >55 years, and recruited through Southampton Eye Unit or research clinics in Guernsey. All participants underwent dilated fundal examination by an experienced retinal specialist. SNP assays were performed using KASP™ biochemistry.
Results: The p.Gly119Arg mutation was identified in 7/521 AMD cases compared to 1/627 age-matched controls (odds ratio [OR] = 8.47, confidence interval [CI] = 1.04–69.00, p = 0.027). There was a varied phenotype among the seven cases with the mutation, which was present in 4/254 (1.6%) cases with active or end-stage wet AMD and 3/267 dry AMD cases (1.1%). The p.Gly188Ala substitution was identified in 1/521 cases and 1/627 controls.
Conclusions: Our results identified a much higher frequency of heterozygosity for p.Gly119Arg in both cases and controls than in previous studies. Of note is that our sub-cohort from Guernsey had a particularly high frequency of p.Gly119Arg heterozygosity in affected individuals (4%) compared to our sub-cohort from the mainland (0.71%). Although these data support the conclusions of van de Ven et al. that the p.Gly119Arg substitution confers a high risk of AMD, our data suggest that this missense mutation is not as rare or as highly penetrant as previously reported. There was no difference in frequency for a second *CFI* variant, p.Gly188Ala, between the cases and the controls.

Age-related macular degeneration (AMD) is the leading cause of blindness in the developed world and is characterized by progressive degeneration of the RPE and secondary photoreceptor loss, resulting in visual loss. AMD is a complex multifactorial disease with a strong genetic component, and it is estimated that more than 50% of the heritability can be accounted for by the genetic loci that have already been identified [1-3], consisting of numerous genes in the complement pathway and a region of chromosome 10.

Complement factor I (CFI) is a serum protease that regulates the classical alternative complement pathways, by cleaving C4b and inactivating C3 [4]. Wang et al. have implicated CFI dysregulation in AMD, potentially resulting in chronic low-grade inflammation, and have shown that amyloid beta, a component of drusen, binds to CFI and interferes with its ability to cleave C3b [5].

Fagerness et al. identified two SNPs (rs10033900 and rs13117504) close to the *CFI* gene (gene ID 3426, OMIM 217030) with a combined haplotype that was strongly associated with AMD, but the span of the linkage disequilibrium included the last two exons of CFI and all four exons of *phospholipase A(2) group 12A* (*PLA2G12A;* gene ID 81579, OMIM 611652) [6]. This latter gene is hypothesized to modulate T helper cell function, and could not be excluded as the source of association with AMD. Kondo et al. demonstrated the association of rs10033900 with neovascular AMD in a Japanese cohort [7], and Qian et al. recently replicated this association in the Han Chinese population [8]. Notably, Cipriani et al. found no association between rs10033900 and AMD in a study of two separate cohorts in the United Kingdom (UK) [9].

Subsequently, Ennis et al. studied six SNPs in the CFI region [10], including four SNPs previously studied by Fagerness et al. Although four of the six SNPs achieved nominal significance in initial analysis, thus implicating *CFI* genomic variation with AMD disease susceptibility, no single SNP maintained marginal significance after Bonferroni correction for multiple testing.

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Seddon et al. sequenced coding regions of all known AMD loci and identified a significantly increased burden in only one gene, *CFI*. Rare missense variants in *CFI* were more common in AMD cases than controls (7.8% versus 2.3%), but despite an in-depth study of 59 variants in the *CFI* gene, these investigators were unable to identify any individual variants associated with AMD, and suggested that this may be due to low population frequency [11]. However, one of these variants, c.355G4A (p.Gly119Arg), was identified by van de Ven et al. as a rare, highly penetrant missense mutation that confers a high risk of AMD due to altered C3b degradation [12]. These authors identified the p.Gly119Arg substitution in 20 of 3,567 AMD cases, versus only one of 3,937 controls. A second *CFI* mutation, p. Gly188Ala, was identified in one patient with AMD and three affected family members, but not in 809 unrelated AMD cases.

In this study, we focused on the *CFI* gene in our cohort of patients with AMD and controls. We screened our cohort for the mutations identified by van de Ven et al. to determine whether these mutations were as rare and highly penetrant in our population as previously described.

METHODS

There were 521 unrelated AMD cases (62% female, mean age at recruitment 78.9 years [SD 7.9]) and 627 unaffected controls (56% female, mean age at recruitment 68.0 years [SD 9.8]) in this case-control study. Recruitment and eligibility of this cohort have been described previously [13]. All participants were Caucasian, aged >55 years and systemically well at the time of recruitment. Participants were recruited through the Southampton Eye Unit or from research clinics undertaken in Guernsey. All participants underwent dilated fundal examination by an experienced retinal specialist (AJL). All AMD patients had an AREDS classification of 2 or greater. Of the 521 AMD cases, 254 had active or end-stage wet AMD; the remaining 267 patients had dry AMD. Control subjects were either spouses or partners of AMD patients, or had attended the eye clinics for an unrelated eye condition. SNP assays were performed using KASP™ biochemistry (see Table 1; LGC). Recruitment was approved by Southampton and Southwest Hants local research ethics committee and followed the tenets of the Declaration of Helsinki. All participants provided informed written consent.

Association testing was performed using a Fisher's exact test, and odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. All statistical analyses were performed using PLINK, the whole genome association analysis toolset [14].

RESULTS

The p.Gly119Arg mutation was identified in 7/521 patients with AMD and 1/627 controls. Of the patients with AMD with this mutation, four patients had evidence of active or end-stage neovascular AMD in at least one eye. The remaining three patients had dry AMD of whom two had central geographic atrophy involving the fovea. The p.Gly188Ala substitution was found in 1/521 patients with AMD and 1/627 controls. The demographic data and clinical phenotypes of the patients and controls positive for either mutation are shown in Table 1.

These data support a significant excess of the allele in *CFI* encoding p.Gly119Arg in the AMD cases compared to the age-matched controls (OR = 8.47, CI = 1.04–69.00, p = 0.027). Table 2 shows the frequency of this mutation in our cases and controls, and compares our results to those reported by van de Ven et al.

These results demonstrate a much higher frequency of heterozygosity for p.Gly119Arg in the cases and the controls than in the cohort reported by van de Ven et al. Of note is that our sub-cohort from Guernsey has a particularly high frequency of p.Gly119Arg heterozygosity in the affected individuals compared to our sub-cohort from the mainland. The prevalence of p.Gly119Arg heterozygosity in each sub-cohort is compared to van den Ven et al.'s findings in Table 2.

DISCUSSION

Our results identified a much higher frequency of heterozygosity for p.Gly119Arg in the cases and the controls than in the cohort reported by van de Ven et al. [12]. Of note is that our sub-cohort from Guernsey has a particularly high frequency of p.Gly119Arg heterozygosity in affected individuals compared to our sub-cohort from the mainland. Although all known relatives, detected by clinical records and/or standard (PLINK) software [14], were excluded, within this isolated island population there are probably levels of population substructure that cannot be detected by these processes; this is the most likely explanation for the higher incidence of the p.Gly119Arg mutation in this subset. Despite an extensive literature review, no epidemiological or genetic data are available that would enable estimation of the prevalence of related individuals in Guernsey compared to the mainland UK population.

We acknowledge that our cohort is a relatively small sample size, and it is therefore difficult to infer the frequency of rare mutations. However, our findings suggest a similar frequency of the p.Gly119Arg mutation in dry AMD and neovascular AMD. A recent clinical trial of intravitreal lampalizumab, a complement factor D inhibitor, identified

TABLE 1. DEMOGRAPHIC DATA AND CLINICAL FEATURES OF CASES AND CONTROLS FOUND TO BE POSITIVE FOR THE CFI VARIANTS P. GLY119ARG (RISK ALLELE A) AND P. GLY188ALA (RISK ALLELE C).

Cohort	CFI p. Gly119Arg	CFI p. Gly188Ala	M/F	Age	Clinical appearance		Family History	CFH rs1061170	C3 rs2230199
					Right Eye	Left Eye			
Southampton	A G	G G	F	64	CNV causing PED, resulting in severe central visual loss	CNV causing PED, treated with anti-VEGF intravitreal injections		C T	C C
Southampton	A G	G G	M	80	Dry AMD	Left CNV, visual loss		C T	G C
Southampton	A G	G G	M	95	GA with foveal involvement	GA with foveal involvement		T T	C C
Guernsey	A G	G G	M	79	Drusen and GA involving fovea	Disciform scar with surrounding hemorrhage		C C	G C
Guernsey	A G	G G	F	63	Disciform scar	Disciform scar	Brother & mother had wet AMD	C T	G C
Guernsey	A G	G G	F	92	GA with foveal involvement	GA with foveal involvement		Not available	Not available
Guernsey	A G	G G	F	87	Dry AMD	Dry AMD		Not available	Not available
Guernsey	G G	G C	M	81	CNV	CNV	Uncle: sight problems	C T	G C
Control	A G	G G	M	58	Normal retinal examination			T T	G C
Control	G G	G C	F	78	Normal retinal examination			C T	C C

Normal retinal examination was defined as the absence of any RPE changes (atrophy or hyperpigmentation) and <5 hard drusen within the macular area. Also shown are the genotypes for the common CFH (risk allele C) and C3 (risk allele G) variants known to be associated with AMD. CNV, Choroidal Neovascular Membrane; PED, Pigment Epithelial Detachment; VEGF, Vascular Endothelial Growth Factor; GA, Geographic Atrophy.

TABLE 2. FREQUENCY OF HETEROZYGOSITY FOR p.GLY119ARG.

Studies	Controls	AMD	Odds Ratio	95% Confidence intervals
van de Ven et al.	1/3937 (0.025%)	20/3567 (0.56%)	22.20	2.98–164.49
This study	1/627 (0.16%)	7/521 (1.34%)	8.47	1.04–69.00
Southampton	1/389 (0.25%)	3/422 (0.71%)	(p=0.027)	
Guernsey	0/238 (0%)	4/99 (4.04%)		

The results of our present study demonstrate a much higher frequency of heterozygosity for p.Gly119Arg in both cases and controls than in the cohort reported by van de Ven. Of note is that our sub-cohort from Guernsey has a particularly high frequency of p.Gly119Arg heterozygosity in affected individuals compared to our sub-cohort from the mainland. The p value was calculated for Fishers Exact Test of the whole cohort.

an undisclosed *CFI* sequence variation in 55% of patients with advanced dry AMD and suggested that this variation was prognostic of treatment response [15]. This indicates the direct clinical significance of genetic variants in *CFI* in patients with AMD. The prevalence of *CFI* mutations found in our study was much lower (1.3%), even when only patients with dry AMD are considered (1.1%).

These data confirm the findings of van de Ven et al. that the p.Gly119Arg substitution confers a high risk of AMD. However, our data suggest that this missense mutation is not as rare or as highly penetrant as previously reported [12]. There was no difference in prevalence for a second *CFI* variant, p.Gly188Ala, between the cases and the controls. With the advent of novel treatments for complement inhibition, these results may have implications for stratifying patients for AMD therapies based on genotype.

ACKNOWLEDGMENTS

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