European Journal of Human Genetics (2012) 20, 1–3

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LETTERS

No evidence of association between complement factor I genetic variant rs10033900 and age-related macular degeneration

European Journal of Human Genetics (2012) **20,** 1–2; doi:10.1038/ejhg.2011.118; published online 12 October 2011

In 2008, an association between age-related macular degeneration (AMD) and single nucleotide polymorphisms (SNPs) on chromosome 4q25 was reported in this journal by Fagerness *et al*[1] studying a large US-based sample of around 1200 cases with advanced AMD and 800 controls. The association signal extended over a region of about 175 kb, the most associated variant ($P<10^{-7}$) being the SNP rs10033900 near the complement factor I (*CFI*) gene. Two replication studies[2,3] published also in this journal provided some additional support for an AMD susceptibility locus in this region. In the course of candidate gene studies of AMD, we had previously investigated SNPs spanning *CFI* including rs10033900 in a UK case–control sample, which shows the expected associations with the well-established AMD-susceptibility loci *CFH*, *ARMS2*, *CFB* and *C3*. No evidence of association with the CFI variants was observed. Following publication of the reports cited above we have typed rs10033900 in additional cases and controls in two independent samples from England and Scotland to investigate this further.

Full details of the phenotyping criteria have been reported previously.[4] The English sample comprised of 859 cases with predominantly advanced AMD, either geographic atrophy (GA) or choroidal neovascularisation (CNV) and 423 examined controls. The Scottish sample consisted of 505 cases with either intermediate disease (age-related maculopathy, ARM) or advanced AMD, and 351 examined controls. Summary demographics of the two cohorts are detailed in Table 1. The English and Scottish samples were genotyped in separate laboratories using different methodologies (ABI Prism SNaPshot Multiplex Kit and Taqman SNP Genotyping Assay respectively, both from Applied Biosystems, Foster City, CA, USA). No departure from Hardy–Weinberg equilibrium was observed either in the English (P=0.92) or in the Scottish (P=0.32) controls, and allele frequencies were almost identical (C allele, 50.1% and 49.6%, respectively). A genetic additive model was assessed using the Cochran–Armitage trend test and corresponding P-values are reported. ORs were calculated using referent C allele as per Fagerness *et al*[1] and are presented with 95% CIs. A pooled OR with corresponding 95% CI and P-value was estimated using both English and Scottish samples using the Mantel–Haenszel (fixed-effects) method of meta-analysis. Heterogeneity between the two studies was assessed using the quantity I^2 and χ^2-test.

Table 2 presents results from an association analysis between rs10033900 and advanced AMD in the English and Scottish samples together with a meta-analysis. The OR estimates showed no evidence of association in either cohort (English: OR=0.94, 95% CI=0.80–1.12; Scottish: OR=0.96, 95% CI=0.76–1.23). No heterogeneity between the two cohorts was observed. The Mantel–Haenszel summary OR (0.95, 95% CI=0.83–1.09, P=0.47) confirmed the lack of evidence of an association at SNP rs10033900 in these two independent samples.

Table 1 Demographic characteristics of the English and the Scottish subjects

	English subjects		*Scottish subjects*	
	Cases (N=859)	*Controls (N=423)*	*Cases (N=505)*	*Controls (N=351)*
Disease status, N				
ARM	29		261	
GA	142		55	
CNV	688		189	
Sex, N (%)				
Female	472 (55.0)	253 (59.8)	316 (62.6)	199 (56.7)
Mean age, year (± SD)				
	78.9 (±7.2)	75.0 (±7.8)	77.8 (±9.2)	78.0 (±8.5)

Abbreviations: ARM, age-related maculopathy; CNV, choroidal neovascularisation; GA, geographic atrophy.

Table 2 Association analysis for SNP rs10033900 comparing cases of advanced AMD (GA or CNV) with controls in the English and the Scottish samples

	Referent allele C frequency[a]		*Genotype counts (TT/TC/CC)*				
	Cases	*Controls*	*Cases*	*Controls*	*OR*	*95% CI*	P-*value*[b]
English subjects	0.48	0.50	211/407/186	102/207/101	0.94	0.80–1.12	0.50
Scottish subjects	0.50	0.50	47/130/45	77/177/80	0.96	0.76–1.23	0.76
Meta-analysis					0.95	0.83–1.09	0.47

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; CNV, choroidal neovascularisation; GA, geographic atrophy; OR, odds ratio.
[a]Referent allele C as per Fagerness *et al.*[1]
[b]Cochran–Armitage trend test for the English and the Scottish subjects; χ^2-test for the meta-analysis (Heterogeneity: I^2=0%; χ^2=0.02, P=0.89).

Adjusting the analysis for age or confining the analysis to cases with either CNV or GA, or including cases with intermediate disease (ARM), did not significantly alter the estimates (results not shown).

Given the strong association signal reported by Fagerness *et al*[1] and the fact that our study is comparably powered, it is surprising we could not replicate this finding. The study by Ennis *et al*[2] is the only previous report based on a UK sample and failed to find significant evidence of association with rs10033900 (P=0.135). Kondo *et al*[3] obtained weak evidence of an association with rs10033900 (P=0.036) in their Japanese sample and argued in favour of a recessive mode of inheritance (P=0.0035). Testing a recessive genetic model did not show significance in either of our UK samples (English sample: P=0.56; Scottish sample: P=0.60).

Recently, Neale *et al*[5] reported a genome-wide association study (GWAS) of advanced AMD using largely the same sample as Fagerness *et al*[1] but with additional controls. As in the earlier study, they found association with rs7690921 ($P<10^{-3}$) in the *CCDC109B* gene 80 kb from rs10033900 (r^2=0.40). Replication samples were studied and meta-analysis gave a stronger signal of association ($P<10^{-8}$). Chen *et al*[6] carried out another genome-wide investigation on a sample of around 2000 cases and 1000 controls, which confirmed previous evidence from Fagerness *et al*[1] of an association with rs2285714 ($P<10^{-6}$) in the *PLA2G12* gene 20 kb from rs10033900 (r^2=0.70). As the genotyping platforms used in these two GWASs did not include rs10033900, no conditional analysis was possible and the signals in *CCDC109B* and *PLA2G12* were interpreted as a proxy for rs10033900. More recently, the GWAS by Kopplin *et al*[7] did not report evidence of association (cut-off $P<10^{-4}$) for the *CFI* locus in two discovery samples.

McCarthy *et al*[8] have enumerated the possible explanations for failure to replicate a reported association as either the original finding being a false positive or the presence of some source of heterogeneity to which the difference in findings can be attributed, such as variable patterns of linkage disequilibrium between the genotyped SNP and untyped causal alleles, differences in the distribution, frequency or effect size of the causal alleles or the impact of non-additive interactions with other genetic variants or environmental exposures. For AMD, there have been several seemingly convincing reports of associations, which have not been replicated by subsequent studies, for example with *SERPING1*[9–11] and *TLR3*.[12–14]

On the available evidence it seems likely that variants at 4q25 do influence susceptibility to AMD. However, the variability observed in the results discussed above together with our complete lack of association for the index variant rs10033900 in two well-characterised independent UK samples leaves uncertainty as to which SNPs/genes are most strongly associated across different populations. Additional studies and a meta-analysis of the data are needed to clarify the nature of the association between AMD and variants in the extended *CFI* region.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work has received funding from the Medical Research Council, United Kingdom (JRWY, ATM, DGC), the Macular Disease Society (JRWY, ATM), Guide Dogs for the Blind Association (ATM, JRWY, DGC, CB), the Wellcome Trust (DGC), the Juvenile Diabetes Research Foundation (DGC), the Macula Vision Research Foundation (AFW), the Chief Scientist Office, Scotland (AFW, BD, CH), the Department of Health's NIHR Manchester Biomedical Research Centre (PB) and the NIHR Biomedical Research Centre for Ophthalmology at Moorfields Eye Hospital and UCL Institute of Ophthalmology (JRWY). The views expressed in the publication are those of the authors and not necessarily those of the Department of Health.

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