Assessment of the contribution of *CFH* and chromosome 10q26 AMD susceptibility loci in a Russian population isolate

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Background/aims: A strong association has been confirmed between age-related macular degeneration (AMD) and variants at two independent loci including Tyr402His in the complement factor H (*CFH*) on 1q32 and Ser69Ala at *LOC387715*, a hypothetical gene on chromosome 10q26. The contribution of both loci to AMD was investigated in an isolated north-west Russian population.

Methods: Together with a *PLEKHA1* variant at 10q26, the *CFH* Tyr402His and *LOC387715* Ser69Ala polymorphisms were genotyped in 155 patients with AMD and 151 age-matched controls. χ^2 and Mantel-Haenszel (M-H) score tests were used to test for association. Sex-adjusted ORs were calculated.

Results: The frequency of the Tyr402His C allele was significantly higher in patients with AMD compared with controls ($p_{M-H}=0.0035$). The increased risk observed in patients homozygous for the C allele ($OR_{HOM}=2.71$, 95% CI 1.25 to 5.90) in this indigenous Russian population was considerably lower than that observed in previous western Caucasian populations. A significant increase in the frequency of the *LOC387715* variant was observed in patients with late-stage AMD compared with controls ($p_{M-H}=0.007$), with a homozygous OR of 3.47 (95% CI 1.01 to 11.9), although this association was not seen with early-stage AMD.

Conclusion: The *CFH* gene contributes to AMD in this Russian population, although the risk conferred is considerably lower in this population than that found in other Western populations. A contribution of *LOC387715* to disease in this population is also likely to be of weak effect.

Age-related macular degeneration (AMD) is the most common cause of visual loss in industrialised countries.^{1–2} Early signs of disease are characterised by the presence of soft drusen, areas of hyperpigmentation and depigmentation. Advanced disease manifests as either choroidal neovascularisation or atrophy of photoreceptors and retinal pigment epithelium.³

In addition to age, a number of environmental factors, most notably smoking, contribute significantly to disease risk.^{4–5} A genetic contribution to AMD susceptibility is well established, with a higher concordance of disease in monozygotic compared with dizygotic twins,^{6–7} and an increased prevalence in first-degree relatives of patients.⁸

Three concurrent papers^{9–11} independently reported an association between AMD and a coding variant (Tyr402His) in the complement factor H (*CFH*) gene. These findings have now been widely replicated, and the *CFH* gene represents a major contributor to the risk of AMD with an approximately 3–7.5-fold increased risk of disease in individuals carrying two copies of the risk variant (reviewed in Haddad *et al*¹²). More recently, a study of the chromosome 10q26 region¹³ confirmed a strong association, previously reported by ourselves and others,^{14–15} between AMD and a common non-synonymous variant Ser69Ala in the hypothetical *LOC387715* gene. These findings have established *LOC387715* as the second major susceptibility locus for AMD. The *LOC387715* and *CFH* susceptibility loci, together with the effect of smoking, may explain up to 61% of the population-attributable risk of AMD.¹³

In this study, we sought to assess the contribution of these two loci in patients with AMD and healthy controls originating from the Norgorodskaja Oblast region of north-west Russia. Nowgorod, the region's capital city with 400 000 inhabitants, lies approximately 600 km north-west of Moscow and is primarily inhabited by indigenous Russians. With little

emigration and almost no immigration into the Norgorodskaja Oblast region for several centuries, there is virtually no admixture with other Russian population groups. This patient sample may therefore represent a genetic population isolate. Three single-nucleotide polymorphisms (SNPs) (*CFH* Y502H (rs1061170), *LOC387715* Ala69Ser (rs10490924) and *PLEKHA1* T320A (rs2421016)) were genotyped in patients and controls, and tested for association with AMD using a case-control study design. Locus-specific disease risks were assessed in the context of previous studies.

MATERIALS AND METHODS

Subjects and clinical assessment

A total of 155 patients (mean (SD) age 72.6 (7.6) years, 27.7% male) were recruited via ophthalmology clinics in hospitals within the Norgorodskaja Oblast region of Russia. Age-matched healthy controls (n = 151) were recruited mainly from among spouses of patients (mean (SD) age 71.1 (7.3) years, 72.2% male). The patients and controls were seen by one of the authors (SP). Each subject underwent a single examination involving visual acuity testing and a clinical ophthalmic examination. The AMD cases presented with early signs of AMD including confluent drusen/areas with hyperplasia (n = 92, 59.4%) or advanced disease either as geographic atrophy or as choroidal neovascularisation/disciform lesions (n = 63, 40.6%). A refined classification of patients with early-stage AMD into those with a low risk of progression to late-stage AMD (n = 17) and those with a high risk to evolve in late-stage manifestations (n = 75) was made, as described previously.¹⁵ All controls were free of macular changes such as drusen, pigmentary alterations or diabetic maculopathy. This

Abbreviations: AMD, age-related macular degeneration; *CFH*, complement factor H; SNP, single-nucleotide polymorphism

study was approved by the ethics committee of the University of Wuerzburg and adhered to the tenets of the Declaration of Helsinki. All subjects were informed about the scientific nature of the study and written consent was obtained.

Genotyping

Genomic DNA was extracted from peripheral blood leucocytes according to established protocols. Genotyping of genetic variants was achieved by resequencing of fragments containing the individual SNP loci using the BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, Foster City, California, USA) according to the manufacturer's instructions. The products were analysed on an ABI Genetic Analyzer 3730 (Applied Biosystems, Foster City, California, USA).

Statistical methods

Hardy–Weinberg equilibrium was assessed using χ^2 statistics. Genotype and allele frequencies were compared between cases and controls by χ^2 tests of 2×2 tables. To avoid the possibility of a confounding effect due to the high proportion of male controls compared with male cases, 2×2 tables of allele frequencies were also stratified by sex and a Mantel–Haenszel score test¹⁶ used to test for association. Significance levels are denoted p_U (unstratified data) and p_{M-H} (Mantel–Haenszel test of gender-stratified data). Mantel–Haenszel sex-adjusted odds ratios (ORs) and confidence intervals (CIs) were calculated for heterozygotes (OR_{HET}) and homozygotes (OR_{HOM}) compared with the non-risk genotype. Linkage disequilibrium (D') and haplotype frequencies were estimated separately in cases and controls using Haploview.¹⁷ Two-sided p values are reported and are uncorrected for multiple testing. Unless stated otherwise, all analyses were carried out using Rv2.2 for Windows (<http://www.r-project.org>).

RESULTS

All genotypes were in Hardy–Weinberg equilibrium in cases and controls ($p > 0.05$). Table 1 shows the genotype frequencies and disease ORs for *CFH* Y402H, *LOC387715* Ala69Ser and *PLEKHA1* rs2421016.

A significantly higher frequency of the Y402H C allele was observed in AMD cases (46.8%) compared with controls (35.7%, $p_U = 0.007$, $p_{M-H} = 0.004$). This association was observed in both late-stage AMD (47.6%, $p_{M-H} = 0.008$), early high-risk AMD (45.3%, $p_{M-H} = 0.0004$) and early low-risk AMD (50%, $p_{M-H} = 0.023$). Genotype-specific sex-adjusted ORs indicated a gene-dosage effect ($OR_{HET} = 2.01$ (95% CI 1.14 to 3.56); $OR_{HOM} = 2.71$ (95% CI 1.25 to 5.90); similar risks were observed in all phenotype subgroups. The risk observed in individuals homozygous for the C allele is considerably lower in this indigenous Russian population than the 6.7-fold risk

observed in our previous study of Caucasian cases and controls from Germany.¹⁵ Interestingly, the frequency of the risk allele in controls was not significantly different between populations (Russian: 35.8% vs German: 37.4%, $p = 0.65$).

The frequency of the risk allele at *LOC387715*, previously shown to best explain the association with the chromosome 10q26 locus in a German cohort,¹⁵ was not significantly different between AMD cases (31.6%) and controls (28.5%, $p_U = 0.45$, $p_{M-H} = 0.22$). However, when cases were stratified by early- or late-stage AMD, an increased frequency of the risk allele was observed in late-stage AMD cases (39.7%), which was significantly different from that of controls ($p_{M-H} = 0.007$). In contrast with our previous findings in a German population,¹⁵ there was no increase in the frequency of the risk allele in early AMD cases classified as at high risk for developing late-stage manifestations (26.7%, $p_{M-H} = 0.83$). In late-stage AMD, a significantly increased risk of disease was observed both in individuals heterozygous ($OR_{HET} = 2.55$ (95% CI 1.24 to 5.24)) and homozygous ($OR_{HOM} = 3.47$ (95% CI 1.01 to 11.88)) for the *LOC387715* risk allele. This compares with disease risk estimates for the TT genotype of between 5.7 and 10.6 in western Caucasian populations.^{13–15} Similar *LOC387715* allele frequencies were observed in patients who carried one or two copies of the Y402H risk variant compared with those who carried none.

In view of the strong linkage disequilibrium across the chromosome 10q26 region,^{14, 15} we additionally genotyped a previously associated SNP (rs2421016) in the *PLEKHA1* gene adjacent to *LOC387715*. Linkage disequilibrium was also observed between these two SNPs in the Russian cohort (controls: $D' = 0.68$; AMD: $D' = 0.73$), although the extent of linkage disequilibrium was considerably lower than that observed in a German cohort ($D' = 1$)¹⁵ due to the presence of the TC haplotype which was not observed in the German population. The frequency of the *PLEKHA1* rs2421016 T allele was not significantly different between AMD cases (60.6%) and controls (58.9%, $p_U = 0.73$, $p_{M-H} = 0.82$). No significant differences were observed in haplotype frequencies estimated in cases and controls (table 2). However, a single haplotype (TT) occurred at higher frequency in cases compared with controls, which may represent linkage disequilibrium between this haplotype and an unobserved population-specific mutation.

DISCUSSION

In the present study, we have shown that *CFH* contributes to AMD susceptibility in an indigenous Russian cohort, although with a considerably weaker risk than has been found in other Western populations. A contribution of *LOC387715* to disease is observed only in late-stage AMD, and disease ORs in this subgroup are lower than those previously reported.

Table 1 Genotype frequencies for complement factor H (*CFH*) and *LOC387715*/*PLEKHA1* single-nucleotide polymorphisms and sex-adjusted ORs in 155 age-related macular degeneration cases and 151 controls* from Nowgorodskaja Oblast region, Russia

Locus	SNP	Phenotype	Genotype†			$OR_{HET}‡$	95% CI	$OR_{HOM}‡$	95% CI
			–/– (%)	–/+ (%)	+/+ (%)				
<i>CFH</i>	Tyr402His	Control	67 (44.7)	59 (39.3)	24 (16)	1.95	1.09 to 3.48	2.45	1.11 to 5.42
		AMD	48 (31)	69 (44.5)	38 (24.5)				
<i>LOC387715</i>	Ala69Ser	Control	75 (49.7)	66 (43.7)	10 (6.6)	1.14	0.67 to 1.95	1.53	0.57 to 4.11
		AMD	73 (47.1)	66 (42.6)	16 (10.3)				
<i>PLEKHA1</i>	rs2421016	Control	22 (14.5)	80 (53)	49 (32.5)	0.97	0.47 to 1.99	1.13	0.53 to 2.43
		AMD	25 (16.1)	72 (46.5)	58 (37.4)				

AMD, age-related macular degeneration; *CFH*, complement factor H; SNP, single-nucleotide polymorphism.

*One control was not genotyped for rs1061170.

†Alleles are denoted as follows: *CFH* Tyr402H: –(T), +(C); *LOC387715* Ala69Ser: –(G), +(C); *PLEKHA1* rs2421016 –(C), +(T).

‡sex-adjusted Mantel–Haenszel odds ratios (ORs) are shown for heterozygous (–/+) and homozygous (+/+) genotypes compared with the baseline non-risk (–/–) genotype.

Table 2 LOC387715/PLEKHA1 haplotype frequencies in age-related macular degeneration cases and controls

Haplotype	Frequency (%)	
	PLEKHA1 rs2421016	AMD Controls
T	T	28.2 25
G	T	32.4 34
G	C	35.9 37.6
T	C	3.4 3.5

AMD, age-related macular degeneration.

The absence of a significant association with LOC387715/PLEKHA1 in patients with early-stage AMD (81.5% of whom are classified as at high risk for developing late-stage manifestations) might be a result of lack of statistical power due to the small number of cases and controls contained herein. However, if the risk due to Ala69Ser was similar in size to that shown in our previous large German study, our sample of approximately 150 case-control pairs would provide over 95% power to detect an effect of this size, based on our observed control frequency. Our sample size would provide 80% power to detect an association if the increased risk associated with each copy of the allele was at least 1.6-fold. The relatively small sample size of our study is therefore unlikely to explain the lack of association with LOC387715, unless the risk is considerably lower than that found in Western populations.

Interestingly, Schmidt *et al*¹³ observed significant evidence for a statistical interaction between LOC387715 and smoking, with an increase in risk in smokers carrying the Ala69Ser variant compared with non-smokers. Unfortunately, we do not have sufficient data on the smoking habit of our Russian patients versus the control group to estimate an influence of smoking on AMD risk. However, a lower frequency of smoking in patients with early AMD might potentially provide an alternative explanation for our lack of association in this subgroup at the LOC387715/PLEKHA1 locus.

Two independent Japanese studies have shown a much lower frequency of the CFH Tyr402His C allele (4%) and no association with AMD in this population.^{18,19} The frequency of the C risk allele in our indigenous Russian control population is similar to that in the Caucasian controls, 35.7% vs 37.4%, respectively,¹⁵ although the risk of disease is lower. Our results are therefore consistent with population differences associated with this locus, and other population-specific risk loci may contribute to disease. Future genetic association studies of AMD should therefore involve careful ethnic matching of controls to avoid the possibility of biased risk estimates and/or false positive association due to population substructure.

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