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No association between the T280M polymorphism of the CX3CR1 gene and exudative AMD

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ABSTRACT

Major genetic factors for age-related macular degeneration (AMD) have recently been identified as susceptibility risk factors. The *CX3CR1* gene has been shown to be associated with AMD in some studies. Our purpose was to analyze the role of the T280M polymorphism of the *CX3CR1* gene in a large French population, in a case-control study. 1093 patients with exudative AMD and 396 controls have been recruited and genotyped for the Y402H of *CFH*, rs10490924 of *ARMS2* and T280M of the *CX3CR1* gene. The distribution of the Y402H of *CFH* and of the rs10490924 of *ARMS2* was significantly different between cases and controls ($p < 0.0001$). The distribution of the T280M genotypes was not significantly different in the AMD patients compared to controls ($p = 0.99$). The Odds Ratio compared to TT individuals was 1.0 (95% CI 0.8–1.3) for TM individuals and 1.0 (95% CI 0.5–2.1) for MM individuals. The M allele frequency was 0.157 in cases and 0.154 in controls ($p = 0.87$).

Our study exclude an association between the T280M of the *CX3CR1* gene and exudative AMD in a French population.

1. Introduction

Age-related macular degeneration (AMD) is the most common cause of irreversible vision loss in the elderly population in Europe and United States (Vingerling et al., 1995; Seddon and Sobrin, 2007). Identification of risk factors is of major importance for the understanding of the origins of the disorder and for establishing strategies to prevent AMD. Risk factors for AMD are both environmental and genetic. Over the past few years, several single nucleotide polymorphisms (SNPs) have been associated with AMD, including variants in the complement factor H gene (*CFH*) (Klein et al., 2005; Edwards et al., 2005; Haines et al., 2005; Souied et al., 2005) and the *ARMS2/HTRA1* locus (*ARMS2*) (Jakobsdottir et al., 2005; Rivera et al., 2005; Leveziel et al., 2007). AMD has been also associated with other polymorphisms of the complement pathway and the inflammatory process such as the complement factor B gene (*CFB*) the complement component 2 gene (*C2*) (Gold et al., 2006; Spencer et al., 2007), the complement component 3 gene (*C3*) (Yates et al., 2007; Spencer et al., 2008; Maller et al., 2007; Zerbib et al., 2010) and CX3C chemokine receptor 1 (*CX3CR1*). Some studies, have associated the polymorphism T280M of the *CX3CR1* gene to AMD (Tuo et al., 2004; Combadière et al., 2007; Chan et al., 2005; Yang et al., 2010). Furthermore features of AMD have been described in *CX3CR1* deficient mice (Chan et al., 2008; Tuo et al., 2007). Our purpose was to test for association between T280M of the CX3CR1 gene and exudative AMD in a large French population.

2. Patients and methods

2.1. Patients

A total of 1093 French AMD patients were recruited in 4 French retinal Centres, at the department of Ophthalmology of Creteil in

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Table 1
Non-genetic characteristics of the AMD patients and controls.

	Cases	Controls	*p*
N	1093	396	
Age, *m* (sd), years	79.0 (7.4)	67.9 (7.6)	<0.0001
Men, *n* (%)	370 (33.9%)	159 (40.2%)	=0.025
Smoking, *n*/*N* (%)	422 (38.6%)	177 (44.7%)	=0.03
Diabetes, *n*/*N* (%)	103/1084 (9.5%)	18/276 (6.5%)	=0.12
BMI, *m* (sd)	25.5 (4.3)	25.4 (4.3)	=0.69

m (sd): mean (standard deviation).
BMI: Body Mass Index, kg/m^2.

collaboration with CHU de Bordeaux, the Quinze-Vingts Hospital and the Centre of Imaging and Laser of Paris, between November 2005 and July 2007. Written informed consent was obtained, as required by the French bioethical legislation and local ethic committee (CPP Henri Mondor), in agreement with the Declaration of Helsinki for research involving human subjects.

Inclusion criteria of the AMD patients were (1) women or men aged 55 or older, and (2) with exudative AMD in at least one eye, (3) no association with other retinal disease (e.g., diabetic retinopathy, high myopia, or macular dystrophies). Patients underwent a complete ophthalmologic examination including best corrected visual acuity measurement, fundus examination, and retinal photographs. Fluorescein angiography (Topcon 50IA camera, Tokyo, Japan) and if needed indocyanine green angiography (HRA, Heidelberg, Germany) and Optical Coherence Tomography (Carl Zeiss Meditec, Inc.) were performed. During the first visit AMD phenotypes in both eyes were analysed independently by each investigator (EHS and NL) prior to genetic testing according to color photographs and FA at presentation. When investigators disagreed on a particular clinical feature this patient was excluded from further analysis. A questionnaire about medical history was completed.

2.2. Controls

Controls were also recruited in our four centres. A total of 396 French women or men over 55 years with a normal fundus examination and a normal aspect of fundus photography were also recruited at the department of Ophthalmology of Creteil between 2002 and 2008. Information about their medical history including smoking was obtained.

2.3. Genotyping methods

Genomic DNA was extracted from 10 mL blood leukocytes using the Illustra® kit according to the manufacturer protocol (GE Healthcare). *CFH* Y402H, *ARMS2* rs10490924 and T280M of *CX3CR1* SNPs were genotyped by quantitative PCR allelic discrimination using reagents and conditions from Custom Taqman SNP Genotyping Assays (Applera Corp., France), using ABI 7900HT (Applied Biosystems).

2.4. Stastitical analysis

Hardy–Weinberg assumption was assessed by the standard method comparing the observed numbers of subjects in different genotype categories with the expected numbers under Hardy–Weinberg equilibrium for the estimated allele frequency, and testing with a Pearson goodness-of-fit chi-square (χ^2) with 1 degree of freedom.

χ^2 test was used to compare allelic and genotype distributions between cases and controls. Logistic regression models were used to estimate Odds Ratio (OR) with 95% confidence interval (95%CI) for AMD risk. A stepwise regression method has been used to select covariates (with $p < 0.15$) of multiple logistic regression model. OR were adjusted for age, gender, tobacco smoking, hypercholesterolemia, *CFH* and *ARMS2* genotypes. Significance levels were set at $p < 0.05$. Analyses were performed with the SAS software release 9.01 (SAS Institute INC, Cary, NC).

3. Results

The population consisted of 1093 exudative AMD cases and 396 controls. The mean age ± SD at AMD diagnosis was 79.0 ± 7.4 years. The non-genetic characteristics of the population are shown in Table 1. Cases were significantly older, were less often men and smokers but often had more hypertension, and hypercholesterolemia than controls. However, on the logistic adjusted model, only age, smoking status, hypercholesterolemia and gender remain significantly different between the two groups. The genotype distributions of the rs1061170 and rs10490924 SNPs within *CFH*, *ARMS2*, respectively, are shown in Table 2. The genotypic distributions of the *CFH* Y402H and *ARMS2* SNPs were significantly different between cases and controls ($p < 0.0001$). Adjusted ORs in individuals carrying 1 at risk allele for *CFH* Y402H was 2.3 (CI 95% 1.7–3.2, $p < 0.0001$) and in individuals with the 2 at risk alleles was 4.6 (CI 95% 3.0–7.1, $p < 0.0001$). Adjusted OR in individuals carrying 1 at risk allele for *ARMS2* was 3.0 (CI 95% 2.2–4.1, $p < 0.0001$) and in individuals with the 2 at risk alleles was 13.9 (CI 95% 7.5–25.7, $p < 0.0001$).

The genotype distribution of the T280M of the *CX3CR1* gene are shown in Table 3. The T280M genotype distributions of the *CX3CR1* gene were in accordance with the Hardy–Weinberg equilibrium ($p = 0.54$ in controls and $p = 0.33$ in cases). The distribution of the

Table 2
Genotype distributions of Y402H of *CFH* and rs10490924 of *ARMS2* among the AMD patients and controls.

	Cases	Controls	Global *p* values	Crude OR CI 95%[a], *p*	Adjusted OR CI 95%[b], *p*
CFH Y402H (rs1061170)					
TT	231 (21.1%)	154 (38.9%)	<0.0001	1 (reference)	1 (reference)
TC	553 (50.6%)	188 (47.5%)		2.0 [1.5–2.6]	2.3 [1.7–3.2]
				$p < 0.0001$	$p < 0.0001$
CC	309 (28.3%)	54 (13.6%)		3.8 [2.7–5.4]	4.6 [3.0–7.1]
				$p < 0.0001$	$p < 0.0001$
ARMS2 (rs10490924)					
GG	343 (31.4%)	252 (63.5%)	<0.0001	1 (reference)	1 (reference)
GT	512 (46.8%)	130 (32.8%)		2.9 [2.3–3.7]	3.0 [2.2–4.1]
				<0.0001	<0.0001
TT	238 (21.8%)	14 (3.5%)		12.5 [7.1–21.9]	13.9 [7.5–25.7]
				<0.0001	<0.0001

[a] Non-adjusted OR.
[b] Adjusted for age, gender, tobacco smoking.