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The genetic variant rs4073 A→T of the *Interleukin-8* promoter region is associated with the earlier onset of exudative age-related macular degeneration

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ABSTRACT.

Purpose: **To study the association of the single nucleotide polymorphism (SNP) rs4073 in the *interleukin-8 (IL-8)* promoter region with the diagnosis and age of onset of exudative age-related macular degeneration (AMD) in association with the known genetic risk factors for AMD and tobacco smoking.**

Methods: **Medical records, smoking history and angiograms or fundus photographs of 301 patients with exudative AMD, 72 patients with dry AMD and 119 control subjects were analysed retrospectively. The associations of *IL-8* rs4073 A→T, *CFH* rs1061170 T→C, *ARMS2* rs10490924 G→T and *C3* rs2230199 C→G SNPs with the presence of AMD and with the age of onset of exudative AMD were analysed.**

Results: **Younger age of exudative AMD onset was associated with the homozygous AA genotype of *IL-8* rs4073 ($p = 0.009$, Mann–Whitney *U*-test), CC genotype of *CFH* rs1061170 ($p = 0.016$), TT genotype of *ARMS2* rs10490924 ($p = 0.001$) and with current smoking ($p = 0.002$). The risk alleles C in *CFH* rs1061170 ($p < 0.0001$, Pearson chi-square) and T in *ARMS2* rs10490924 ($p < 0.0001$), as well as smoking ($p < 0.0001$), were more prevalent in AMD patients compared with controls. No association was found between the *IL-8* rs4073 genotype and the presence of AMD.**

Conclusion: **Out of the factors associated with the earlier onset of exudative AMD, only the genotype of *IL-8* rs4073 did not appear as a risk factor for AMD in general. IL-8 may have a role in accelerating the development of the choroidal neovascularization in exudative AMD.**

Key words: exudative age-related macular degeneration – genetic associations – interleukin-8 – single nucleotide polymorphism

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Introduction

Inflammation plays an important role in the pathogenesis and progression of age-related macular degeneration (AMD). Higher circulatory levels of inflammatory mediators C-reactive protein (CRP) and interleukin-6 (IL-6) have been shown to be associated with the progression of AMD (Seddon et al. 2005; Hong et al. 2011). Genetic association studies have emphasized the role of individual variations in the alternative complement cascade in increasing the risk of AMD development (Edwards et al. 2005; Haines et al. 2005; Klein et al. 2005; Gold et al. 2006; Maller et al. 2006, 2007; Yates et al. 2007; Seddon et al. 2013). The role of interleukin-8 (IL-8) is more controversial. Single nucleotide polymorphisms (SNPs) in the *IL-8* gene have been variably associated with the prevalence of atrophic and exudative AMD (Goverdhan et al. 2008; Tsai et al. 2008; Ricci et al. 2013). The intraocular IL-8 concentrations, however, have been elevated in patients with exudative AMD (Jonas et al. 2012) and correlated with the size of an active choroidal neovascularization (CNV) and with the macular volume after bevacizumab treatment (Roh et al. 2009; Miao et al. 2012).

Promoter region SNP *IL-8* rs4073 A→T (-251A/T) has been connected to the regulation of transcriptional activity of the *IL-8* gene and to the levels of IL-8 production (Hull et al. 2000; Ohyauchi et al. 2005; Taguchi et al. 2005; Hildebrand et al. 2007). In our previous studies in patients with exudative AMD treated with bevacizumab, the *IL-8* rs4073 genotype was associated with both the initial anatomic treatment response and with the persistence of intra- and subretinal fluid in macular optical coherence tomographies (OCTs) during 2-year follow-up (Hautamäki et al. 2013, 2014). In that data, the risk alleles of *IL-8* rs4073 A→T and *CFH* rs1061170 T→C were also associated with earlier onset of exudative AMD (data not published). Therefore, we wanted to study further the association of the *IL-8* SNP with the prevalence of AMD and with the age of onset of exudative AMD in a larger patient material, in association with the known AMD risk SNPs *CFH* rs1061170 T→C (Y402H), *ARMS2* rs10490924 G→T (*LOC387715*, A69S) and *C3* rs2230199 C→G (R102G) previously found to be associated with AMD also in the Finnish population (Seitsonen et al. 2006, 2008).

Materials and Methods

The total number of 492 Finnish subjects was included: 301 patients with exudative AMD, 72 with dry AMD and 119 control subjects. The patients with exudative AMD, 90 control subjects and 28 patients with dry AMD were recruited to our three previous studies on AMD genetics and treatment response in exudative AMD from the Medical Retina (cases) and Cataract Surgery (controls) units of the Departments of Ophthalmology of Helsinki (n = 368), Oulu (n = 6), and Tampere (n = 1) University Hospitals, or private offices and outpatient clinics (n = 44), between January 2003 and April 2010 (Seitsonen et al. 2006; Hautamäki et al. 2013, 2014). Additionally, 44 diabetic patients with large drusenoid macular deposits visible in fundus photographs taken for screening of diabetic (DM) retinopathy between January 2006 and February 2007 were included in the analysis of *IL-8* rs4073 as having dry AMD, and 29 men recruited between October 2004 and June 2007 to an ongoing study of risk factors for AMD in a cohort of male executives or academic professionals, originally examined for cardiovascular health, cognition and quality of life (Strandberg et al. 2004), were included as additional control subjects.

The control group consisted of 119 subjects (74 female, 45 male) who were >69 years of age, with no large drusen, and no, or minimal focal pigmentary abnormalities within the radius of one disc diameter from the fovea in fundus photographs graded according to the AREDS classification (Davis et al. 2005). The maximum total area of pigment abnormalities corresponded to a circle with diameter of 250 μm, and a maximum of five hard small (<63 μm) drusen was allowed. Sixty-five subjects had neither pigment abnormalities nor drusen, 50 had only pigment abnormalities, three had only small drusens, and one subject had both drusens and pigment abnormalities.

Patients with dry AMD were >50 years of age (43 female, 29 male), had at least 10 soft drusen >125 μm, a confluent area of drusen >1 disc area within the radius of one disc diameter from the fovea or a central geographic atrophy related to AMD.

The diagnosis of exudative AMD (204 female, 97 male) was made by an experienced medical retina specialist. The age of onset of exudative AMD was determined as the age at the diagnosis in cases with a recent onset of symptoms based on the medical records, and no substantial subretinal fibrosis detected in clinical examination or angiograms. The age of onset of the exudative AMD in the first affected eye was possible to determine in 259 patients (259/303, 85%). In 42 patients, the diagnosis was considered to be delayed: patients had been symptomatic for longer than a year, or the diagnosis was made only in a late stage of the disease with subretinal fibrosis. In these patients, the age of onset of the exudative AMD in the first eye could not be estimated, and their data were included only in the genetic analyses comparing AMD patients with the control group. The diagnosis of exudative AMD was based on fluorescein angiograms (FA) in 253 patients. The CNV lesions were classified according to the TAP-study criteria (Barbazetto et al. 2003); 39 were predominantly classic (15.1%), 129 occult (50.0%), 53 minimally classic (20.5%), 1 polypoidal (0.4%) and 23 not defined (>50% of the lesion area composed of haemorrhage or serous pigment epithelial detachment, 8.9%). In eight patients (3.1%), the angiograms were recorded, but were not available for retrospective analysis, and no classification of the lesion was found in the medical records. Five patients had received no treatment (poor visual acuity in four patients and a pigment epithelial tear in one patient), and FAs were, therefore, not recorded.

The patients were classified as never, ex- or current smokers. Never smokers were determined as having smoked less than one pack-year, ex-smokers as having quit smoking at least 10 years ago. If a participant had ever smoked, the smoking pack-years were calculated (pack-year = [cigarettes per day] × [years of smoking]/[20 cigarettes per pack]).

The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee. A written informed consent was obtained from all participants.

SNP genotyping

The blood DNA samples of the patients and control subjects (301 exudative AMD, 28 dry AMD patients and 119 controls) were analysed for the SNPs *IL-8* rs4073 A→T, *CFH* rs1061170 T→C, *ARMS2*rs10490924 G→T and *C3* rs2230199 C→G (Seitsonen et al. 2008; Hautamäki et al. 2013), except for the additional 44 samples of dry AMD patients recruited from photographic screening of DM retinopathy, which were analysed only for the *IL-8* rs4073.

The SNP analyses were carried out using phenol–chloroform extraction for DNA and PCR-based genotyping with Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) as described before. (Seitsonen et al. 2008; Hautamäki et al. 2013). The DNA fragments were purified from excess dye terminators with Performa® DTR v3 filter plates (Edge Biosystems, Gaithersburg, MD, USA) and resolved on an ABI 3730 capillary sequencer. All the methods were used as recommended by the manufacturer.

Statistical analysis

IBM SPSS Statistics version 22 (IBM Corp., Armonk, NY, USA) was used for statistical testing. Non-parametric tests were used in univariate comparisons between the groups. The simultaneous effects of genetic markers and smoking on the diagnosis and the age of onset of exudative AMD were estimated first with multivariate models built with the generalized linear model (GLZ) procedure in SPSS 22. To take into account the differences in the age distributions in the patients and control subjects, the effects of the studied variables were also estimated with the Cox regression analysis as suggested by van der Net and associates (2008). The use of Cox regression in cross-sectional genetic association studies is based on the principle that as the genotype status does not change over time, age at event can be considered as follow-up time (van der Net et al. 2008). A two-tailed p-value <0.05 was considered statistically significant, no correction for multiple comparisons was used. We estimated that with this sample size 13% differences in allele frequencies between the groups, and difference of 3 years in the age of onset could be detected with a power of 0.8 and a p-value of 0.05 (calculator for sample size provided at https://www.statstodo.com/).

Results

We first analysed the associations of the genotypes of *IL-8* rs4073, *CFH* rs1061170, *ARMS2* rs10490924 and *C3* rs2230199 and smoking behaviour with the diagnosis of any AMD or exudative AMD in the whole study population.

As reported before for a subgroup of the patients included in the analyses, also in this combined sample the risk alleles C in *CFH* rs1061170 ($p < 0.0001$, Pearson chi-square) and T in *ARMS2* rs10490924 ($p < 0.0001$), as well as smoking ($p < 0.0001$), were more prevalent both in the exudative AMD group and in all AMD patients compared with the control group (Table 1) (Seitsonen et al. 2006, 2008). No significant differences were found in the frequencies of *IL-8* rs4073 or *C3* rs2230199 genotypes between the groups. The effects of the studied SNPs and smoking remained unchanged in multivariate modelling (GLZ, data not shown).

We then analysed the associations of the studied factors with the age of onset of exudative AMD including only patients with exudative AMD in the analyses. The effects of the homozygous risk genotypes AA in IL-8 rs4073, CC in CFH rs1061170 and TT in ARMS2 rs10490924, as well as smoking were significant both in the univariate comparisons between the groups (Table 2) and in the conventional multivariate modelling with the GLZ (Table 3).

Table 1. Genotype frequencies and smoking history in exudative age-related macular degeneration (AMD) patients and all AMD patients (dry or exudative) compared with control subjects.

Polymorphisms	Controls (n = 119)	Exudative AMD (n = 301)	Pearson chi-square	Any AMD (n = 373)	Pearson chi-square
IL-8 rs4073 A→T					
AA	26 (21.8%)	45 (15.0%)	p = 0.120	61 (16.4%)	p = 0.275
AT	52 (43.7%)	161 (53.5%)		190 (50.9%)	
TT	41 (34.5%)	95 (31.6%)		122 (32.7%)	
NA	0	0		0	
CFH rs1061170 T→C					
CC	13 (11.5%)	109 (36.2%)	p < 0.0001	129 (39.2%)	p < 0.0001
CT	59 (52.2%)	154 (51.2%)		162 (49.2%)	
TT	41 (36.3%)	38 (12.6%)		38 (11.6%)	
NA	6	0		44	
ARMS2 rs10490924 G→T					
TT	2 (1.8%)	63 (21.1%)	p < 0.0001	70 (21.5%)	p < 0.0001
TG	42 (36.8%)	157 (52.7%)		170 (52.1%)	
GG	70 (61.4%)	78 (26.2%)		86 (26.4%)	
NA	5	3		47	
C3 rs2230199 C→G					
GG	4 (3.6%)	9 (3.0%)	p = 0.437	11 (3.4%)	p = 0.422
GC	27 (24.3%)	92 (30.8%)		101 (30.9%)	
CC	80 (72.1%)	198 (66.2%)		215 (65.7%)	
NA	8	2		46	
Tobacco					
Current smoker	3 (2.6%)	50 (17.5%)	p < 0.0001	64 (18.0%)	p < 0.0001
Ex-smoker	25 (21.9%)	81 (28.3%)		95 (26.8%)	
Never smoker	86 (75.4%)	155 (54.2%)		196 (55.2%)	
NA	5	15		18	

NA = data not available.

Table 2. Age of onset of exudative age-related macular degeneration (AMD).

Polymorphisms	Age of Onset of Exudative AMD Years, median (range)	Mann–Whitney U test
IL-8 rs4073 A→T		
AA	71.7 (55.5–84.3)	AA versus AT and TT
AT	75.5 (56.1–91.2)	p = 0.009
TT	75.6 (53.1–87.6)	
CFH rs1061170 T→C		
CC	73.8 (53.1–91.2)	CC versus CT and TT
TC	76.3 (59.4–87.6)	p = 0.016
TT	76.2 (63.9–87.0)	
ARMS2 rs10490924 G→T		
TT	71.3 (55.5–87.6)	TT versus TG and GG
GT	76.2 (53.1–91.2)	p = 0.001
GG	75.8 (54.5–88.1)	
C3 rs2230199 C→G		
GG	74.7 (63.1–85.1)	CC versus CG and GG
CG	74.8 (55.4–87.6)	p = 0.267
CC	75.8 (53.1–91.2)	
Tobacco		
Current smoker	70.1 (54.5–82.3)	Current versus never and ex-smokers
Ex-smoker	76.5 (62.8–88.1)	
Never Smoker	75.0 (53.1–91.2)	p = 0.002

Table 3. Multivariate model built with the generalized linear model procedure of SPSS 22 to estimate the effects of studied variables on the age of onset of exudative AMD.

			95% Wald CI		Hypothesis Test	
Parameter	B	SE	Lower	Upper	Wald chi-square	p-value
IL-8 rs4073 A→T						
AA	−2.502	1.1448	−4.746	−0.258	4.776	0.029
AT or TT	Reference					
CFH rs1061170 T→C						
CC	−2.171	0.8170	−3.773	−0.570	7.064	0.008
CT or TT	Reference					
ARMS rs10490924 G→T						
TT	−2.973	0.9811	−4.896	−1.050	9.181	0.002
TG or GG	Reference					
Smoking						
Current smoker	−3.885	1.0613	−5.965	−1.805	13.398	0.0003
Non- or ex-smoker	Reference					
Intercept	2.240	0.5966	1.071	3.409	14.100	<0.0001

Dependent variable: age – 75.0 years (median age of the exudative study patients).

To take into account the difference in the age distribution in the groups of exudative AMD patients and control subjects, the Cox proportional hazards models were used (Fig. 1) as described in the methods section. First, both patients with exudative AMD and control subjects were included to estimate the effects of the studied variables on the prevalence of exudative AMD (Fig. 1, left column). Age was used as time scale: age at the onset of exudative AMD for the cases and age at data collection for the controls. Patients with dry AMD were not included. The estimated effects remained similar compared to the univariate analyses and multivariate models built with the GLZ procedure, significant effects seen with the *CFH* rs1061170 [TC versus TT, hazard ratio (HR) = 1.7, p = 0.021 and CC versus TT, HR = 2.6, p < 0.0001], *ARMS2* rs10490924 (GT versus GG, HR = 1.6, p = 0.024 and TT versus GG, HR = 3.3, p < 0.0001) and smoking (ex- versus never smokers, HR = 1.4, CI 1.06 –1.90, p = 0.020 and current versus never smokers, HR = 2.4, p < 0.0001). Then, the effects of the studied factors on the age of onset of exudative AMD were analysed separately in the group of patients with the exudative disease (Fig. 1, middle column). The homozygous risk genotypes AA in *IL-8* rs4073 (HR = 1.7, p = 0.005), CC in *CFH* rs1061170 (HR = 1.3, p = 0.05) and TT in *ARMS2* rs10490924 (HR = 1.8, p = 0.0003) were associated with the younger onset of exudative AMD, whereas the effects of the heterozygous genotypes did not differ from the non-risk homozygous genotypes. Also, current smoking was significantly associated with the younger age at the onset of the disease (HR = 1.6, p = 0.005), whereas ex-smokers did not differ from never smokers. No significant associations were detected with the genotypes of *C3* rs2230199.

We considered the possibility of a more delayed diagnosis of exudative AMD before the era of widely used OCTs and availability of more effective treatments and analysed the data of patients diagnosed during the last 5 years of data collection (n = 101, between April 2005 and April 2010) separately. The effects of the studied SNPs and tobacco smoking on the diagnosis of exudative AMD remained the same when the subgroup was analysed with the control subjects (data not shown). When only the patients with exudative AMD were included, the effect of the *IL-8* rs4073 on the age at diagnosis, however, was even more pronounced (HR = 2.9, p = 0.00031), and became the most significant of the factors associated with the earlier onset of the exudative AMD (Fig. 1, right column).

The homozygous risk genotypes and current smoking had also a cumulative effect on the age of onset of exudative AMD. The best correlation with the age of onset was found when the sum of the homozygous risk genotypes of *IL-8*, *CFH* and *ARMS2*, and smoking (current smoker = 1 point, ex- or non-smoker = 0 points) were calculated for each patient (Spearman's rho −0.348, p < 0.0001). Significant difference in the age of onset existed between the patients with zero risk factors (n = 84, median age 77.6 years, range 62.8–87.6 years) compared with the patients with one (n = 107, 74.5 years, 53.1–91.2 years, p = 0.0001), two to four (n = 52, 71.8 years, 54.5–83.8 years, p < 0.0001) or three to four (n = 6, 68.3 years, 63.3–69.8 years, p = 0.0002) risk factors. A significant difference existed also between the patients with one and two (n = 46, 72.9 years, 54.5–83.8 years, p = 0.015) risk factors (Fig. 2).

No clear associations were found between the CNV lesion types and studied SNPs or tobacco smoking. Minimally classic lesions, however, were less frequent in patients with the homozygous TT risk genotype of *ARMS2* (p = 0.001). The type of the exudative lesion had no effect on the age of onset of exudative AMD. Gender had no effect in any of the analyses. No interactions between the studied factors were found.

Discussion

In this study, we could show the earlier onset of exudative AMD to be associated not only with current smoking and the homozygous risk genotypes of the *CFH* rs1061170 and *ARMS2* rs10490924 as reported earlier in other populations (Shuler et al. 2007, 2008; Keilhauer et al. 2013; Lechanteur et al. 2015), but also with the risk genotype AA of the *IL-8* rs4073. Out of these factors, only the AA genotype of *IL-8* rs4073 is not a well-established risk factor for AMD and did not associate with the overall prevalence of AMD in our material. The *IL-8* rs4073 genotype may thus be connected with the mechanisms leading to the growth of CNV in the AMD disease process.

The *IL-8* rs4073 A→T has been associated with variations in IL-8 production, and significantly higher levels of circulating and mucosal IL-8 have been detected in patients carrying the A allele or the AA homozygous genotype in *IL-8* rs4073 (Hull et al. 2000; Ohyauchi et al. 2005; Taguchi et al. 2005; Hildebrand et al. 2007). In addition to being a powerful inflammatory cytokine, a chemotactic factor for migratory immune cells and an activating factor for neutrophilic granulocytes,

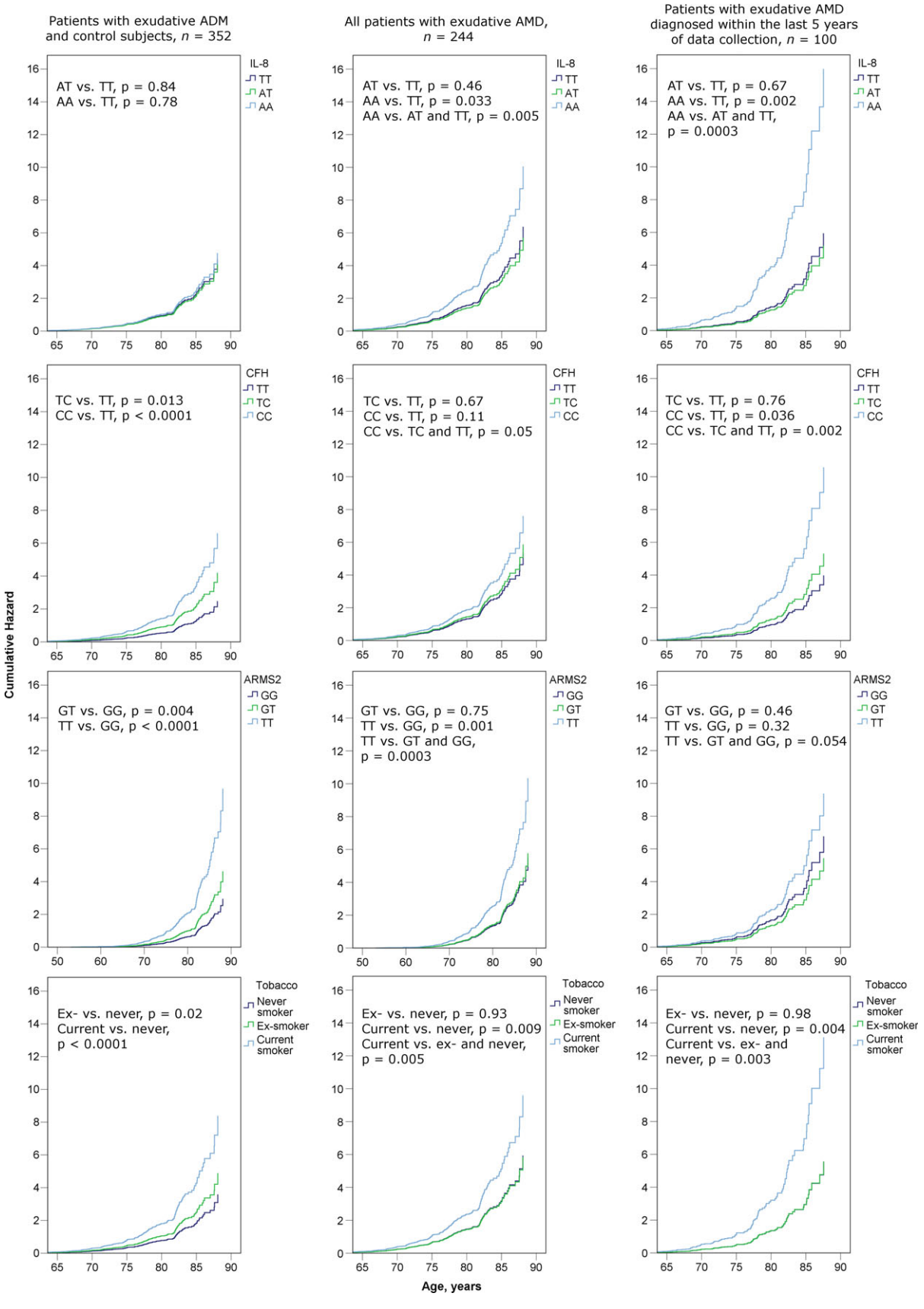

Fig. 1. The effects of single nucleotide polymorphisms (SNPs) *IL-8* rs4073, *CFH* rs1061170 and *ARMS2* rs10490924, as well as smoking, were estimated with Cox proportional hazards models in exudative AMD patients together with control subjects (left column), in all patients with exudative AMD (middle column) and in exudative AMD patients diagnosed within the last 5 years of data collection (right column). *n*, number of cases available in the analysis (no missing values).

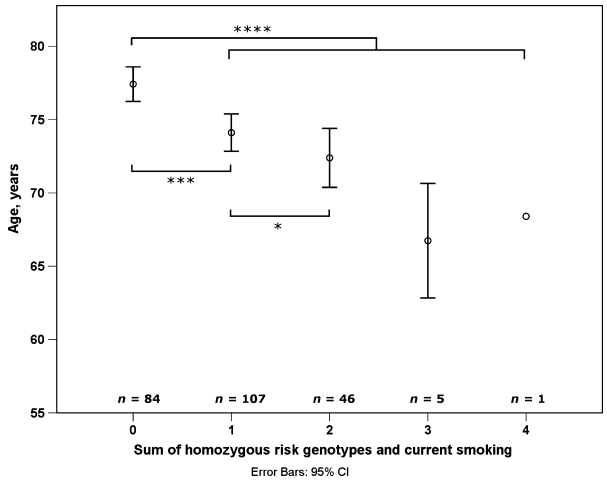

Fig. 2. The sum of homozygous risk genotypes in *IL-8*, *CFH* and *ARMS2*, and current smoking on the age of onset of exudative AMD (*p < 0.05, ***p < 0.001, ****p < 0.0001, Mann–Whitney *U*-test).

IL-8 is a potent proangiogenic factor. IL-8 is expressed not only by the cells of immune system but also by vascular endothelial and retinal pigment epithelial (RPE) cells. It has a critical role in vascular formation both in physiological and pathological conditions (Belperio et al. 2000). Expression of IL-8 is upregulated in response to various stimuli, including CRP (Wang et al. 2010), oxidative stress (DeForge et al. 1993), saturated fatty acids (Stentz & Kitabchi 2006), preoxidized photoreceptor outer segments (Higgins et al. 2003), amyloid-β (Kurji et al. 2010) and angiopoietin-1 (Abdel-Malak et al. 2008). IL-8 induces angiogenesis through both VEGF-dependent and VEGF-independent pathways and increases endothelial permeability (Mizukami et al. 2005; Martin et al. 2009). IL-8 receptors CXCR1 and CXCR2 are expressed in endothelial cells. The activation of the CXCR2-receptor elevates the levels of intracellular VEGF and enhances VEGF secretion (Martin et al. 2009). The activated CXCR2 is also capable of forming a complex with the VEGF receptor, VEGFR2, and causes transactivation of the VEGF receptor without the presence of VEGF (Petreaca et al. 2007). In aged cells, disturbance of calcium homeostasis may lead to induced *IL-8* gene expression and IL-8 secretion (Yang et al. 2015). Chronic oxidative stress has also been found to trigger the RPE cells to produce higher levels of IL-8 stimulating both inflammation and angiogenesis, which, in turn, may lead to the development of CNV and exudative AMD (Zhu et al. 2009).

In our data, the associations found between the presence of AMD lesions and the known genetic risk factors for AMD, *CFH* rs1061170, *ARMS2* rs10490924 and current smoking were clear (Christen et al. 1996; Seddon et al. 1996, 2013; Edwards et al. 2005; Haines et al. 2005; Jakobsdottir et al. 2005; Klein et al. 2005; DeWan 2006; Yang et al. 2006; Maller et al. 2007; Yates et al. 2007). We could not, however, show any association between the prevalence of AMD and the risk genotype of *IL-8* rs4073. The A allele in *IL-8* SNP rs4073 has previously been associated with an increased risk of AMD in Caucasian population in Britain (Goverdhan et al. 2008). Also, the *IL-8* rs2227306 T→C (+781 C/T) has been associated with an increased risk of developing exudative AMD in Taiwan Chinese and in Italian populations (Tsai et al. 2008; Ricci et al. 2013). In Europeans, a strong linkage disequilibrium exists between the *IL-8* SNPs rs4073 and rs2227306 (Hull et al. 2001; Ricci et al. 2013), and therefore, we would have expected to find a same kind of association with the *IL-8* rs4073 in our material. Our sample size, however, is limited, and with the power of 0.8 only differences over 13% in allele frequencies between the groups could be detected. The differences reported earlier have been less than that both in the British and in the Italian populations (Goverdhan et al. 2008; Ricci et al. 2013). The *IL-8* rs2227306 may be the SNP that is, after all, the main functional polymorphism responsible for the enhanced interaction with the transcription factor binding complex and the higher levels of secreted IL-8 and may therefore show somewhat stronger associations with pathologies than the *IL-8* rs4073 (Hacking et al. 2004). Despite our limited sample size, however, the younger age of onset of the exudative AMD was quite clearly associated not only with the known risk genotypes in the *CFH* rs1061170 and *ARMS2* rs10490924 and current smoking but also with the AA genotype of *IL-8* rs4073.

The younger age of onset of the disease in the exudative AMD patients was associated only with the homozygous, not heterozygous risk genotypes in the *IL-8* rs4073, *CFH* rs1061170 and *ARMS2* rs10490924. It was also associated with only current smoking, not ex-smoking compared with non-smoking behaviour. The associations with the prevalence of AMD, on the other hand, resemble more of a dose–response pattern with higher hazard ratios associated also with heterozygous genotypes and ex-smoking compared with non-risk homozygous genotypes and non-smoking. The same kind of phenomenon has also been seen in the previous studies showing the homozygous risk genotypes of *CFH* rs1061170 and *ARMS2* rs10490924 and tobacco smoking to be associated with the younger onset of exudative AMD (Shuler et al. 2007, 2008; Keilhauer et al. 2013; Lechanteur et al. 2015). One possible explanation could be that when in the analyses of the age of onset only diseased subjects are included, the protective effects of the non-risk genotypes and non-smoking behaviour are not fully seen when healthy controls are not represented.

The limitations of this study are its retrospective nature, the relatively small sample size, the estimate of onset of the disease being based on the medical records and the diagnosis being based on clinical findings and angiograms in the era before OCTs. The study population also represents a combined sample of subjects of our previous studies. The results, especially the distinct difference between the effects of *IL-8* rs4073 on the prevalence of AMD and on the age of onset of

exudative disease, need to be confirmed in larger and independent patient materials.

The association of the *IL-8* rs4073 A→T with the earlier onset of exudative AMD increases the evidence of the role of IL-8 in the exudative AMD disease process. The results of this study suggest that the *IL-8* polymorphism may have a stronger effect on the age of onset of exudative AMD than on the risk of developing AMD. An explanation for that could be that the IL-8 enhances vascular leakage. An increased tendency to develop retinal oedema with higher IL-8 levels could lead to more symptomatic lesions and earlier diagnosis of the disease. Also, increased local IL-8 production may augment the proinflammatory effects of the established AMD risk factors and link inflammation and angiogenesis in the transformation of a dry AMD lesion into the exudative phenotype.

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