Genetic, Behavioral, and Sociodemographic Risk Factors for Second Eye Progression in Age-Related Macular Degeneration

Yara T. E. Lechanteur,[1] *Johannes P. H. van de Ven,*[1] *Dzenita Smailhodzic,*[1] *Camiel J. F. Boon,*[1] *B. Jeroen Klevering,*[1] *Sascha Fauser,*[2] *Joannes M. M. Groenewoud,*[3] *Gert-Jan van der Wilt,*[3] *Anneke I. den Hollander,*[1,4] *and Carel B. Hoyng*[1]

Purpose. This study was conducted to investigate the correlation of genetic, sociodemographic, and behavioral risk factors with second eye progression to end-stage AMD.

Methods. One hundred and eight patients with end-stage AMD in one or both eyes were included in a retrospective time-to-event analysis of the onset of end-stage AMD in the second eye. Multivariate Cox regression survival analysis was performed for sex, age, smoking, body mass index (BMI), education, and 16 single nucleotide polymorphisms (SNPs) associated with AMD.

Results. Except for education, all sociodemographic and behavioral risk factors analyzed were significantly associated with a more rapid progression toward second eye involvement. Hazard ratios (HRs) were 2.6 (95% confidence interval [CI], 1.4–5.0) for female sex; 5.0 (95% CI, 2.0–12.5) for age >80; 2.2 (95% CI, 1.1–4.1) for BMI >30; and 4.4 (95% CI, 1.4–14.3) for >40 pack years, compared with the referent groups. Carriers of the lipoprotein lipase (*LPL*; rs12678919) risk alleles were at risk for more rapid progression to end-stage AMD in the second eye compared with the referent wild-type genotype (HR 2.0; 95% CI, 1.0–3.6). For complement factor I (*CFI*; rs10033900), homozygous carriers of the risk allele progressed faster than wild-type individuals (HR 2.2; 95% CI, 1.1–4.3).

Conclusions. Sociodemographic, behavioral, and genetic risk factors are associated with the rate of second eye progression toward end-stage AMD. The findings of this study underline the importance of lifestyle factors and the complement pathway in AMD progression and suggest a role of the high-density-lipoprotein metabolism in second eye progression. (*Invest Ophthalmol Vis Sci.* 2012;53:5846–5852) DOI:10.1167/iovs.11-7731

AMD is a multifactorial disease of the central retina and the most prevalent cause of progressive vision loss in the elderly in the developed world, with a prevalence of 30% after the age of 75 years.[1] With the rapid increase of the elderly population, AMD is considered a major and growing health problem.

Several studies have investigated the contribution of behavioral, genetic, sociodemographic, and ocular risk factors to the incidence and progression of AMD, as well as the development of choroidal neovascularization (CNV) or foveal geographic atrophy (GA), viewed as the end stages of AMD.[2–10]

Patients with unilateral advanced AMD are at high risk of developing end-stage AMD in their fellow eye.[11] Cumulative incidence rates have been reported to be 10% to 14%, 28% to 31%, and 36% to 37% after 1, 3, and 4 years of follow-up, respectively.[12,13] Some studies have tried to identify risk factors for progression to advanced AMD in the second eye, but results in the different studies are not unanimous. Smoking, age, body mass index (BMI), and systemic hypertension—as well as ocular characteristics such as drusen size, presence of ≥5 drusen, focal hyperpigmentation, and nonfoveal GA—have been suggested as possible risk factors.[12,14–17]

A variety of single nucleotide polymorphisms (SNPs) have been reported to be associated with AMD.[18] It has been shown that complement factor H (*CFH*) Y402H; age-related maculopathy susceptibility 2 (*ARMS2*) A69S; complement component 2 (*C2*) E318D; and complement component 3 (*C3*) R102G risk alleles are associated with progression toward bilateral advanced AMD.[15,16] Carrying all these genetic risk factors, together with modifiable risk factors such as smoking and high BMI, increases the risk of developing advanced AMD by a factor of 19.[15]

The purpose of this study is to determine the correlation of genetic, sociodemographic, and behavioral risk factors with second eye progression to end-stage AMD. This will not only allow the selection of patients with a high risk for development and progression of AMD in the second eye, but also provide more insight into the development of AMD. Furthermore, it will provide patients with additional and more accurate information regarding their individual risk profile.

Methods

Study Population

All 108 subjects were selected by means of chart review from the European Genetic Database (EUGENDA, www.eugenda.org [in the

From the [1]Department of Ophthalmology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; the [2]Department of Vitreo-Retinal Surgery, Centre of Ophthalmology, University of Cologne, Cologne, Germany; the [3]Department of Epidemiology, Biostatistics and Health Technology Assessment, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; and the [4]Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.

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Corresponding author: Carel B. Hoyng, Department of Ophthalmology, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB, Nijmegen, the Netherlands; C.Hoyng@ohk.umcn.nl.

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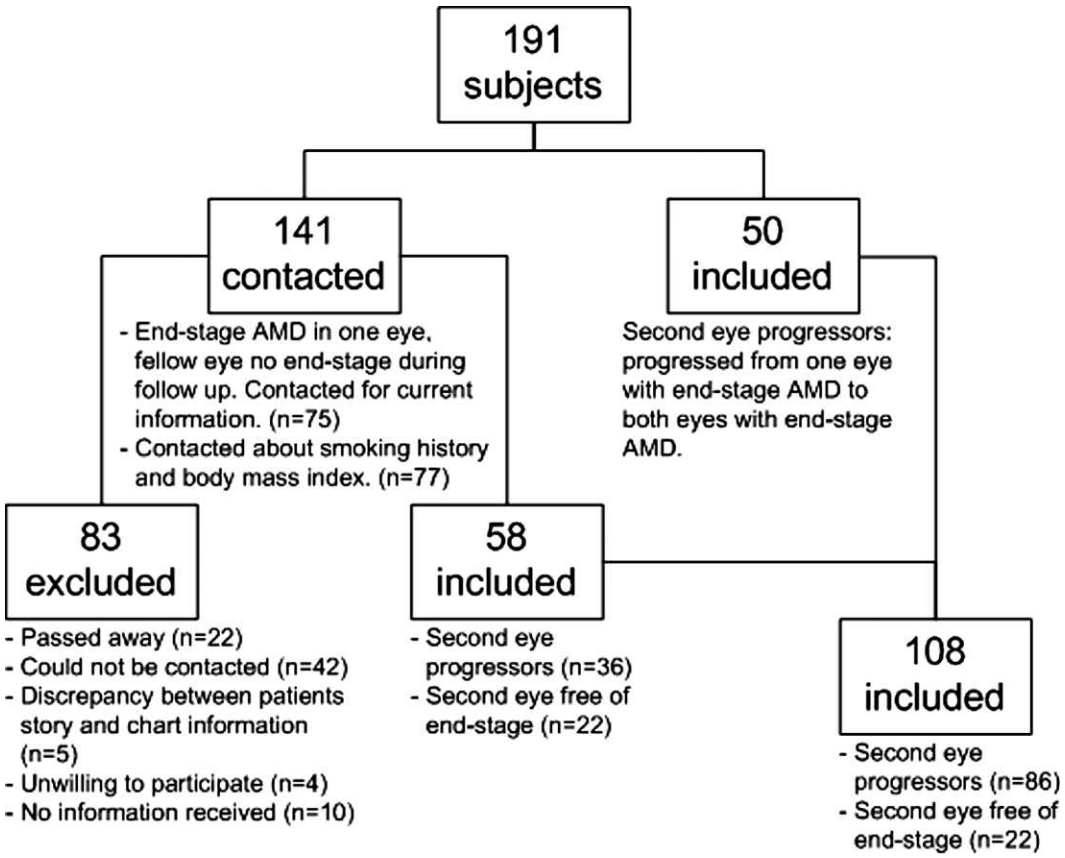

Figure 1. Flow chart of patient inclusion.

public domain]) and were entered into the database between January 1997 and December 2006. EUGENDA is a multicenter database of AMD patients and control subjects founded by the Radboud University Nijmegen Medical Centre and the University of Cologne Medical Centre. Before enrollment in the database, written informed consent was obtained from all participants after receiving information about the study objectives and methods.

Information about smoking, BMI, and education were obtained by a questionnaire. For smoking, patients were asked if they had ever smoked, how long they had smoked, if and how long they had quit, and how many cigarettes a day were smoked on average. For each patient, the number of pack years was calculated, where one pack year was the equivalent of smoking 20 cigarettes a day for 1 year. BMI was calculated from self-reported weight and height of patients. Education was classified into four levels: primary school, high school, higher professional education, and university. For statistical analysis, the four categories were collapsed into high school or lower and higher than high school.

Color fundus photographs and fluorescein angiography images were taken with a digital fundus camera (Topcon TRC 50IX; Topcon Corporation, Tokyo, Japan). For inclusion, end-stage AMD had to be present in at least one eye. End-stage AMD was defined as either choroidal neovascularization within the central 6 mm ETDRS grid or geographic atrophy of an area of at least 175 μm including the fovea.[19,20] Development of advanced AMD in the first eye was taken as starting-point (T[0]) and had to be known with an accuracy range of 1 month; an accuracy range of 6 months was accepted if the second eye did not develop end-stage AMD within 4 years. Progression time until the development of end-stage AMD in the fellow eye was calculated in months after T(0). The following exclusion criteria were used: no end-stage AMD in both eyes; unknown or unclear time of end-stage AMD in one or both eyes; other retinal diseases that interfered with the diagnosis of end-stage AMD, such as central serous chorioretinopathy; laser treatment or radiotherapy for a retinal disease or treatment for AMD in a stage that could not be determined as end-stage (e.g., laser therapy for extensive drusen). Patients were excluded at baseline. If patients met one of the exclusion criteria during follow-up, they were included in the study until the moment of exclusion and data were entered as censored values.

A group of 191 patients was selected according to the in- and exclusion criteria. Seventy-five patients with end-stage AMD in one eye remained free of CNV or foveal GA in their second eye during the period of follow-up at our department. Patients lost to follow-up for a period of more than 4 months were contacted by phone to determine if the second eye had developed advanced AMD during this period. The status of the second eye was verified either by inviting the patients to our outpatient clinic for clinical evaluation (if the patient reported that the second eye still had clear vision) or by contacting the patient's current ophthalmologist to request conformational data (if the patient was unable to visit our outpatient clinic or if the patient reported that the second eye was also lost to AMD). Only if patients reported that they had developed wet AMD, for which they had received intraocular injections with anti-VEGF medication, was this information considered reliable and were the patient-reported data used without further supportive evidence. An additional 77 patients were contacted for additional information about smoking history and BMI. In total, 83 patients were excluded because they could not be contacted, had passed away, were not willing to participate, or because the patients' information was inconsistent with the information available from the charts. A total of 86 patients were classified as "second eye progressors," whereas 22 patients did not develop end-stage AMD in the fellow eye during follow-up. Data of this last group were entered as censored values. Figure 1 represents a flow chart of patient inclusion. All individuals in the current study were from the Nijmegen (the Netherlands) area.

This study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Committee on Research Involving Human Subjects at the Radboud University Nijmegen Medical Centre (Nijmegen, the Netherlands) and University Eye Clinic of Cologne (Cologne, Germany).

Genetic Analysis

DNA was isolated from venous blood leukocytes and analyzed for 16 SNPs known to be associated with AMD: rs1061170/*CFH* Y402H,[6,21–23] rs10490924/*ARMS2* A69S,[7,24–26] rs4151667/*CFB* H9L,[27,28] rs9332739/*C2* E318D,[27–29] rs2230199/*C3* R102G,[27,30–33] rs10033900/*CFI*,[34,35] rs1410996/*CFH* IVS14,[16,36,37] rs2511989/*SERPING1*,[38,39] rs1883025/*ABCA1*,[40–42] rs3775291/*TLR3* L412F,[43,44] rs7412/*APOE,* E2 allele,[45–47] rs429358/*APOE,* E4 allele,[45–47] rs3764261/*CETP*,[40,41,48] rs12678919/*LPL*,[40,41,48] rs10468017/*LIPC*,[40,41] and rs174547/*FADS1_3*.[41,42] Genotyping of SNPs in all the genes analyzed except for the *CFH* variant Y402H was carried out as described elsewhere.[49] The *CFH* variant Y402H (rs1061170) was analyzed by direct sequencing of PCR products using forward primer TCATTGTTATGGTCCTTAGG and reverse primer AAAGACATGAACATGCTAGG. Polymerase chain reaction (PCR) amplification was conducted following standard protocols (primers sequences and PCR conditions available upon request). All genotyping was performed in the same lab.

Statistical Analysis

Variables were entered in a Cox regression model for survival analysis and were first analyzed in a univariate model. Statistically significant variables ($P < 0.05$) were analyzed in a multivariate model. To correct for possible confounding, important nonsignificant variables were also included in the multivariate model. For each AMD-associated SNP, a Cox regression model was made, controlling for the statistically significant baseline variables. Survival timing started at T(0), the moment of end-stage AMD in the first eye. The event was defined as the development of advanced AMD in the second eye. A two-sided *P* value of 0.05 was considered to be statistically significant. For smoking, we analyzed the total number of pack years up to T(0) and BMI was calculated at T(0). All analyses were conducted using statistical analysis software (SPSS 16.0; IBM Corporation, Armonk, NY).

Results

Analysis of Sociodemographic Factors

Mean age was 74.3 years (range 54.3–93.4; standard deviation ±7.2) in our studied cohort. There were 37 males (34.3%) and 71 females (65.7%). The type of end-stage AMD in the first eye was CNV in 82.4% and GA in 3.7% of cases. Of those who progressed toward end-stage in the fellow eye, 68.5% had a CNV and 9.3% had GA in the second eye.

Sex, age, and BMI were significantly associated with second eye progression in the univariate analysis. For pack years and education, no association could be observed. Since both factors have been implied to be involved in (second eye) progression in AMD, they were also added to the multivariate model. Table 1 shows the results from the multivariate analysis. Sex, age, pack years, and BMI were all associated with the risk of developing end-stage AMD in the second eye. For sex and pack years, these findings reached statistical significance. For age, the risk increases with each age category. Only for the two highest age categories was significance reached. Obese patients (BMI ≥30) are more at risk for second eye progression as compared with patients with normal weight (BMI 18–25; $P = 0.020$). For overweight patients (BMI 25–30), there was a trend toward an increased risk, but this did not reach a conventional level of statistical significance. This study used self-reported data on weight and height. It has been shown that people may overstate their height and underreport their weight.[50–53] This would result in an underestimation of the prevalence of obesity. It has therefore been suggested to evaluate BMI as a continuous variable rather than using predefined BMI categories.[52,53] We repeated our analyses with this modification and this did not affect our results in any way.

Table 1. Multivariate Association between Sociodemographic Risk Factors and Progression toward End-Stage AMD in the Fellow Eye of Patients with Unilateral Advanced AMD

Variable	*n* (%)	HR* (95% CI)	*P*
Sex			
Male	37 (34)	1.0	
Female	71 (66)	2.6 (1.4–5.0)	0.004
Age†			
<65	17 (16)	1.0	
65 to 70	15 (14)	1.2 (0.5–2.7)	0.704
70 to 75	23 (21)	1.5 (0.7–3.1)	0.255
75 to 80	37 (34)	2.6 (1.3–5.3)	0.010
≥80	16 (15)	5.0 (2.0–12.5)	0.001
BMI			
Normal weight (18–25)	52 (48)	1.0	
Overweight (25–30)	40 (37)	1.3 (0.8–2.1)	0.375
Obese (≥30)	16 (15)	2.2 (1.1–4.1)	0.020
PY‡			
0 to 1	45 (42)	1.0	
1 to 40	54 (50)	2.4 (1.3–4.5)	0.005
≥40	9 (8)	4.4 (1.4–14.3)	0.014
Education§			
≤ High school	59 (61)	1.0	
> High school	38 (39)	0.6 (0.4–1.1)	0.128

PY, pack years.
* Corrected for sex, age, BMI, and PY.
† Age at moment of end-stage AMD in the first eye.
‡ Total amount of pack years smoked.
§ Analysis restricted to 97 patients.

Information about education was available for all study subjects, except for 11 patients. In this study, education was not related to second eye progression, but a trend for higher education being protective was observed.

Additional analyses revealed that there were more females and more older persons in the group with 0 to 1 pack years. This explains why smoking was only significant in the multivariate analysis. With the inclusion of smoking in the multivariate analysis, the effects of age and sex also became more pronounced.

Analysis of Genetic Variants

Genotype data of the SNPs were available for at least 87% of all patients. Because of the clear stepwise increase in hazard ratios with each age category, age was entered as a continuous variable. Results are shown in Table 2. There was an increased risk for homozygous carriers of the *CFI* risk allele with a hazard ratio of 2.2 ($P = 0.028$). Heterozygous and homozygous carriers of the *LPL* risk allele had a higher hazard ratio compared with homozygous individuals for the nonrisk alleles (HR 2.0; $P = 0.036$). Other evaluated risk alleles did not make a significant contribution to development of end-stage AMD in the second eye.

To test for gene-gene interaction of the *CFH* Y402H and *ARMS2* A69S genotypes, separate analyses for each possible allele combination of these two SNPs were performed. Comparison of subjects who were homozygous for both risk alleles with subjects carrying the wild-type alleles for both genotypes yielded no significant results (results not shown

Table 2. Multivariate Association between 21 SNPs and Progression toward End-Stage AMD in the Fellow Eye of Patients with Unilateral Advanced AMD

SNP	*n* (%)	HR* (95% CI)	*P*
CFI/rs10033900			
CC	24 (24.5)	1	
CT	46 (46.9)	1.2 (0.6–2.4)	0.519
TT	28 (28.6)	2.2 (1.1–4.3)	0.028
LPL/rs12678919			
AA	88 (84.6)	1	
AG/GG	16 (15.4)	2.0 (1.0–3.6)	0.036
CFH IVS14/rs1410996			
TT	5 (4.7)	1	
CT	35 (32.7)	2.2 (0.7–6.7)	0.177
CC	67 (62.6)	2.6 (0.9–7.6)	0.081
LIPC/rs10468017			
CC	49 (49.5)	1	
CT	42 (42.4)	1.5 (0.9–2.4)	0.125
TT	8 (8.1)	0.9 (0.3–2.3)	0.760
ARMS2 S69A/rs10490924			
GG	24 (23.5)	1	
GT	49 (48.0)	0.8 (0.5–1.5)	0.565
TT	29 (28.4)	0.7 (0.4–1.4)	0.339
CFH Y402H/rs1061170			
TT	15 (14.6)	1	
TC	44 (42.7)	1.2 (0.6–2.6)	0.571
CC	44 (42.7)	1.3 (0.6–2.6)	0.476
CFB H9L/rs4151667			
TT	91 (94.8)	1	
TA/AA	5 (5.2)	1.0 (0.4–2.6)	0.966
C3 R102G/rs2230199			
CC	53 (52.0)	1	
CG	40 (39.2)	1.3 (0.8–2.0)	0.363
GG	9 (8.8)	1.4 (0.6–3.1)	0.477
C2 E318D/rs9332739			
GG	101 (95.3)	1	
GC/CC	5 (4.7)	1.1 (0.4–2.9)	0.873
SERPING1/rs2511989			
GG	36 (37.5)	1	
GA	49 (51.0)	1.1 (0.7–1.9)	0.697
AA	11 (11.5)	0.9 (0.4–2.0)	0.801
TLR3 L412F/rs3775291			
CC	49 (48.0)	1	
CT	40 (39.2)	1.0 (0.6–1.6)	0.878
TT	13 (12.7)	0.8 (0.4–1.8)	0.658
APOE4/rs429358			
TT	75 (79.8)	1	
TC/CC	19 (20.2)	1.1 (0.6–1.9)	0.736
APOE2/rs7412			
CC	82 (82.8)	1	
CT/TT	17 (17.2)	0.8 (0.4–1.6)	0.596
CETP/rs3764261			
CC	42 (39.6)	1	
CA	46 (43.4)	1.1 (0.7–1.8)	0.680
AA	18 (17.0)	0.9 (0.5–1.7)	0.724
FADS1_3/rs174547			
TT	50 (47.2)	1	
TC	48 (45.3)	1.2 (0.8–2.0)	0.343
CC	8 (7.5)	1.4 (0.6–3.2)	0.490
ABCA1/rs1883025			
CC	67 (63.2)	1	
CT/TT	39 (36.8)	0.8 (0.5–1.3)	0.355

Genotype data of the analyzed SNPs were available for at least 87% of all patients.

* Corrected for sex, age, BMI, and pack years.

here). Since the *LPL* and *CFI* SNPs were significantly related to second eye progression, gene-gene interaction between these two genotypes was also analyzed. Subjects with two or more risk alleles had a higher risk of second eye progression (HR 2.7; 95% CI 1.3–5.6; $P = 0.008$) when compared with subjects with no risk alleles. No interaction was found between the *LPL* and *CFI* genotypes.

Discussion

The primary goal of our study was to determine sociodemographic, behavioral, and genetic risk factors that contribute to the progression toward end-stage AMD in the fellow eyes of patients who already have developed end-stage AMD in their first eye. Our results show that the evaluated baseline characteristics of sex, age, smoking status, and to a lesser extent BMI, all individually contribute to second eye progression. Of the genetic risk alleles that were analyzed, *CFI* (rs10033900) and *LPL* (rs12678919) were found to confer an increased risk for second eye progression.

There is only one other study that reported a significantly higher risk of progression to bilateral advanced AMD for higher age, current smoking, and higher BMI.[16] Other studies have not reported these associations, although trends have been observed.[12,15,17] The *CFH* Y402H (rs1061170), *ARMS2* A69S (rs10490924), *C3* R102G (rs2230199), and *C2* E318D (rs9332739) risk alleles were also reported to have an influence on the progression of the fellow eye to end-stage AMD.[15,16] Sex has not previously been associated with second eye progression to advanced AMD.

One possible explanation for the differences between the studies previously performed as well as between these studies and our study might be found in the different criteria used for inclusion. The submacular surgery trials (SST) group could not find any significant contribution of age, sex, or smoking history.[17] Only patients with a CNV in their first eye were included in the SST study, regardless of the presence of foveal GA. This could have resulted in the inclusion of patients who already had preexisting foveal GA before the CNV occurred. In our study, these patients would have been included at the time foveal GA developed and therefore would have a longer progression time. Another study did not only include patients with new CNV, but also patients with recurrent CNV, which also influences the progression time.[12] In this study, no statistically significant differences or trends were found for sex and cigarette smoking.[12] However, this study showed a strong trend for age to influence the incidence of CNV in the fellow eye.[12]

Remarkably, this study demonstrates a relation between sex and second eye progression not previously observed. However, a recent meta-analysis on AMD prevalence shows that the prevalence of late AMD is higher in women compared with men.[54] More specifically, the diagnosis of neovascular AMD occurred more frequently in women compared with men, while for GA, no specific sex difference was observed.[54] We are not the first to observe a possible effect of sex on progression in AMD, despite the fact that we are dealing with a relatively small sample size, which may affect the validity of this outcome. Moreover, population differences may further contribute to the observed inconsistencies between studies.

Education was not related to second eye progression in the current study. However, there appears to be a trend for higher education to be protective. This trend is in line with other studies that have associated education with AMD and AMD progression.[2,16]

Two recent studies showed an association of the *CFH* Y402H, *ARMS2* A69S, *C3* R102G, and *C2* E318D risk alleles

Table 3. Comparison of Multivariate Associations between Genetic Risk Factors and Second Eye Progression to End-Stage AMD or Incident Bilateral Advanced AMD

	Our Study		Seddon (2009)		Seddon (2007)	
Genetic Risk Factors	**HR* (95% CI)**	***P***	**OR† (95% CI)**	***P***	**OR† (95% CI)**	***P*‡**
CFH Y402H/rs1061170						
TT	1		1		1	
TC	1.2 (0.6–2.6)	0.571	1.1 (0.6–2.1)	0.77	1.5 (0.8–2.7)	
CC	1.3 (0.6–2.6)	0.476	1.5 (0.7–3.1)	0.33	2.3 (1.3–4.2)	
ARMS2 S69A/rs10490924						
GG	1		1		1	
GT	0.8 (0.5–1.5)	0.565	2.0 (1.2–3.3)	0.007	2.5 (1.5–4.1)	
TT	0.7 (0.4–1.4)	0.339	4.6 (2.6–8.2)	<.001	5.4 (3.0–9.7)	
C3 R102G/rs2230199						
CC	1		1			
CG	1.3 (0.8–2.0)	0.363	1.6 (1.0–2.4)	0.044		
GG	1.4 (0.6–3.1)	0.477	1.6 (0.7–3.6)	0.24		
C2 E318D/rs9332739						
GG	1		1			
GC/CC	1.1 (0.4–2.9)	0.873	0.2 (0.1–0.8)	0.021		
CFI/rs10033900						
CC	1					
CT	1.2 (0.6–2.4)	0.519				
TT	2.2 (1.1–4.3)	0.028				
LPL/rs12678919						
AA	1					
AG	2.1 (1.1–3.9)	0.027				
GG	1.1 (0.1–8.7)	0.914				

* Corrected for sex, age, BMI, and pack years.
† Adjusted for age, sex, education, smoking, baseline AMD grade, BMI, treatment groups, and six genetic variants and associated genotypes.
‡ *P* values not mentioned in article.

with progression toward bilateral end-stage AMD.[15,16] We could not confirm this, although for the *C3* R102G (rs2230199) risk allele, our hazard ratios are comparable to the odds ratios found by Seddon in 2009.[16] Table 3 compares the results of our SNP analyses and the two studies by Seddon et al.

A possible explanation for these differences may be due to different definitions used. In both studies, progressors were defined as patients with early or intermediate AMD at baseline who progressed toward end-stage AMD as well as patients with end-stage AMD in one eye at baseline who progressed toward end-stage AMD in the fellow eye.[15,16] Only the latter group is comparable to our second eye progressors. The subcategory "bilateral progressors" included patients without advanced AMD at baseline, progressing toward end-stage AMD in both eyes, as well as those with end-stage AMD in one eye at baseline who progressed toward end-stage AMD in the fellow eye. Again, this definition is broader than the definition of second eye progressors employed in the current study. Another difference is that in these studies, the progressors were compared with a group of nonprogressors with regard to genotype and sociodemographic risk factors,[15,16] whereas we started with a group of progressors (the first eye had already progressed towards end-stage AMD) and searched for second eye progressors within this high-risk group.

The implications of these differences become clear if we look at the distribution of the risk alleles of the genotypes across the study groups. In the aforementioned studies, 43% to 44% of the progressors carried the *CFH* Y402H risk allele homozygously, compared with 23% to 24% of the nonprogressors. For *ARMS2* A69S, this was 25% to 27% vs. 9%; and for *C3* R102G, 9% vs. 5%.[15,16] Since all of the subjects in our study are progressors (they all have end-stage AMD in at least one eye), they have the same genotype distribution (see Table 2) as those in the progressor groups in the studies above, showing the homogeneous character of our study group. This suggests that these SNPs influence disease progression only at an earlier stage of the disease. Furthermore, only one paper reported an association between the *CFH* Y402H genotype and the incidence of bilateral advanced AMD[15]; in the other paper, this association could not be confirmed.[16] We do realize, however, that we have a relatively small sample size, and that we cannot exclude that the *CFH* Y402H and *ARMS2* A69S genotypes have an influence on second eye progression. To test this hypothesis, these findings should be replicated in a larger and independent cohort.

In our study, we also looked at SNPs that have not previously been investigated for their relationship with second eye progression toward end-stage AMD. By exploring new candidate SNPs, we found new and interesting associations. The *CFI* (rs10033900) and *LPL* (rs12678919) risk alleles were associated with second eye progression. The *CFI* gene encodes complement factor I, one of the complement pathway regulatory proteins involved in the cleavage of C3b.[34,55,56] The complement system plays a major role in the pathogenesis of AMD and these findings suggest that the complement system may also play a role in the progression of earlier-stage AMD toward end-stage AMD in the second eye.[55] The lipoprotein lipase (*LPL*) gene is involved in the high density–lipoprotein metabolism (HDL metabolism) and is associated with a decrease in HDL-c levels in blood.[40,48] Besides its role in the pathogenesis and progression of AMD,[40,48] our findings suggest that HDL metabolism may also play a role in second eye progression in AMD.

Limitations

Because of the relatively small sample size, we were not able to correct for multiple testing and cannot rule out that some of

the correlations we found are based on coincidence. Therefore, our findings need to be confirmed in future studies with a larger sample size.

Because of the small number of patients, it was not possible to perform additional subanalyses with regard to different phenotypic characteristics. It would, for example, be interesting to evaluate the influence of the type of end-stage in the first eye (GA or CNV) on second eye progression. The presence of reticular macular disease would also be an interesting feature to look at. Reticular pseudodrusen are best visible on autofluorescence and infrared images[57]; however, most patients were collected in a time when these imaging modalities were not used in a standard examination setting. These points will be addressed in future studies with larger cohort sizes and more advanced imaging modalities.

A relatively large group of patients was excluded from this study after initial selection. To exclude the possibility that we were dealing with a biased sample, we used multiple imputation analysis for missing values to compare the included and excluded groups. Pooled results showed that the patients in the excluded group were slightly older and that there were slightly more nonsmokers as well as heavy smokers in the excluded group. For all significant variables, we therefore repeated our analyses with the excluded and included group combined using the imputated data for the excluded group. This did not lead to any significant changes in the results, suggesting that our findings are not affected by selection bias.

Conclusions

The findings of this study give us further insight into the progression of AMD, and they can be a guideline for preventive measures to decrease the risk of second eye progression toward end-stage AMD. By reducing modifiable risk factors such as smoking and BMI, patients may be able to influence their individual risk for progression, thereby preserving their remaining eyesight for a longer period. It would also be possible to select those patients who are at high risk for second eye progression, based on their genetic profile, for future therapeutic trials in research.

The goal of this study was to identify risk factors for the progression of AMD toward end-stage in the fellow eye of patients who had already developed end-stage AMD in their first eye. We found that female sex, age, pack years, and BMI $\geq$ 30 contribute to this second eye progression. In addition, we have comprehensively investigated the most important AMD-associated SNPs for their relationship with second eye progression to end-stage AMD. In this study, the *CFH* Y402H and *ARMS2* polymorphisms do not play a role in the second eye progression of AMD. The *LPL* and *CFI* risk alleles turned out to be genetic predictors for second eye progression. However, these results were observed in the context of a relatively small sample size, and should therefore be replicated in a larger and independent cohort.

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