Published in final edited form as:

Ophthalmology. 2010 March ; 117(3): 500–511. doi:10.1016/j.ophtha.2009.08.032.

The Complement Component 5 gene and Age-related Macular Degeneration

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Abstract

Objective—To investigate the association between variants in the complement component 5 (C5) gene and age-related macular degeneration (AMD).

Design—Separate and combined data from three large AMD case-control studies and a prospective population-based study (The Rotterdam Study).

Participants—A total of 2599 AMD cases and 3458 ethnically matched controls.

Methods—Fifteen single nucleotide polymorphisms (SNPs) spanning the C5 gene were initially genotyped in 375 cases and 199 controls from the Netherlands (The AMRO-NL study population). Replication testing of selected SNPs was performed in the Rotterdam Study (NL) and study populations from Southampton, United Kingdom (UK) and New York, United States (US).

Main Outcome Measures—Early and late stages of prevalent and incident AMD, graded according to (a modification of) the international grading and classification system of AMD.

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Results—Significant allelic or genotypic associations between eight *C5* SNPs and AMD were found in the AMRO-NL study and this risk appeared independently of *CFH* Y402H, *LOC387715* A69S, age and gender. None of these findings could be confirmed consistently in three replication populations.

Conclusions—Although the complement pathway, including *C5*, plays a crucial role in AMD, and the *C5* protein is present in drusen, no consistent significant associations between *C5* SNPs and AMD were found in all studies. The implications for genetic screening of AMD are discussed.

Introduction

Age-related macular degeneration (AMD) is the leading cause of severe visual impairment in the elderly in the Western world and is therefore a major public health issue.^{1,2} Typically, AMD is classified in early and late forms. Early AMD is characterized by the presence of soft drusen and pigmentary changes in the macular area. Late AMD is further divided into a “dry” form, geographic atrophy (GA), and a “wet” form, choroidal neovascularization (CNV). The overall prevalence of early and late AMD among Americans over 40 years of age is estimated to be respectively 7.3 million and 1.8 million. The prevalence of late AMD is estimated to increase to 3.0 million in 2020.^{1,2} AMD has a multifactorial etiology. Both environmental and genetic factors contribute to disease susceptibility.³ Environmental risk factors include cigarette smoking, undue sunlight combined with low antioxidant dietary intake, insufficient physical activity and poor cardiovascular health. ⁴ Molecular studies have been successful in dissecting the genetic susceptibility for AMD in the last few years. Genetic association studies for at least four loci (complement factors H (*CFH*), B (*CFB*), 2 (*C2*), 3 (*C3*)), and the *LOC387715* (*HTRA1/ARMS2*) locus suggested that the complement system and, possibly, oxidative stress pathways play a major role in the molecular pathology of AMD.⁵⁻¹³ Most importantly, at least 74% of AMD cases can be explained by polymorphisms in *CFH*, *CFB* and *C2* genes.⁷⁻¹⁰

In general, the complement system plays a highly effective role in destructing invading microorganisms and in immune complex elimination. The three activation pathways of complement are the classical, the alternative and the mannose-binding lectin pathway. *CFH* and *CFB* are key components of the alternative complement pathway and *C2* plays a role in the classical pathway. The three pathways converge where *C3*-convertase cleaves and activates component *C3*, creating *C3a* and *C3b*. Binding of the latter increases pathogen-immune complex clearance and initiates the formation of the lytic membrane attack complex (MAC), consisting of *C5b*-*C9*.^{14,15} Complement component 5 (*C5*) is cleaved by *C5*-convertase into the anaphylatoxin *C5a* and *C5b*, with *C5a* being an effective leukocyte chemoattractant and a powerful inflammatory mediator.¹⁶ Next to the suggestion that AMD may be a result of local activation of the alternative pathway provoked by chronic inflammatory processes, Scholl et al (2008) showed that genetic variants of *CFH* could lead to systemic activation of the complement pathway, and thus implying AMD as a systemic disease with a local disease manifestation.¹⁷

Studies on the molecular composition of drusen have also implicated the complement system in AMD. More specifically, *CFB* and *C5* proteins were found in drusen, whereas *CFH*, *C3* and MAC (*C5b*-9) protein complexes were detected in both basal laminar deposits (BLD) and drusen.^{8,18-26} Recently, Nozaki et al (2006) observed immunolocalization of *C3a* and *C5a* in both hard and soft drusen.²⁶ Preliminary genetic association studies for *C3* and *C5* have, so far, implicated a role for *C3* in the pathogenesis of AMD, but not for *C5*.^{11,13}

In summary, based on the crucial role of *C5* in the formation of the MAC complex of the complement system, its role as chemoattractant regulating local inflammatory processes, and its localization in drusen, we hypothesized that genetic variants in *C5* mediate AMD-

susceptibility. Therefore, we performed an extensive association analysis to test whether complement C5 gene variants are associated with AMD.

Methods

Cases and controls

Altogether, we utilized three case-control studies and one prospective, population-based study, consisting of 2599 AMD cases and 3458 ethnically- and age-matched control subjects. All studies were approved by the Ethics Committees of the Academic Medical Center Amsterdam, the Erasmus Medical Center Rotterdam, Southampton Local Research Ethics Committee (approval no. 347/02/t) and the Institutional Review Board of Columbia University. All studies followed the tenets of the Declaration of Helsinki. All participants provided signed informed consent for participation in the study.

The initial population screened for C5 variants, the AMRO-NL study population, consisted of 375 unrelated individuals with AMD and 199 control individuals. All subjects were Caucasian and recruited from the Netherlands Institute of Neuroscience (NIN) Amsterdam and Erasmus University Medical Centre Rotterdam, by newsletters, via patient organizations, and nursing home visits. Controls were at least 65 years old, and were usually unaffected spouses or non-related acquaintances of cases or individuals who attended the ophthalmology department for reasons other than retinal pathology.

The second population, the Rotterdam study is a prospective, population-based study of chronic diseases in the elderly.²⁷ The eligible population comprised all 10 275 inhabitants aged 55 years or older of a middle-class suburb of Rotterdam, the Netherlands, of whom 7983 (78%) participated. Because the ophthalmologic part of the study became operational after the pilot phase of the study had started, 6780 (66%) took part in the ophthalmic examinations. Baseline examinations, including a home interview and physical examinations at the research center, took place from 1990 until 1993 and were followed by three examinations from 1993 through 1994, from 1997 through 1999, and from 2000 through 2004.

The third population, the United Kingdom (UK) study population, consisted of 564 cases and 640 age matched control subjects from the same clinic population as described elsewhere.^{28, 29}

The fourth population, the Columbia University, United States (US) study population, consisted of 644 unrelated individuals with AMD and 368 unrelated controls of European American descent, recruited at Colombia University as previously described.³⁰

Diagnosis of AMD

Study subjects from the AMRO-NL, Rotterdam and US study population underwent ophthalmic examination and fundus photography covering a 35° field centered on the macula after pupil dilatation at each visit (Topcon TRV-50VT fundus camera, Topcon Optical Co, Tokyo, Japan). Signs of AMD in those study populations were graded according to (a modification of) the international classification and grading system for AMD.³¹ Cases and controls from the UK study were classified as having or not having disease on the basis of the Age-Related Eye Disease Study (AREDS) classification system.³² While AREDS uses slightly different criteria for classification of early AMD and GA, the grading criteria were very similar for the four studies. Controls showed no, or less than five small hard drusen, and no other macular pathology. Early AMD cases had either soft distinct drusen with pigmentary irregularities or soft indistinct drusen without pigmentary irregularities and soft indistinct drusen with pigmentary irregularities. Late AMD cases presented with geographic atrophy (GA), choroidal neovascularization (CNV), or a combination of both (mixed AMD).

SNP selection

Fifteen SNPs were selected to span and tag the entire *C5* gene.

SNP data were used from the Centre d'Étude du Polymorphisme Humain (CEPH) population (Utah residents with ancestry from northern and western Europe) by use of the International HapMAP Project. Available at: <http://www.hapmap.org/>, NCBI build 36, dbSNP b126. Accessed January 7, 2009. SNP selection was based on criteria like functional relevance, minor allele frequency (MAF)>10%, coverage of the main linkage disequilibrium (LD) blocks and tagging of the most common haplotypes. Tag SNPs were selected by use of Tagger, an option of Haploview³³ (all SNPs were captured with a LD tagging criteria of $r^2>0.8$).

Genotyping

Genomic DNA was isolated from peripheral leukocytes after venous puncture according to standard protocols. The AMRO-NL study population was genotyped using an Illumina GoldenGate assay on a BeadStation 500 GX (Illumina Inc., San Diego, CA, US).

The Rotterdam Study was genotyped with the Illumina HumanHap 550K array (Illumina Inc., San Diego, CA, US). Quality control was performed using PLINK (version 1.01).^{34,35} The UK study population was genotyped by the KASPar SNP genotyping system. Available at: <http://www.kbioscience.co.uk> (KBiosciences, Unit 7, Maple Park, Essex Road Hoddesdon, Herts, UK). Accessed December, 2008. The US study population was genotyped with TaqMan SNP Genotyping Assays performed on ABI 7300 Real-Time PCR systems (Applied Biosystems, Foster City, California, US) according to the supplier's recommendations. Genotyping data from the Rotterdam study, and the UK and US study populations were used as source of replication.

Statistical analysis

Baseline characteristics of cases and controls were compared using the independent samples T-test for continuous variables and χ^2 test statistic for categorical variables.

The χ^2 test was also employed to test SNP distributions for conformity with Hardy—Weinberg equilibrium (HWE), which was present if the observed homozygote and heterozygote frequencies did not differ significantly ($p<0.05$) from the expected frequencies. Allele frequencies between cases and controls were compared using the Fisher's exact test statistic. Odds ratios (ORs) and 95% confidence intervals (CI) for the risk of early and late AMD were estimated with logistic regression analysis with the major alleles as reference. For the AMRO-NL study population and the Rotterdam study, all ORs were adjusted for age and gender. Interaction with the eight associated *C5* SNPs, found in the AMRO-NL study, on early and late AMD was determined for *CFH* Y402H, *LOC387715* A69S, age and gender. All analyses were performed in SPSS for windows (release 16.0; SPSS, Inc). In the Rotterdam study, association testing was performed using logistic regression in the PLINK software package (version 1.01).^{34,35}

Results

The AMRO-NL study population: association between *C5* SNPs and AMD

Baseline characteristics of the cases and controls of the AMRO-NL study population are given in Table 1. Early AMD was found in 24.8% of all AMD subjects, whereas wet AMD, GA and mixed AMD were found in 50.4, 14.7 and 10.1% respectively. The age distribution was significantly different between cases and controls, which was corrected for in the logistic regression model. The distribution of gender and smoking were not significantly different. For both early and late AMD, we also calculated OR's with logistic regression using only the 70

+ controls as reference. After all, 65+ controls may be too young and develop late AMD in the future. However, using only 70+ controls did not essentially change the OR's (data not shown).

Initially, 15 SNPs spanning the *C5* gene (Figure 1a) were genotyped in 375 unrelated AMD patients and 199 controls of the AMRO-NL study population. The LD plot and the distinct haplotype blocks for the 15 selected SNPs, as generated by Haploview, are presented in Figure 1b³³. Corresponding LD scores (D' and R^2) for each selected marker of the *C5* gene with a MAF>10% are also given (Figure 1c).

All genotype frequencies of the controls followed HWE (Table 2), except for one SNP (rs7027797; $P=0.04$). However, HWE calculations for this SNP may not be completely accurate since one of the genotype groups contains less than five individuals and as multiple HWE tests were conducted this may constitute a false positive finding.^{36,37} We therefore left rs7027797 in the analysis.

Allelic P -values and genotype distributions of the association analyses are presented in Table 2. We found significant allelic associations between AMD and eight out of 15 *C5* SNPs distributed over three LD blocks. The strongest allelic associations were found for rs17611, rs7026551 and rs7037673 (respectively: $P=0.0015$, $P=0.0065$ and $P=0.0009$). The corresponding ORs for these eight *C5* SNPs in early or late AMD, adjusted for *CFH* Y402H, age and gender, are given in Table 3.

In the early AMD group, we observed association for three SNPs, which are in complete LD (Figure 1c): heterozygous carriers of rs2269066 and rs7026551 had a significantly increased risk (respectively OR 2.20; 95%CI 1.12-4.38) and (OR 1.75; 95%CI 1.01-3.06) compared to the wild type genotype. For rs7037673, we observed a protective effect for the homozygotes (OR 0.34; 95%CI 0.15-0.77).

In the late AMD group SNPs, we observed protective effects for five SNPs, four of which occur (also) in heterozygous genotypes: heterozygotes for rs17611, rs25681 and rs7040033 had lower risks for late AMD compared to the other genotypes. Rs7037673 also showed a protective effect for late AMD but a lower risk was seen in the homozygotes for the minor allele of this SNP. The ORs for the heterozygotes were respectively 0.53 (95%CI 0.34-0.82), 0.54 (95%CI 0.35-0.84), 0.63 (95%CI 0.41-0.96) and 0.58 (95%CI 0.37-0.90). These 4 SNPs, while tagging 3 different haplotype blocks (Figure 1b and 1c), most likely identify the same protective effect, since they are in almost complete LD ($D'>0.92$). Homozygotes for the minor alleles of rs1468673 showed the lowest statistically significant protective effect for late AMD.

Interaction studies of the eight significantly associated *C5* SNPs found in the AMRO-NL study with 4 prominent AMD risk factors: *CFH* Y402H, *LOC387715* A69S, age and gender, did not yield significant interaction for one of the *C5* SNPs with either risk factor. This implies that these risk factors did not modify the relation of one of the *C5* SNPs with AMD and that *C5*, *CFH* Y402H, *LOC387715* A69S, gender and age are independent risk factors for AMD. Based on the observed allelic and genotypic associations for some of the SNPs in the *C5* gene with early and late AMD (Table 2 and 3) replication analyses were performed in one other Dutch population, one UK population and one US population.

The Rotterdam, UK and US replication populations: replication does not confirm association

Baseline characteristics of the cases and controls of the Rotterdam, UK and US replication populations are given in Table 1. In all replication populations, the distribution of age was significantly different between cases and controls. In the AMRO-NL and Rotterdam study we used logistic regression to corrected for this. In the other replication populations (UK and US), this may have reduced power by potentially diluting the control sample with (as yet

undeveloped) cases. This discrepancy would therefore be expected to make the strength of findings more conservative since any observed association tests would be an underestimation.

Three *C5* SNPs, which were associated with AMD in the AMRO-NL study population, were selected for further screening in the Rotterdam Study: rs17611, rs7026551, and rs7037673. These three gene variants spanned two different haplotype blocks of the *C5* gene, and were not in complete LD with each other (Figure 1b and 1c). The results of the replication screening of the three *C5* SNPs in early and late AMD cases of the Rotterdam population are presented in Table 4a. Genotypes frequencies for all SNPs followed HWE (data not shown). None of the significant associations found in the AMRO-NL study population could be independently confirmed for these three SNPs.

Two SNPs, rs17611 and rs7037673, which were already screened in both the AMRO-NL and Rotterdam populations, were also screened in the two (case-control) studies from the UK and the US. The results from the genotype analysis in the cases and controls are shown in Table 4b and 4c. Again, all the genotype frequencies followed HWE (data not shown) but no significant associations between the SNPs and AMD were found.

Data pooling of four study populations

Pooling of populations with the same genetic background—Pooling the data of the two Dutch study populations (still) resulted in significantly decreased ORs for carriers of the minor allele of rs17611 and rs7037673 (Table 5).

We observed a protective effect for the heterozygous genotype for both SNPs with ORs of respectively 0.74 (95%CI 0.59-0.92) and 0.79 (95%CI 0.64-0.99). We also observed a protective effect for homozygotes for the minor alleles of rs7037673 with an OR of 0.67 (95% CI 0.49-0.93).

Pooling of populations with the same study design—Pooling the data from the Dutch, UK and US case-control studies did not lead to significant associations for either rs17611 or rs7037673 (Table 6).

Finally, combining all data from the four studies did not result in statistically significant association (data not shown).

Discussion

We tested the association between complement *C5* gene variants and AMD in four independent studies (three case-control and one prospective, population-based study), consisting of 2334 AMD cases and 3460 ethnically matched controls. Despite the established involvement of the complement system, including *C5*, in AMD, and *C5* protein localization in drusen,^{7-11,13,18, 21,25} we were unable to find consistent association between common SNPs in *C5* and AMD in all study populations.

Our data confirms the preliminary data of Yates et al. (2007) who did not find association between *C5* SNPs (rs17611, rs7026551 and rs7033790) and AMD.¹³ This study also corroborates previous findings that replication of initial associations found in one or more cohort(s), even when it involves very plausible candidate genes, is essential to determine true genetic disease susceptibility.

A limitation of the current study is that we initially selected for relatively common SNPs, with MAFs>10%, in order to haplotype-tag the whole coding region of the *C5* gene. Consequently, we could have missed both variants with MAFs<10% and outside the coding region which

might influence the disease phenotype. Strengths of the study include the large number of AMD cases and controls screened across populations of European descent, and the relatively uniform clinical classification of the cases and controls.

Data pooling of four cohorts

After our initial finding of a statistically significant association between several SNPs in *C5* and AMD in the AMRO-NL study population, we extended our study with three other independent cohorts. Although the AMRO-NL study population and the Rotterdam Study have a different design, the populations have the same genetic background. Therefore, we pooled data from these two populations first. Pooling the data resulted in decreased, but still significant ORs for carriers of the minor allele of rs17611 and rs7037673 (Table 5). The effect seen may be largely attributable to the findings in the AMRO-NL study population. Nonetheless, the data of the two populations individually did not contradict each other; i.e., the CIs of the associated genotypes overlapped substantially (Table 3, 4a,5). Even more interestingly, the homozygote minor alleles of rs17611 did not show a protective effect for “late AMD” in both populations if analyzed individually, but almost reached statistical significance (OR 0.76 with 95% CI 0.56-1.02) in the pooled data set (Table 5). The potential AMD risk modifying effect of the rs7026551 allele (AMRO-NL) disappeared in the pooled data set.

Next, we pooled the AMRO-NL, UK and US data (Table 6), since these studies, despite their different genetic backgrounds, have the same case-control study design. Then, we also added the Rotterdam population data again. Pooling the data in these ways abolished all associations that were initially found in the AMRO-NL study population.

C5 variants and AMD

The complement factor 5 is very likely involved in processes leading to AMD, since it is a key component of the (alternative) complement system and is present in drusen.^{18,21,25} Nevertheless, we could not find robust, consistently statistically significant, association between *C5* SNPs and AMD among the populations tested. How can this be explained?

The simplest explanation is that the findings in the AMRO-NL population are due to chance, and that *C5* gene variants do not contribute at all to genetic susceptibility for AMD. In other words, the screened *C5* sequence variants may result in a (mildly) altered *C5* function but, these possibly functional changes have no role in the etiology of AMD even if they regulate, limit or alter the total activity of the complement cascade.

But is it that simple? If our findings are *not* due to chance (alone), we find a possible protective effect for the *C5* variant rs17611 in the Dutch population, which cannot be replicated in two UK or USA populations. Interestingly, Hillebrandt et al (2005) and Gressner et al (2007) found that this same rs17611 variant is both possibly associated with elevated *C5* and Gc globulin serum concentrations in man, and with enhanced liver fibrosis in mice.^{38,39} While, to our knowledge, further experimental evidence is lacking, one could speculate that elevated *C5* serum levels *increase* the risk for AMD through further activation of the complement pathway. In contrast, increased fibrosis capabilities, if it occurs in the eye also, could perhaps *decrease* the risk of advanced AMD, by accelerating scar formation after retinal injury. In conclusion, these data suggest that *C5* may have multiple roles in AMD pathology, thereby complicating potential correct risk assessment of *C5* sequence variants.

Non-replication of C5 variants

Whether *C5* SNPs are associated with AMD in the Dutch population, or not, our current study presents another example where initial significant associations found in one study cannot be replicated in others. So far, only very strong AMD risk factors such as *CFH*^{7,9,10} or *ARMS2*/

HTRA1^{6,12} have been consistently replicated. These “strong” risk factors are rather an exception than rule. Initial associations between “weaker” AMD risk factors, such as the *TLR4* gene,⁴⁰ the *SERPING1* gene,²⁸ the *TLR3* gene⁴¹ and even the *ApoE2* and *4* alleles^{42, 43} could not be replicated in several other case-control cohorts.⁴⁴⁻⁴⁷ Also, in a GWAS on the AREDS study, only 1 (rs2230199; *C3*) out of 57 initially AMD associated SNPs was replicated in a second case-control cohort.⁴⁵ Since most investigators or scientific journals are reluctant to publish negative associations, most non-replication studies are never published.

Non-replications in different populations have been frequently explained by chance, variation in study design, by phenotypic AMD (grading) differences, genotype errors^{48,49} or by genetic variability between populations.³⁶ In our current *C5* association study, we used two different study designs which may have affected the outcome in the individual studies. Phenotypic grading differences may not account for a very large variability in our study, since the fundi of all patients and controls were graded according to (a modification of) the international classification system of AMD^{31,32} under supervision of the same ophthalmologists (CK, PdJ) in three out of four populations. Moreover, Colhoun and coworkers (2003) calculated that 5% clinical misclassification has only a small effect, comparable to a reduction of the sample size by 10%.⁵⁰ Potential genotype errors in our study are also unlikely, since both positive and negative associations between *C5* variants and AMD are confirmed by at least one second variant in LD with the first one.

Finally, association studies in populations that are genetically heterogeneous can yield large numbers of spurious associations if population subgroups are unequally represented among cases and controls. Especially case-control designs have been described as being susceptible to population stratification when population subtypes show variation in their baseline disease risk (the risk not attributable to the gene of interest) and evident heterogeneity in allele frequency for the candidate gene studied. While all participants in our study were of European descent, we did observe marked variation in MAFs of almost all *C5* SNPs between the populations. For example, in controls, we found MAFs for rs7037673 varying from 0.38 (UK) to 0.45 (NL). In the AMD cases, we found for the same SNP MAFs of, respectively, 0.35 (NL) to 0.46 (US). Family-based association studies have the advantage that they are less prone to the effect of population structure because of matched genetic background among study participants. Unfortunately, due to the lack of family data from all the case-control populations used here, we cannot correct for this phenomenon.

Interestingly, there are at least two (other) marked examples in which geographically determined genetic variation was clearly implicated in differences in disease susceptibility: the *CFH* Y402H variant confers a very strong risk for AMD in western populations, while it has only a marginal effect in Chinese⁵¹ or Japanese.^{52,53} In addition, The Wellcome Trust Case Control Consortium⁵⁴ suggested, on the basis of a GWA of 14,000 cases and 3000 shared controls, that geographic differences in *TLR1* receptor allele frequencies within the UK coincide with natural selection and susceptibility against *TLR1* mediated (auto-) immune disease.

Apart from differences in *C5* MAF, population-specific genetic variations in one or more other genes of the complement pathway and/or the fibrosis pathways may also affect AMD disease outcome. These variants may determine the capability of *C5* SNPs to influence or regulate the activity of the complement cascade or fibrotic pathways and thus determine their potential effect on the AMD phenotype.

In conclusion, common SNPs in the *C5* gene confer either no, or limited risk for AMD, which may be dependent on genetic differences between populations. While, given its crucial role in the complement cascade, and the presence of *C5* protein in drusen, *C5* is very likely involved

in AMD. The tested genetic variation in *C5* has a very limited, if any, effect on the AMD phenotype.

Acknowledgments

Financial Support This study was in part financed by an unrestricted research grant from Merck Sharpe and Dohme and the Nederlandse Vereniging ter Voorkoming van Blindheid (both to A.A.B.)

The generation and management of GWAS genotype data for the Rotterdam Study is supported by the Netherlands Organisation of Scientific Research NWO Investments (nr. 175.010.2005.011, 911-03-012). This study is funded by the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO) project nr. 050-060-810, and funding from the Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam.

The US study is supported in part by the grants from the National Eye Institute EY13435 and EY017404; the Macula Vision Research Foundation; Kaplen Foundation; Wigdeon Point Charitable Foundation and an unrestricted grant to the Department of Ophthalmology, Columbia University, from Research to Prevent Blindness, Inc.

References

1. Eye Diseases Prevalence Research Group. Causes and prevalence of visual impairment among adults in the United States. *Arch Ophthalmol* 2004;122:477–85. [PubMed: 15078664]
2. Eye Diseases Prevalence Research Group. Prevalence of age-related macular degeneration in the United States. *Arch Ophthalmol* 2004;122:564–72. [PubMed: 15078675]
3. de Jong PT. Age-related macular degeneration. *N Engl J Med* 2006;355:1474–85. [PubMed: 17021323]
4. Coleman HR, Chan CC, Ferris FL III, Chew EY. Age-related macular degeneration. *Lancet* 2008;372:1835–45. [PubMed: 19027484]
5. Chen W, Xu W, Tao Q, et al. Meta-analysis of the association of the *HTRA1* polymorphisms with the risk of age-related macular degeneration. *Exp Eye Res*. In press.
6. Dewan A, Liu M, Hartman S, et al. *HTRA1* promoter polymorphism in wet age-related macular degeneration. *Science* 2006;314:989–92. [PubMed: 17053108]
7. Edwards AO, Ritter R III, Abel KJ, et al. Complement factor H polymorphism and age-related macular degeneration. *Science* 2005;308:421–4. [PubMed: 15761121]
8. Gold B, Merriam JE, Zernant J, et al. AMD Genetics Clinical Study Group. Variation in factor B (*BF*) and complement component 2 (*C2*) genes is associated with age-related macular degeneration. *Nat Genet* 2006;38:458–62. [PubMed: 16518403]
9. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science* 2005;308:419–21. [PubMed: 15761120]
10. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science* 2005;308:385–9. [PubMed: 15761122]
11. Maller JB, Fagerness JA, Reynolds RC, et al. Variation in complement factor 3 is associated with risk of age-related macular degeneration. *Nat Genet* 2007;39:1200–1. [PubMed: 17767156]
12. Yang Z, Camp NJ, Sun H, et al. A variant of the *HTRA1* gene increases susceptibility to age-related macular degeneration. *Science* 2006;314:992–3. [PubMed: 17053109]
13. Yates JR, Sepp T, Matharu BK, et al. Genetic Factors in AMD Study Group. Complement C3 variant and the risk of age-related macular degeneration. *N Engl J Med* 2007;357:553–61. [PubMed: 17634448]
14. Walport MJ. Complement. First of two parts. *N Engl J Med* 2001;344:1058–66. [PubMed: 11287977]
15. Walport MJ. Complement. Second of two parts. *N Engl J Med* 2001;344:1140–4. [PubMed: 11297706]
16. Gerard C, Gerard NP. C5A anaphylatoxin and its seven transmembrane-segment receptor. *Annu Rev Immunol* 1994;12:775–808. [PubMed: 8011297]

17. Scholl, HP.; Issa, P Charbel; Walier, M., et al. Systemic complement activation in age-related macular degeneration; PLoS One [serial online]. [Accessed July 31, 2009]. 2008 p. e2593 Available at: <http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0002593>
18. Anderson DH, Mullins RF, Hageman GS, Johnson LV. A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol* 2002;134:411–31. [PubMed: 12208254]
19. Crabb JW, Miyagi M, Gu X, et al. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *Proc Natl Acad Sci U S A* 2002;99:14682–7. [PubMed: 12391305]
20. Hageman GS, Luthert PJ, Chong NH Victor, et al. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog Retin Eye Res* 2001;20:705–32. [PubMed: 11587915]
21. Johnson LV, Ozaki S, Staples MK, et al. A potential role for immune complex pathogenesis in drusen formation. *Exp Eye Res* 2000;70:441–9. [PubMed: 10865992]
22. Johnson LV, Leitner WP, Staples MK, Anderson DH. Complement activation and inflammatory processes in drusen formation and age related macular degeneration. *Exp Eye Res* 2001;73:887–96. [PubMed: 11846519]
23. Lommatzsch A, Hermans P, Weber B, Pauleikhoff D. Complement factor H variant Y402H and basal laminar deposits in exudative age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol* 2007;245:1713–6. [PubMed: 17704937]
24. Lommatzsch A, Hermans P, Muller KD, et al. Are low inflammatory reactions involved in exudative age-related macular degeneration? Morphological and immunohistochemical analysis of AMD associated with basal deposits. *Graefes Arch Clin Exp Ophthalmol* 2008;246:803–10. [PubMed: 18414889]
25. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. *FASEB J* 2000;14:835–46. [PubMed: 10783137]
26. Nozaki M, Raisler BJ, Sakurai E, et al. Drusen complement components C3a and C5a promote choroidal neovascularization. *Proc Natl Acad Sci U S A* 2006;103:2328–33. [PubMed: 16452172]
27. Hofman A, Breteler MM, van Duijn CM, et al. The Rotterdam Study: objectives and design update. *Eur J Epidemiol* 2007;22:819–29. [PubMed: 17955331]
28. Ennis S, Jomary C, Mullins R, et al. Association between the *SERPINE1* gene and age-related macular degeneration: a two-stage case-control study. *Lancet* 2008;372:1828–34. [PubMed: 18842294]
29. Lotery AJ, Baas D, Ridley C, et al. Reduced secretion of fibulin 5 in age-related macular degeneration and cutis laxa. *Hum Mutat* 2006;27:568–74. [PubMed: 16652333]
30. Hageman GS, Anderson DH, Johnson LV, et al. A common haplotype in the complement regulatory gene factor H (*HFI/CFH*) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci U S A* 2005;102:7227–32. [PubMed: 15870199]
31. Bird AC, Bressler NM, Bressler SB, et al. International ARM Epidemiological Study Group. An international classification and grading system for age-related maculopathy and age-related macular degeneration. *Surv Ophthalmol* 1995;39:367–74. [PubMed: 7604360]
32. Age-Related Eye Disease Study Research Group. The Age-Related Eye Disease Study system for classifying age-related macular degeneration from stereoscopic color fundus photographs: the Age-Related Eye Disease Study Report number 6. *Am J Ophthalmol* 2001;132:668–81. [PubMed: 11704028]
33. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263–5. [PubMed: 15297300]
34. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559–75. [PubMed: 17701901]
35. Richards JB, Rivadeneira F, Inouye M, et al. Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. *Lancet* 2008;371:1505–12. [PubMed: 18455228]
36. Attia J, Ioannidis JP, Thakkinian A, et al. How to use an article about genetic association: B: Are the results of the study valid? *JAMA* 2009;301:191–7. [PubMed: 19141767]

37. Trikalinos TA, Salanti G, Khoury MJ, Ioannidis JP. Impact of violations and deviations in Hardy-Weinberg equilibrium on postulated gene-disease associations. *Am J Epidemiol* 2006;163:300–9. [PubMed: 16410351]
38. Gressner O, Meier U, Hillebrandt S, et al. Gc-globulin concentrations and C5 haplotype-tagging polymorphisms contribute to variations in serum activity of complement factor C5. *Clin Biochem* 2007;40:771–5. [PubMed: 17428459]
39. Hillebrandt S, Wasmuth HE, Weiskirchen R, et al. Complement factor 5 is a quantitative trait gene that modifies liver fibrogenesis in mice and humans. *Nat Genet* 2005;37:835–43. [PubMed: 15995705]
40. Zarepari S, Buraczynska M, Branham KE, et al. Toll-like receptor 4 variant D299G is associated with susceptibility to age-related macular degeneration. *Hum Mol Genet* 2005;14:1449–55. [PubMed: 15829498]
41. Yang Z, Stratton C, Francis PJ, et al. Toll-like receptor 3 and geographic atrophy in age-related macular degeneration. *N Engl J Med* 2008;359:1456–63. [PubMed: 18753640]
42. Baird PN, Guida E, Chu DT, et al. The epsilon2 and epsilon4 alleles of the apolipoprotein gene are associated with age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2004;45:1311–5. [PubMed: 15111582]
43. Baird PN, Richardson AJ, Robman LD, et al. Apolipoprotein (*APOE*) gene is associated with progression of age-related macular degeneration (AMD). *Hum Mutat* 2006;27:337–42. [PubMed: 16453339]
44. Despriet DD, Bergen AA, Merriam JE, et al. Comprehensive analysis of the candidate genes *CCL2*, *CCR2*, and *TLR4* in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2008;49:364–71. [PubMed: 18172114]
45. Edwards, AO.; Fridley, BL.; James, KM., et al. Evaluation of clustering and genotype distribution for replication in genome wide association studies: the Age-Related Eye Disease Study; PLoS ONE [serial online]. [Accessed July 31, 2009]. 2008 p. e3813 Available at: <http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0003813>
46. Park, KH.; Ryu, E.; Tosakulwong, N., et al. Common variation in the *SERPING1* gene is not associated with age-related macular degeneration in two independent groups of subjects; *Mol Vis* [serial online]. [Accessed July 31, 2009]. 2009 p. 200-7. Available at: <http://www.molvis.org/molvis/v15/a19/>
47. Allikmets R, Bergen AA, Dean M, et al. Geographic atrophy in age-related macular degeneration and TLR3 [letter]. *N Engl J Med* 2009;360:2252–4. author reply 2255-6. [PubMed: 19469038]
48. Kang SJ, Gordon D, Finch SJ. What SNP genotyping errors are most costly for genetic association studies? *Genet Epidemiol* 2004;26:132–41. [PubMed: 14748013]
49. Miller MB, Schwander K, Rao DC. Genotyping errors and their impact on genetic analysis. *Adv Genet* 2008;60:141–52. [PubMed: 18358319]
50. Colhoun HM, McKeigue PM, Smith G Davey. Problems of reporting genetic associations with complex outcomes. *Lancet* 2003;361:865–72. [PubMed: 12642066]
51. Lau LI, Chen SJ, Cheng CY, et al. Association of the Y402H polymorphism in complement factor H gene and neovascular age-related macular degeneration in Chinese patients. *Invest Ophthalmol Vis Sci* 2006;47:3242–6. [PubMed: 16877387]
52. Gotoh N, Yamada R, Hiratani H, et al. No association between complement factor H gene polymorphism and exudative age-related macular degeneration in Japanese. *Hum Genet* 2006;120:139–43. [PubMed: 16710702]
53. Okamoto H, Umeda S, Obazawa M, et al. Complement factor H polymorphisms in Japanese population with age-related macular degeneration. *Mol Vis* [serial online]. 2006 12:156–8. Available at: <http://www.molvis.org/molvis/v12/a17/>.
54. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–78. [PubMed: 17554300]

Figure 1A.

Single Nucleotide Polymorphisms (SNPs) screened per study population. 1B. Linkage disequilibrium (LD) display in Haploview of SNPs encompassing the complement component 5 (*C5*) gene with minor allele frequency >10% screened in this study and illustrating the 3 distinct haplotype blocks. All SNPs on the top row showed significant allelic association with age-related macular degeneration (AMD). Seven of those SNPs (marked with an asterix) also showed genotypic association with AMD in the Amsterdam study population. 1C. LD scores (D' and R^2) between markers genotyped. Note D' above the diagonal and R^2 scores below the diagonal.

Table 1
Basic Demographic Characteristics of the Amsterdam, Rotterdam, United Kingdom and United States Study Populations

Diagnosis	AMSTERDAM			ROTTERDAM			UNITED KINGDOM			UNITED STATES		
	AMD cases	Controls	p-value	AMD cases	Controls	p-value	AMD cases	Controls	p-value	AMD cases	Controls	p-value
No. of subjects (%)	N= 375	N= 199		N=1016	N=2251		N=564	N= 640		N= 644	N= 368	
No AMD		199 (100. 0)			2251 (100.0)			640(100.0)			368 (100. 0)	
Early AMD	93 (24.8)			858 (84.4)			221 (39.2)			276 (42.8)		
Neovascular AMD	189 (50.4)			59 (5.8)			243 (43.1)			276 (42.8)		
Geographic atrophy	55 (14.7)			63 (6.2)			71 (12.6)			92 (14.4)		
Mixed AMD	38 (10.1)			36 (3.6)			29 (5.1)			92 (14.4)		
Age- y			<0.001*			<0.001*			<0.001*			<0.001*
Mean	78	74		72	67.8		78	68		76.2	74.8	
SD	9	6		8.9	8.5		9	10		8.7	7.1	
Gender												
Female	223 (59.5)	110 (55.3)		592 (58.3)	1268 (56.3)		349 (61.9)	360 (56.3)		396 (61.5)	204 (55.4)	
Male	152 (40.5)	89 (44.7)		424 (41.7)	983 (43.7)		215 (38.1)	280 (43.7)		248 (38.5)	164 (44.6)	
Smoking												
Never	87 (23.2)	43 (21.6)		351 (35.2)	712 (32.0)		204 (36.2)	275 (43.0)				
Former smokers	138 (36.8)	49 (24.6)		423 (42.5)	964 (43.3)		277 (49.1)	291 (45.5)				
Current smokers	44 (11.7)	13 (6.5)		222 (22.3)	549 (24.7)		79 (14.0)	59 (9.2)			NA	
CFH Y402, minor allele frequency	0.5	0.35		0.45	0.33		0.46	0.38		0.54	0.34	
LOC387115 A69S, minor allele frequency	0.4	0.18		0.25	0.18		0.40	0.21		0.48	0.22	

AMD= age- related macular degeneration; NA= not available; SD= standard deviation. Due to missings, the % of smokers does not equal 100%.

* p-value represents significance of independent samples T- test

† p-value represent significance of Chi square test

Table 2

Allelic Association Between Age-related Macular Degeneration and 15 Selected Complement Component 5 Single Nucleotide Polymorphisms in the AMRO-NL Study Population

SNP ID	No AMD (controls)				AMD (cases)			
	AA	Aa number (%)	aa	MAF	AA	Aa number (%)	aa	MAF
<i>rs10818495</i>	32 (18.8)	82 (48.2)	56 (32.9)	0.57	79 (22.1)	187 (52.2)	92 (25.7)	0.52
<i>rs10985126</i>	129 (67.5)	55 (28.8)	7 (3.7)	0.18	228 (62.5)	109 (29.9)	28 (7.7)	0.23
<i>rs1468673</i>	17 (13.1)	56 (43.1)	57 (43.8)	0.65	66 (21.0)	139 (44.3)	109 (34.7)	0.57
<i>rs1548782</i>	83 (42.8)	82 (42.3)	29 (14.9)	0.36	142 (38.5)	166 (45.0)	61 (16.5)	0.39
<i>rs16910280</i>	143 (73.7)	49 (25.3)	2 (1.0)	0.14	267 (72.2)	94 (25.4)	9 (2.4)	0.15
<i>rs17611</i>	53 (27.7)	99 (51.8)	39 (20.4)	0.46	145 (39.4)	161 (43.8)	62 (16.8)	0.39
<i>rs1978270</i>	82 (42.5)	82 (42.5)	29 (15.0)	0.36	140 (38.1)	166 (45.2)	61 (16.6)	0.39
<i>rs2269066</i>	120 (82.8)	23 (15.9)	2 (1.4)	0.09	236 (73.8)	74 (23.1)	10 (3.1)	0.15
<i>rs25681</i>	54 (28.3)	99 (51.8)	38 (19.9)	0.46	144 (39.0)	164 (44.4)	61 (16.5)	0.39
<i>rs7026551</i>	132 (69.1)	50 (26.2)	9 (4.7)	0.18	210 (57.5)	127 (34.8)	28 (7.7)	0.25
<i>rs7027797</i>	160 (83.8)	27 (14.1)	4 (2.1)	0.09	281 (76.2)	77 (20.9)	11 (3.0)	0.13
<i>rs7031128</i>	122 (62.9)	60 (30.9)	12 (6.2)	0.22	218 (59.6)	128 (35.0)	20 (5.5)	0.23
<i>rs7037673</i>	57 (29.8)	95 (49.7)	39 (20.4)	0.45	156 (42.3)	167 (45.3)	45 (12.5)	0.35
<i>rs7040033</i>	53 (27.9)	97 (51.1)	40 (21.1)	0.47	140 (38.0)	167 (45.4)	61 (16.6)	0.39
<i>rs92670</i>	52 (29.9)	79 (45.4)	43 (24.7)	0.47	87 (24.4)	155 (43.5)	114 (32.0)	0.54

AMD = age-related macular degeneration; SNP = single nucleotide polymorphism; MAF = minor allele frequency; HWE = Hardy-Weinberg Equilibrium. "A" indicates common allele, "a" minor allele. Because of genotyping errors, not all subject data is obtainable. Genotype frequencies are given as a percentage of subjects genotyped. The allelic *P*-value is calculated with Fisher's exact test. The χ^2 test was used to test SNP distributions for conformity with HWE.

Table 3
Odds Ratios and 95% Confidence Intervals of Early and Late Age-related Macular Degeneration Cases Versus Unrelated Controls of the AMRO-NL Study Population for Single Nucleotide Polymorphisms in the Complement Component 5 Gene

	No AMD (controls)			Early AMD			Late AMD		
	N=130	N=77		N=237			N=237		
<i>rs1468673</i>	No. (%)	No. (%)		No. (%)	OR (95% CI)		No. (%)	OR (95% CI)	
Genotype									
Noncarrier (AA)	17 (13.1)	14 (18.2)		1			52 (21.9)		1
Heterozygous (Aa)	56 (43.1)	31 (40.3)			0.68 (0.29-1.58)		108 (45.6)		0.67 (0.35-1.29)
Homozygous (aa)	57 (43.8)	32 (41.6)			0.65 (0.28-1.50)		77 (32.5)		0.46 (0.24-0.90)
MAF(%)	0.65	0.62					0.55		
<i>Rs17611</i>	N=191	N=93		N=275					
Genotype	No. (%)	No. (%)		No. (%)	OR (95% CI)		No. (%)	OR (95% CI)	
Noncarrier (AA)	53 (27.7)	33 (35.5)		1			112 (40.7)		1
Heterozygous (Aa)	99 (51.8)	46 (49.5)			0.66 (0.37-1.19)		115 (41.8)		0.53 (0.34-0.82)
Homozygous (aa)	39 (20.4)	14 (15.1)			0.50 (0.23-1.10)		48 (17.5)		0.66 (0.38-1.15)
MAF(%)	0.46	0.4					0.38		
<i>rs2269066</i>	N=145	N=86		N=234					
Genotype	No. (%)	No. (%)		No. (%)	OR (95% CI)		No. (%)	OR (95% CI)	
Noncarrier (AA)	120 (82.8)	58 (67.4)		1			178 (76.1)		1
Heterozygous (Aa)	23 (15.9)	24 (27.9)			2.20 (1.12-4.38)		50 (21.4)		1.38 (0.78-2.41)
Homozygous (aa)	2 (1.4)	4 (4.7)			3.16 (0.54-18.50)		6 (2.6)		1.86 (0.34-10.17)
MAF(%)	0.09	0.19					0.13		
<i>rs25681</i>	N=191	N=93		N=276					
Genotype	No. (%)	No. (%)		No. (%)	OR (95% CI)		No. (%)	OR (95% CI)	
Noncarrier (AA)	54 (28.3)	37 (39.8)		1			112 (40.6)		1
Heterozygous (Aa)	99 (51.8)	42 (45.2)			0.72 (0.40-1.28)		117 (42.4)		0.54 (0.35-0.84)
Homozygous (aa)	38 (19.9)	14 (15.1)			0.54 (0.25-1.17)		47 (17.0)		0.68 (0.39-1.20)
MAF(%)	0.46	0.38					0.38		

No AMD (controls)		Early AMD		Late AMD	
N=191		N=92		N=273	
Genotype		No. (%)	No. (%)	No. (%)	OR (95% CI)
Rs7026551					
Noncarrier (AA)		132 (69.1)	49	1	161 (59.0) 1
Heterozygous (Aa)		50 (26.2)	35 (38.0)	92 (33.7)	1.32 (0.86-2.03)
Homozygous (aa)		9 (4.7)	8 (8.7)	20 (7.4)	1.50 (0.63-3.55)
MAF(%)		0.18	0.28	0.24	
Rs7027797					
Noncarrier (AA)		160 (83.8)	74 (79.6)	1	1
Heterozygous (Aa)		27 (14.1)	17 (18.3)	60 (21.7)	1.49 (0.89-2.49)
Homozygous (aa)		4 (2.1)	2 (2.2)	9 (3.3)	1.75 (0.49-6.19)
MAF(%)		0.09	0.11	0.14	
Rs7037673					
Noncarrier (AA)		57 (29.8)	38 (40.9)	1	1
Heterozygous (Aa)		95 (49.7)	45 (48.4)	122 (44.2)	0.63 (0.41-0.96)
Homozygous (aa)		39 (20.4)	10 (10.8)	36 (13.0)	0.51 (0.29-0.92)
MAF(%)		0.45	0.35	0.35	
rs7040033					
Noncarrier (AA)		53 (27.9)	31 (33.3)	1	1
Heterozygous (Aa)		97 (51.1)	48 (51.6)	119 (43.3)	0.58 (0.37-0.90)
Homozygous (aa)		40 (21.1)	14 (15.1)	47 (17.1)	0.65 (0.37-1.13)
MAF(%)		0.47	0.41	0.39	

AMD = age-related macular degeneration; CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism; MAF = minor allele frequency. "A" indicates common allele, "a" minor allele. Genotype frequencies are given as a percentage of subjects genotyped. Percentages not always 100% because of rounding. ORs are estimated with logistic regression analysis (with the noncarriers as reference group and respectively early and late AMD as outcome variable). Adjusted for age and gender.

Table 4

Odds Ratios and 95% Confidence Intervals of Age-related Macular Degeneration Early and Late Cases Versus Unrelated Controls of Three Replication Populations: 4A. the Rotterdam Study, 4B. United Kingdom study Population and 4C. the United States Population for Single Nucleotide Polymorphisms in the Complement Component 5 Gene

	^A Rotterdam study				^B United Kingdom study population				^C United States study population			
	No AMD	Early AMD	No. (%)	OR (95% CI)	No AMD	Early AMD	No. (%)	OR (95% CI)	No AMD	Early AMD	No. (%)	OR (95% CI)
<i>Rs17611</i>	N= 2251	N= 858	N= 158	N= 635	N= 218	N= 335	N= 365	N= 274	N= 367	N= 274	N= 367	N= 367
Genotype												
Noncarrier (AA)	702 (31.2)	246 (28.7)	51 (32.3)	1	213 (33.5)	75 (34.4)	1	110 (32.8)	120 (32.9)	77 (28.3)	105 (28.6)	1
Heterozygous (Aa)	1141 (50.7)	437 (50.9)	82 (51.9)	1.10 (0.91-1.32)	316 (49.8)	103 (47.2)	0.93 (0.66-1.31)	159 (47.5)	157 (43.0)	143 (52.2)	174 (47.4)	1.42 (0.99-2.04)
Homozygous (aa)	408 (18.1)	175 (20.4)	25 (15.8)	1.21 (0.96-1.52)	106 (16.7)	40 (18.3)	1.07 (0.68-1.68)	66 (19.7)	88 (24.1)	54 (19.7)	88 (24.0)	0.96 (0.61-1.49)
MAF(%)	0.43	0.46	0.42		0.42	0.42		0.43	0.46	0.46	0.48	
<i>Rs7037673</i>	N= 2234	N= 849	N= 157	N= 633	N= 218	N= 333	N= 364	N= 274	N= 362	N= 274	N= 362	N= 362
Genotype												
Noncarrier (AA)	781 (35.0)	282 (33.2)	59 (37.6)	1	234 (37.0)	78 (35.8)	1	121 (36.3)	130 (35.7)	78 (28.5)	110 (30.4)	1
Heterozygous (Aa)	1092 (48.9)	415 (48.9)	77 (49.0)	1.06 (0.89-1.27)	311 (49.1)	101 (46.3)	0.97 (0.69-1.37)	151 (45.3)	156 (42.9)	147 (53.6)	174 (48.1)	1.57 (1.10-2.25)
Homozygous (aa)	361 (16.2)	152 (17.9)	21 (13.4)	1.15 (0.91-1.45)	88 (13.9)	39 (17.9)	1.33 (0.84-2.10)	61 (18.3)	78 (21.4)	49 (17.9)	78 (21.5)	1.05 (0.66-1.65)
MAF(%)	0.41	0.42	0.38		0.38	0.41		0.41	0.43	0.45	0.46	
<i>Rs7026551</i>	N= 2247	N= 858	N= 159									
Genotype												
				OR (95% CI)								

	^A Rotterdam study			^B United Kingdom study population			^C United States study population		
	No AMD	Early AMD	Late AMD	No AMD	Early AMD	Late AMD	No AMD	Early AMD	Late AMD
Noncarrier (AA)	1384 (61.6)	536 (62.5)	100 (62.9)	1					
Heterozygous (Aa)	770 (34.3)	277 (32.3)	54 (34.0)	1.00 (0.70- 1.11)					
Homozygous (aa)	93 (4.1)	45 (5.2)	5 (3.1)	1.28 (0.88- 1.86)					
MAF(%)	0.21	0.21	0.2						

AMD = age- related macular degeneration; CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism; MAF = minor allele frequency. “A” indicates common allele, “a” minor allele. Percentages not always 100% because of rounding. ORs are estimated with logistic regression analysis (with the noncarriers as reference group and respectively early and late AMD as outcome variable). Adjustment for age and gender only in the Rotterdam study.

Odds Ratios and 95% Confidence Intervals of Early and Late Cases Versus Unrelated Controls For Pooled Data of the AMRO-NL Study Population and the Rotterdam Study for Single Nucleotide Polymorphisms in the Complement Component 5 Gene

Table 5

	No AMD (controls)			Early AMD			Late AMD		
	N=2442	No. (%)	N=951	OR (95% CI)	No. (%)	OR (95% CI)	N=433	No. (%)	OR (95% CI)
Genotype									
<i>Rs17611</i>	Noncarrier (AA)	755 (30.9)	279 (29.3)	1	163 (37.6)	1			
	Heterozygous (Aa)	1240 (50.8)	483 (50.8)	1.05 (0.89-1.25)	197 (45.5)	0.74 (0.59-0.92)			
	Homozygous (aa)	447 (18.3)	189 (19.9)	1.15 (0.92-1.43)	73 (16.9)	0.76 (0.56-1.02)			
<i>Rs7026551</i>									
	N=2438		N=950		N=432				
Genotype									
	Noncarrier (AA)	1516 (62.2)	585 (61.2)	1	261 (60.4)	1			
	Heterozygous (Aa)	820 (33.6)	312 (32.8)	0.99 (0.84-1.15)	146 (33.8)	1.03 (0.83-1.29)			
	Homozygous (aa)	102 (4.2)	53 (5.6)	1.35 (0.95-1.90)	25 (5.8)	1.42 (0.90-2.25)			
<i>Rs7037673</i>									
	N=2425		N=942		N=433				
Genotype									
	Noncarrier (AA)	838 (34.6)	320 (34.0)	1	177 (40.9)	1			
	Heterozygous (Aa)	1187 (48.9)	460 (48.8)	1.01 (0.86-1.20)	199 (46.0)	0.79 (0.64-0.99)			
	Homozygous (aa)	400 (16.5)	162 (17.2)	1.06 (0.85-1.33)	57 (13.2)	0.67 (0.49-0.93)			

AMD = age-related macular degeneration; CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism; MAF= minor allele frequency. "A" indicates common allele, "a" minor allele. Percentages not always 100% because of rounding. ORs are estimated with logistic regression analysis (with the noncarriers as reference group and respectively early and late AMD as outcome variable). Adjusted for age and gender.

Odds Ratios and 95% Confidence Intervals of Age-related Macular Degeneration Early and Late Cases Versus Unrelated Controls For Pooled Data of the AMRO-NL, United Kingdom and United States study populations for Single Nucleotide Polymorphisms in the Complement Component 5 Gene

Table 6

No AMD (controls)			Early AMD		Late AMD	
<i>Rs17611</i>	N=1191	N=585	N=977			
	No. (%)	No. (%)	OR (95% CI)	No. (%)	OR (95% CI)	
Genotype						
Noncarrier (AA)	386 (32.4)	185 (31.6)	1	327 (33.5)	1	
Heterozygous (Aa)	572 (48.0)	292 (49.9)	1.07 (0.85-1.33)	448 (45.9)	0.92 (0.76-1.12)	
Homozygous (aa)	233 (19.6)	108 (18.5)	0.97 (0.73-1.29)	202 (20.7)	1.02 (0.81-1.30)	
<i>Rs7037673</i>	N=1188	N=585	N=972			
	No. (%)	No. (%)	OR (95% CI)	No. (%)	OR (95% CI)	
Genotype						
Noncarrier (AA)	421 (35.4)	194 (33.2)	1	349 (35.9)	1	
Heterozygous (Aa)	562 (47.3)	293 (50.1)	1.13 (0.91-1.41)	448 (46.1)	0.96 (0.80-1.16)	
Homozygous (aa)	205 (17.3)	98 (16.8)	1.04 (0.77-1.39)	175 (18.0)	1.03 (0.80-1.32)	

AMD = age- related macular degeneration; CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism; MAF = minor allele frequency. "A" indicates common allele, "a" minor allele. Percentages not always 100% because of rounding. ORs are estimated with logistic regression analysis (with the noncarriers as reference group and respectively early and late AMD as outcome variable).