Evidence of association of APOE with age-related macular degeneration: a pooled analysis of 15 studies.

McKay, G. J., Patterson, C. C., Chakravarthy, U., Dasari, S., Klaver, C. C., Vingerling, J. R., Ho, L., de Jong, P. T. V. M., Fletcher, A. E., Young, I. S., Seland, J. H., Rahu, M., Soubrane, G., Tomazzoli, L., Topouzis, F., Vioque, J., Hingorani, A. D., Sofat, R., Dean, M., ... Silvestri, G. (2011). Evidence of association of APOE with age-related macular degeneration: a pooled analysis of 15 studies. *Human Mutation*, *32*(12), 1407-16. https://doi.org/10.1002/humu.21577

Published in:
Human Mutation

Document Version:
Peer reviewed version

Published in final edited form as:
Hum Mutat. 2011 December ; 32(12): 1407–1416. doi:10.1002/humu.21577.

Evidence of association of *APOE* with age-related macular degeneration - a pooled analysis of 15 studies

Gareth J. McKay[1], **Chris C. Patterson**[1], **Usha Chakravarthy**[2], **Shilpa Dasari**[2], **Caroline C. Klaver**[3], **Johannes R. Vingerling**[4], **Lintje Ho**[4], **Paulus T.V.M. de Jong**[5,6], **Astrid E. Fletcher**[7], **Ian S. Young**[1], **Johan H. Seland**[8], **Mati Rahu**[9], **Gisele Soubrane**[10], **Laura Tomazzoli**[11], **Fotis Topouzis**[12], **Jesus Vioque**[13,14], **Aroon D. Hingorani**[15,16], **Reecha Sofat**[15,16], **Michael Dean**[17], **Julie Sawitzke**[17], **Johanna M. Seddon**[18,19], **Inga Peter**[20], **Andrew R. Webster**[21,22], **Anthony T. Moore**[21,22], **John R.W. Yates**[21,22,23], **Valentina Cipriani**[21,22], **Lars G. Fritsche**[24], **Bernhard H.F. Weber**[24], **Claudia N. Keilhauer**[25], **Andrew J. Lotery**[26,27], **Sarah Ennis**[28], **Michael L. Klein**[29], **Peter J. Francis**[29], **Dwight Stambolian**[30], **Anton Orlin**[30], **Michael B. Gorin**[31], **Daniel E. Weeks**[32], **Chia-Ling Kuo**[32], **Anand Swaroop**[33,34], **Mohammad Othman**[33], **Atsuhiro Kanda**[34], **Wei Chen**[35], **Goncalo R. Abecasis**[35], **Alan F. Wright**[36], **Caroline Hayward**[36], **Paul N. Baird**[37], **Robyn H. Guymer**[37], **John Attia**[38,39,40], **Ammarin Thakkinstian**[41], and **Giuliana Silvestri**[2]

[1]Centre for Public Health, Royal Victoria Hospital, Queen's University Belfast, Belfast, Northern Ireland BT12 6BA [2]Centre for Vision and Vascular Science, Royal Victoria Hospital, Queen's University Belfast, Belfast, Northern Ireland BT12 6BA [3]Department of Ophthalmology, Erasmus Medical Centre, Rotterdam, The Netherlands [4]Department of Epidemiology, Erasmus Medical Centre, Rotterdam, The Netherlands [5]The Netherlands Institute for Neuroscience, KNAW, Amsterdam, The Netherlands [6]Department of Ophthalmology AMC, Amsterdam, The Netherlands [7]Department of Epidemiology & Population Health, London School of Hygiene & Tropical Medicine, London, United Kingdom [8]Eye Department, Stavanger University Hospital, University of Bergen, Stavanger, Norway [9]Department of Epidemiology and Biostatistics, National Institute for Health Development, Tallinn, Estonia [10]Clinique Ophthalmologique, Universitaire de Creteil, Paris, France [11]Clinica Oculistica, Unversita degli studi di Verona, Verona, Italy [12]Department of Ophthalmology, Aristotle University of Thessaloniki, Thessaloniki, Greece [13]Departamento Salud Publica University Miguel Hernandez, Alicante, Spain [14]CIBERESP, Alicante, Spain [15]University Centre for Clinical Pharmacology [16]Department of Medicine, University College London, London, United Kingdom [17]Cancer and Inflammation Program, National Cancer Institute, Frederick, Maryland [18]Department of Ophthalmology, Tufts University School of Medicine, Boston, Massachusetts [19]Tufts Medical Center, Boston, Massachusetts [20]Department of Genetics & Genomic Sciences, Mount Sinai School of Medicine, New York, New York [21]Institute of Ophthalmology, University College London, London, United Kingdom [22]Moorfields Eye Hospital, London, United Kingdom [23]Department of Medical Genetics, University of Cambridge, Cambridge, United Kingdom [24]Institute of Human Genetics, University of Regensburg, Regensburg, Germany [25]Department of Ophthalmology, University Hospital Würzburg, Würzburg, Germany [26]Clinical Neurosciences Division, School of Medicine, University of Southampton, Southampton, United Kingdom [27]Southampton Eye Unit, Southampton General Hospital, Southampton, United Kingdom [28]Genetic Epidemiology & Bioinformatics Group Human Genetics Division, University of Southampton, United Kingdom [29]Macular Degeneration Center, Casey Eye Institute, Oregon Health and Science University, Oregon [30]Department of Ophthalmology, University of Pennsylvania, Philadelphia, Pennsylvania [31]Departments of Ophthalmology and

Corresponding author: Dr Gareth J. McKay, Phone: 44 (0)28 9063 5232, Fax: 44 (0)28 9023 5900, g.j.mckay@qub.ac.uk.

Supporting Information for this preprint is available from the *Human Mutation* editorial office request (humu@wiley.com)

Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, California [32]Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania [33]Department of Ophthalmology and Visual Sciences, Kellogg Eye Center, University of Michigan, Ann Arbor, Michigan [34]Neurobiology-Neurodegeneration and Repair Laboratory, National Eye Institute, NIH, Bethesda, Maryland [35]Center for Statistical Genetics, Biostatistics, University of Michigan, Ann Arbor, Michigan [36]MRC Human Genetics Unit, Western General Hospital, Edinburgh, United Kingdom [37]Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, University of Melbourne, Melbourne, Australia [38]Centre for Clinical Epidemiology and Biostatistics, University of Newcastle, Newcastle, Australia [39]Hunter Medical Research Institute, Newcastle, Australia [40]Department of General Medicine, John Hunter Hospital, Newcastle, Australia [41]Section for Clinical Epidemiology and Biostatistics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Abstract

Age-related macular degeneration (AMD) is the most common cause of incurable visual impairment in high-income countries. Previous studies report inconsistent associations between AMD and apolipoprotein E (APOE), a lipid transport protein involved in low-density cholesterol modulation. Potential interaction between APOE and sex, and smoking status, has been reported. We present a pooled analysis (n=21,160) demonstrating associations between late AMD and *APOε4* (OR=0.72 per haplotype; CI: 0.65–0.74; $P=4.41\times10^{-11}$) and *APOε2* (OR=1.83 for homozygote carriers; CI: 1.04–3.23; P=0.04), following adjustment for age-group and sex within each study and smoking status. No evidence of interaction between *APOE* and sex or smoking was found. Ever smokers had significant increased risk relative to never smokers for both neovascular (OR=1.54; CI: 1.38–1.72; $P=2.8\times10^{-15}$) and atrophic (OR=1.38; CI: 1.18–1.61; $P=3.37\times10^{-5}$) AMD but not early AMD (OR=0.94; CI: 0.86–1.03; P=0.16), implicating smoking as a major contributing factor to disease progression from early signs to the visually disabling late forms. Extended haplotype analysis incorporating rs405509 did not identify additional risks beyond*ε2* and *ε4* haplotypes. Our expanded analysis substantially improves our understanding of the association between the *APOE* locus and AMD. It further provides evidence supporting the role of cholesterol modulation, and low-density cholesterol specifically, in AMD disease etiology.

Keywords

age-related macular degeneration; AMD; apolipoprotein E; APOE; case-control association study

Introduction

Age-related macular degeneration (AMD; MIM# 603075) is in its late form, the leading cause of incurable visual impairment among individuals of European descent over the age of 50 [Centers for Disease Control and Prevention, 2004], accounting for more than half of all new cases of registered blindness [Evans and Wormald, 1996]. The socioeconomic burden associated with AMD is increasing in our aging societies with almost 30% of those aged 75 years and above showing early signs of disease [Klein et al., 1992; Vingerling et al., 1995]. AMD is a common disorder of complex etiology with multiple genetic, environmental and lifestyle factors contributing to the phenotype, although the specifics of the etiology remain largely unresolved [Swaroop et al., 2007]. AMD by definition affects the macular region of the retina which is associated with detailed central vision. AMD is commonly divided into early (eAMD) and late AMD, and the vision impairing late AMD is subdivided into geographic atrophic (GA) and neovascular AMD (NV) or mixed GA and NV together (GANV). eAMD is characterized by drusen formation and pigmentary changes at the level

of the retinal pigment epithelium (RPE), and can progress through atrophy of the pigment epithelium to the visually disabling late atrophic and/or neovascular phenotypes.

Advances in our understanding of the genetic basis of the disease have identified risk and protective variants in several genes associated with the complement pathway and chronic inflammation such as factor H (*CFH*) [Edwards et al., 2005, Hageman et al., 2005; Haines et al., 2005; Hughes et al., 2006; Klein et al., 2005], component 2 (*CC2*)/factor B (*CFB*) region [Gold et al., 2006; McKay et al., 2009], component 3 (*C3*) [Maller et al., 2007; McKay et al., 2010; Park et al., 2009; Yates et al., 2007] and complement factor I (*CFI*) [Fagerness et al., 2009]. Beyond the complement pathway, chromosome 10q26 and specifically the Age Related Maculopathy Susceptibility 2 (*ARMS2*) locus has been implicated as a second major genetic contributor to the AMD disease process [Dewan et al., 2006; Jakobsdottir et al., 2005; Riveria et al., 2005; Yang et al., 2006]. The high linkage disequilibrium that exists between *ARMS2* and the serine protease *HTRA1* makes it difficult to determine the source of the genetic effect at this locus, although data have been reported supporting mitochondrial involvement through interaction with translocase of outer mitochondrial membrane proteins and co-localization to the mitochondrial-rich ellipsoid region of the photoreceptors [Fritsche et al., 2008; Kanda et al., 2007]. Mitochondrial dysfunction and oxidative stress have been implicated in AMD disease etiology with reports of increased mitochondrial damage in the neural retina and RPE with ageing. Reports of mitochondrial genomic variation and associated increased AMD risk [Canter et al., 2008; San Giovanni et al., 2009] provide an excellent rationale for the involvement of oxidative stress in the disease pathway. More recently, *TIMP3*, a metalloproteinase involved in degradation of the extracellular matrix, hepatic lipase C (*LIPC*) and cholesterylester transfer protein (*CETP*), key genes involved in the metabolism of triglycerides and high density lipoproteins (HDL), were implicated in AMD pathogenesis through genome-wide association studies (GWAS) [Chen et al., 2010; Neale et al., 2010].

One of the first reported genetic associations with AMD was the protective effect exerted by the *ε4* haplotype of Apolipoprotein E (APOE; MIM# 107741), a lipid transport protein that acts as a ligand for the low density lipoprotein receptor which is involved in the maintenance and repair of neuronal cell membranes [Klaver et al., 1998; Souied et al., 1998]. Association of APOE with AMD pathogenesis is supportive of mechanisms such as immunoregulation and cell signalling [Zarbin, 2004]. Variation at two single nucleotide polymorphisms (SNPs) within the coding sequence of the *APOE* gene, rs429358 and rs7412, results in different isoforms reported to attenuate binding affinity to the low density lipoprotein (LDL)-receptor. For example, *ε2* has a much reduced binding affinity leading to lower total cholesterol levels with respect to *ε3* and *ε4*, which reveal a higher binding affinity with higher total cholesterol levels [Siest et al., 1995]. Three allelic variants derived from these SNPs commonly referred to as *ε2*, *ε3* and *ε4* are differentiated on the basis of cysteine (Cys) and arginine (Arg) residue interchanges at positions 112 (rs429358) and 158 (rs7412) in the amino acid sequence and give rise to 6 diplotypes (*ε3/ε3*,*ε3/ε4*, *ε2/ε3*, *ε4/ε4*, *ε2/ε4* and*ε2/ε2*, ranked from most to least common amongst European populations [Corbo and Scacchi, 1999]). The *ε2* haplotype has a Cys residue at positions 112 and 158 in the receptor-binding region of APOE. The *ε3* haplotype has residues Cys-112 and Arg-158 and the *ε4* haplotype has Arg residues at both positions, with these amino acid substitutions having a strong physiological consequence on protein function. *ε3* is by far the most common haplotype in all human populations, but considerable variation in the frequency distribution of all haplotypes have been reported across different ethnic groups [Corbo and Scacchi, 1999] and with human longevity [McKay et al., 2011].

Many independent replications of AMD and *APOE* have been undertaken and while the protective effect associated with*ε4* was confirmed in some studies, the significance of the

increased risk associated with*ε2* has not been consistent [Baird et al., 2004, 2006; Bergeron-Sawitzke et al., 2009; Bojanowski et al., 2006; Conley et al., 2005; Francis et al., 2009; Fritsche et al., 2009; Schmidt et al., 2002, 2005; Thakkinstian et al., 2006; van Leeuwen et al., 2004; Zareparsi et al., 2004]. Some studies have reported increased risk of AMD associated with*ε2* [Baird et al., 2004; Schmidt et al., 2002; van Leeuwen et al., 2004; Zareparsi et al., 2004] while others have suggested that effect modification by sex [Baird et al., 2004; Schmidt et al., 2002] or smoking status may occur [Schmidt et al., 2005]. Previously, pooled data and meta analyses in much smaller sample sizes indicated a significant protective effect associated with *ε4* and a non-significant increased risk associated with *ε2* [Bojanowski et al., 2006; Thakkinstian et al., 2006]. In our investigation, we invited contributing studies to pool individual sample data to assess *APOE* genotype in AMD with respect to accompanying clinical phenotype data and to test for interaction with sex, age and smoking status.

Materials and methods

Study Population, Risk Factors and Clinical Phenotypes

Initially 18 research groups likely to have *APOE* genetic data in a well-phenotyped dataset were invited to participate. The analysis originated from 15 of these studies in 40 centers based in 11 countries which had data on *APOE* in AMD patients and suitable controls: nine European countries (United Kingdom, Germany, the Netherlands, Norway, Estonia, Italy, France, Greece and Spain), six centers in the United States and one in Australia [Augood et al., 2004; Baird et al., 2006; Bergeron-Sawitzke et al., 2009; Conley et al., 2005; Dandekar et al., 2006; Ennis et al., 2008; Francis et al., 2009; Fritsche et al., 2009; Haan et al., 2006; Hadley et al., 2010; McKay et al., 2009; Vingerling et al., 1995; Yates et al., 2007; Zareparsi et al., 2004]. Study demographics are summarized (n=21,160; Table 1). Three studies, the Rotterdam Study [Vingerling et al., 1995], the Women's Health Initiative (WHI) [Haan et al., 2006] and the EUREYE study [Augood et al., 2004], provided data derived from samples acquired through population based surveys (n=10,253). The remainder of the data originated from case-control (association) studies. Individual participant data for age at examination, sex, smoking status (ever smoker or never smoker), AMD phenotype and *APOE* haplotype (SNPs rs429358, rs7412) were necessary from participating studies to facilitate a pooled data analysis in order to be eligible for this study. *APOE* genotypes were determined using several methods: TaqMan genotyping technology (Applied Biosystems, Foster City, CA): Portland, AREDS, Philadelphia, Edinburgh, Women's Health Initiative, Cambridge, London; *APOE*-specific polymerase chain reaction followed by restriction digest: Michigan, Rotterdam, Melbourne, Los Angeles, Cambridge; KASPar genotyping (KBioscience, Hertfordshire, UK): EUREYE, Southampton; Sequenom (San Diego, CA): Regensburg; BigDye Terminator DNA Cycle Sequencing analysis (Applied Biosystems): Belfast. Where available, genotypes were also requested for rs405509, a SNP located in the promoter region of *APOE* (n=7,568). Extended haplotype frequencies were estimated using UNPHASED, where data were available for rs405509 [Dudbridge, 2008].

Control subjects were classified on the basis of direct clinical examination or by retinal imaging and were graded as having either no signs of AMD in either eye, or had fewer than five hard drusen of diameter ≤ 63um and no focal pigmentary irregularities such as hyperpigmentation or hypopigmentation (i.e., grades 0a and 0b using the definitions of the Wisconsin Age Related Maculopathy Grading System; n=10,623). Cases were classified according to AMD diagnosis in the worst eye. Participants with drusen > 63um and/or focal pigmentary irregularities but had not progressed to late AMD, were classified as early AMD (eAMD; i.e. grades 1a–3; n=4,143). Late AMD was defined as those individuals with geographic atrophy (grade 4a) and/or exudative AMD (grade 4b) in at least one eye. Samples with late AMD were sub-categorized to those with NV without GA (n=3,935); GA

without NV (n=1,370) and both NV and GA in the same or fellow eye (GANV; n=1,089). Cases of macular disease due to other primary causes that mimic NV AMD, such as myopic maculopathy, adult vitelliform, any retinal scarring and idiopathic macular telangiectasia, were excluded.

All participants provided prior written informed consent, and the protocols were reviewed and approved by local institutional review boards. Recruitment procedures and detailed AMD grading methods for each study have been described previously [Augood et al., 2004; Baird et al., 2006; Bergeron-Sawitzke et al., 2009; Conley et al., 2005; Dandekar et al., 2006; Ennis et al., 2008; Francis et al., 2009; Fritsche et al., 2009; Haan et al., 2006; Hadley et al., 2010; McKay et al., 2009; Vingerling et al., 1995; Yates et al., 2007; Zareparsi et al., 2004].

Statistical Analysis

Analysis was restricted to samples derived from participants of self-reported European descent and who had been assessed for AMD through retinal photography or clinical examination. Call rates for all SNPs were verified and minor allele frequencies assessed; departure from Hardy-Weinberg Equilibrium (HWE) was determined separately in cases and controls, using the χ^2 goodness-of-fit test ($P<0.01$). Associations between haplotype and AMD and possible effect modification by smoking, age and sex were assessed using likelihood ratio χ^2 tests in the logistic regression model. Sex and age were considered to be potential confounding variables, particularly in population surveys where cases of AMD were older and more often female than those who were AMD free. To allow for this and also for the different control group selection criteria employed in the various case-control studies, sex and age (categorized in five year groups) were included in all logistic regression models as were interactions between sex and study and between age-group and study. Similar findings were obtained with age treated as a continuous variable rather than catergorized.

To assess the most appropriate genetic model two diplotype analyses were explored separately with *ε3ε3* assigned as the reference group in each: (i) *ε2ε2*, *ε2ε3*, and *ε3ε3* and (ii) *ε4ε4*, *ε3ε4*, and *ε3ε3*. The diplotype effects were estimated using the model-free approach [Minelli et al., 2005], which does not assume that the underlying genetic model is known in advance and makes use of the information available on all diplotypes. Odds ratios (ORs), OR_1 (comparing *ε2ε2* with*ε3ε3*), OR_2 (*ε2ε3* vs. *ε3ε3*), OR_3 (*ε4ε4* vs. *ε3ε3*), and OR_4 (*ε3ε4* vs. *ε3ε3*) were estimated, adjusting for study, age-group, sex, and smoking status, using methods previously described [Minelli et al., 2005; Thakkinstian et al., 2006]. These ORs were modeled on a logarithmic scale accounting for both between and within study variation. The ratios $\lambda_1=\log OR_2/\log OR_1$ and $\lambda_2=\log OR_4/\log OR_3$ were estimated. These parameters capture information about the genetic model, as follows; the model is recessive if $\lambda = 0$, dominant if $\lambda = 1$ and additive if $\lambda = 0.5$. A homozygous or heterosis model is appropriate for $\lambda > 1$ or $\lambda < 0$. Additionally, logistic regression was used to fit various genetic models and the Akaike Information Criteria was calculated to choose the most appropriate genetic model. ORs with 95% confidence intervals (CIs) were then estimated after first checking that there was no evidence of heterogeneity in genetic effects between studies. This was done using likelihood-ratio tests to assess the significance of interactions in the logistic model.

Multinomial logistic regression was used to test if the contribution of *APOE* and smoking to AMD varied between the 3 late AMD sub-phenotypes (GA, NV and GANV) and between eAMD and late AMD. In these analyses the various disease subgroups were compared with a common control group. The multinomial logistic regression was fitted using the mlogit command in STATA (StataCorp, College Station, Tex) while the remainder of the analysis was performed in SPSS (SPSS Inc, Chicago, Ill).

Results

APOE Haplotype and Diplotype Frequencies

Of the 10,537 classified cases, 4,143 (39%) were categorized as eAMD and 6,394 (61%) as late AMD (Table 1). Late AMD cases were further categorized by sub-phenotype: GA: n=1370 (21%); NV: n=3935 (62%) and GANV: n=1089 (17%). *APOE* haplotype frequencies in controls varied between studies ranging from 6.7–10.8% for *ε2*; 70.8–81.8% for *ε3* and 9.5–20.8% for *ε4* (Supp. Table S1). *APOE* haplotype frequencies also differed by phenotype with less variation in*ε2* (7.9–10.2%) and *ε3* (78.0–80.9%) than in*ε4* (9.1–14.1%) (Supp. Table S2). No evidence for departure from HWE was detected in participating studies for any SNP and genotyping quality control metrics are provided (Supp. Table S3).

Assessment of Genetic Model

The most appropriate genetic models for the *APOE* diplotypes were assessed from parameters λ_1 andλ_2 representing the ratio of risks in specific diplotypes [Minelli et al., 2005]. The parameter, λ_2, estimated as 0.49 (CI: 0.29–0.89) was strongly indicative of an additive model for *ε4* (λ_2 =0.5). The parameter λ_1 was estimated at 0.14 (CI: 0.01–0.89) suggesting a recessive model for*ε*2 was likely (λ_1=0) although an additive model (λ_1 =0.5) could not be excluded (Table 2). This choice of model was further supported by the Akaike Information Criteria which attained its minimum value for the recessive model (12807.37) compared to the values obtained for the general (12809.37), additive (12811.34) and dominant models (12811.84). This model is most in keeping with the diplotype comparisons shown in Figure 1 (*ε2ε3* and*ε2ε4* versus*ε3ε3*) which clearly indicate minimal increase in risk associated with a single copy of *ε2*.

Association of *APOE* with Late AMD

Initial comparison of diplotype frequencies between late AMD and controls used the *ε3ε3* diplotype as a reference for calculating ORs, CI and P values. ORs were estimated after adjustment for age-group and sex within each study and for smoking status (ever versus never). The *ε2ε2* diplotype showed a significantly increased risk for late AMD after adjustment for age-group and sex within each study and for smoking status (OR=1.83; CI: 1.04–3.23; P=0.04), with non-significant differences in risk associated with the two heterozygous *ε2* diplotypes (*ε2ε3*: OR=0.97; CI: 0.85–1.10; P=0.62; *ε2ε4*: OR=0.88; CI: 0.64–1.20; P=0.41) relative to the reference*ε3ε3* diplotype. Both the heterozygous *ε3ε4* diplotype (OR=0.71; CI: 0.64–0.80; $P=5.54 \times 10^{-9}$) and the homozygous *ε4ε4* diplotype (OR=0.45; CI: 0.29–0.69; $P=2.97 \times 10^{-4}$) showed significantly decreased risks compared to the reference *ε3ε3* diplotype for late AMD (Figure 1).

The estimate of the risk per *ε4* haplotype with late AMD was OR=0.72; CI: 0.65–0.79; $P=4.41 \times 10^{-11}$. Addition of interaction terms to the logistic model suggested no evidence of heterogeneity of the *ε4* haplotype risk between studies (χ^2=15.0, df=14, P=0.38; Figure 2). The risk estimate for the *ε2ε2* diplotype relative to the *ε3ε3*diplotype was OR=1.83; CI: 1.04–3.23; P=0.036, with little evidence of heterogeneity between studies (χ^2=19.8, df=14, P=0.14).

Analyses of *APOE* Diplotype and AMD Sub-Phenotype

Tests for heterogeneity of the OR for *ε4* between studies were not significant for any of the four AMD sub-phenotypes (NV: χ^2=12.0, df=14, P=0.61; GA: χ^2=9.90, df=14, P=0.77; GANV:χ^2=15.2, df=14, P=0.37; eAMD: χ^2=19.4, df=14, P=0.15). These results indicate that risk estimates per *ε4* haplotype were homogeneous across studies and so can be validly pooled. Corresponding tests for the *ε2ε2* diplotype were not considered robust because of the very low frequency of this diplotype and consequently are not presented.

Assessment of *ε2ε2* risk by AMD sub-phenotype (Table 2) resulted in similar non-significant increases in risk after adjustment for age-group, sex, smoking status and *ε4* (NV: OR=1.86; CI: 1.00–3.48; GA: OR=1.59; CI: 0.68–3.69; GANV: OR=1.82; CI: 0.68–4.85; eAMD: OR=1.59; CI: 0.96–2.65). Multinomial logistic regression analysis showed no significant difference in the *ε2ε2* effect between the three late AMD sub-phenotypes (χ^2=0.35, df=2, P=0.84) or between all late AMD combined and eAMD (χ^2=0.01, df=1, P=0.91) (Figure 3a).

There was a significant decrease in risk associated with each copy of *ε4* (Table 2) in all AMD sub-phenotypes (NV: OR=0.74; CI: 0.66–0.83; GA: OR=0.65; CI: 0.55–0.77; GANV: OR=0.71; CI: 0.59–0.85; eAMD: OR=0.84; CI: 0.77–0.92) after adjustment for age-group, sex, smoking status and *ε2*. The multinomial logistic model tests for differences in the *ε4* effects between the three late AMD sub-phenotypes (χ^2=4.33, df=2, P=0.11) and between all late AMD and eAMD (χ^2=2.52, df=1, P=0.11) were not significant (Figure 3b).

Sex and *APOE* Haplotype

Due to variability in recruitment procedures, including the use of some spouse controls in several of the case-control studies, sex was treated primarily as a confounder rather than an independent risk factor in the analysis. However, tests for differences between sexes were estimated in the late AMD risk associated with *APOE* by fitting interaction terms within the logistic regression model. These indicated no effect modification by sex for either *ε2* (χ^2=0.56, df=1, P=0.46) or *ε4* (χ^2=0.47, df=1, P=0.49). Analysis of *APOE* diplotype and late AMD in males and females separately is presented in Supp. Figure S1.

Smoking Status and AMD

Smoking status (ever smoker versus never smoker) was associated with a highly significant increased risk for late AMD after adjustment for age-group and sex (OR: 1.50; CI: 1.36–1.65: $P=7.92 \times 10^{-17}$; Table 2). Tests for differences between ever smokers and never smokers in the late AMD risk associated with *APOE* were obtained by fitting interaction terms. These indicated no effect modification by smoking for either *ε2* (χ^2=0.46, df=1, P=0.50) or *ε4* (χ^2=2.27, df=1, P=0.13).

Although smoking status was not significantly associated with increased risk in eAMD (OR=0.94; CI: 0.86–1.03; P=0.16), it was significantly associated with each of the late AMD sub-phenotypes (NV: OR=1.54; CI: 1.38–1.72; GA: OR=1.38; CI: 1.18–1.61; GANV: OR=1.51; CI: 1.27–1.79) after adjustment for age-group, sex and *APOE* diplotype (Figure 4).

The test for differences in the effect of smoking between each late AMD sub-phenotype was not significant (χ^2=3.4, df=2, P=0.18), but a difference in the effect of smoking was detected between late AMD and eAMD (χ^2=66.5, df=1, P<0.00001; Figure 4). Significant heterogeneity in the effect of smoking was also detected between studies (Figure 4) (eAMD: χ^2=34.0, df=14, P=0.002; NV: χ^2=48.9, df=14, P<0.001; Late AMD:χ^2=52.4, df=14, P<0.001). Moderate but non-significant heterogeneity was detected for the effect of smoking in GA (χ^2=21.9, df=14, P=0.08) but none in GANV (χ^2=11.7, df=14, P=0.63).

Test for differences between ever smokers and never smokers in late AMD risk associated with *APOE* were obtained by fitting interaction terms within the logistic regression model. These indicated no significant effect modification by smoking status for either*ε2* (χ^2=0.08, df=1, P=0.78) or *ε4* (χ^2=1.91, df=1, P=0.17). Analysis of *APOE* diplotype and late AMD in never smokers and ever smokers separately is presented in Supp. Figure S2.

Extended *APOE* Haplotype

Genotype data to estimate extended haplotype frequencies (rs405509, rs429358 and rs7412) with adjustment for age, sex and smoking were available for 1,739 late AMD cases and 4,725 controls. Haplotype frequency estimates using UNPHASED [Dudbridge, 2008] are presented in Table 3 and linkage disequilibrium values in Supp. Figure S3. Comparison of ORs between the different haplotypes based on the genotype at rs405509 (i.e. G-*ε2* v T-*ε2*, G-*ε3* v T-*ε3*, G-*ε4* v T*ε4*) showed no significant difference in effect between G-*ε2* and T-*ε2* (OR=1.58; CI: 0.85–2.95; P=0.15), between G-*ε3* and T-*ε3* (OR=0.99; CI: 0.88–1.11; P=0.85) or between G-*ε4* and T-*ε4* (OR=1.07; CI: 0.71–1.62; P=0.75) following adjustment for age, sex and smoking status. Comparison of extended haplotypes against a reference category G-*ε3*/G-*ε3* did not identify significant variation in risk beyond G-*ε3*/G-*ε4* (OR=0.61; CI: 0.38–0.99; P=0.04) and T-*ε4*/T-*ε4* (OR=0.27; CI: 0.08–0.85; P=0.03).

Discussion

The results generated for *ε4* from this pooled data analysis (OR=0.72 per haplotype; CI: 0.65–0.79; $P=4.41 \times 10^{-11}$) provide support for previous studies which identified a protective role with late AMD [Bojanowski et al., 2006; Klaver et al., 1998; Souied et al., 1998]. Previous studies of *APOE* were dominated by smaller reports involving multiple comparisons which tend to be more liable to publication biases. By conducting a pooled analysis in a large dataset of both published and previously unreported studies, we attempted to clarify the relationship between AMD risk and *APOE*. Variation in geographic distribution of *APOE* haplotype frequencies as measured in this large study also highlights the limitations of small studies whose power to detect associations diminishes with decreased haplotype frequency; for example, the frequency of the *ε4* haplotype in the control samples from the Edinburgh study (9.5%) was less than half that observed in the Melbourne study (20.8%) and a study in Edinburgh would thus have lower power than a study in Melbourne for any given sample size.

While the significantly increased risk associated with *ε2* for AMD has been reported previously [Fritsche et al., 2009], most studies have found non-significant increases in risk. This study has demonstrated a significant increased risk associated with *ε2ε2* for late AMD only (OR=1.83; CI: 1.04–3.23; P=0.04), with a corresponding low population attributable risk. The wide confidence intervals and imprecise estimates associated with the rare *ε2ε2* diplotype meant that, although our results were most consistent with a recessive genetic model, we could not entirely exclude an additive genetic model. Little heterogeneity was detected between studies for the genetic effect exerted by either *ε2* or*ε4* and, indeed, the genetic effects were not significantly different across all sub-phenotypes of AMD.

Previous reports have suggested that *APOE* effects may be stronger in women [Baird et al., 2004; Schmidt et al., 2002]. Our analyses did not find evidence of interaction between *APOE* haplotype risk and sex. Adjustment for both age and sex within studies was made using a logistic regression model, to adjust for possible confounding, but the differing designs of the studies included in this analysis were such that we could not use the data to assess the effect of age and sex on AMD risk. This would be best addressed in a population-based longitudinal study. An earlier meta-analysis examining the effect of *APOE* haplotype on coronary risk found no effect modification by sex in more than 100,000 participants [Bennet et al., 2007]. Similarly, we did not find evidence to support *APOE* effect modification by sex in AMD.

Smoking status (ever smoker versus never smoker) showed an increased risk associated with late AMD (OR=1.50; CI: 1.36–1.65) after adjustment for age-group, sex and *APOE* diplotype, and showed a non-significant difference in effect size between late AMD sub-

phenotypes. Our finding of no significant association between smoking status and eAMD suggests that smoking exacerbates early symptoms leading to the progression from eAMD to late AMD, supporting previous epidemiology-based findings [Clemons et al., 2005; Klein et al., 2008; Smith et al. 1996; Vingerling et al., 1996; Xu et al., 2006]. Cigarette smoke may influence macular pigment concentrations [Hammond et al., 1996; Stryker et al., 1988], increase oxidative stress [Beatty et al., 2000] and impair choroidal microcirculation [Suner et al., 2004], all processes hypothesized to be involved in the pathogenesis of AMD. Significant heterogeneity in smoking effect was detected across studies for late AMD, which may be attributable in part to variation in smoking criteria definitions. Standardization of definitions could not be imposed retrospectively beyond the level of ever smoker versus never smoker. As such, this study is limited in its ability to estimate the effect of smoking on disease outcome. Previous reports of effect modification of *APOE* haplotype by smoking status were not supported in this study suggesting that *APOE* haplotype and smoking status are independent risk factors.

Previously, rs405509 has been implicated in attenuating *APOE* promoter activity and subsequent expression [Artiga et al., 1998; Campillos et al., 2003; Ramos et al., 2005] with possible consequences in attenuating AMD risk [Fritsche et al., 2009]. Results reported in this analysis, however, do not support an influence of rs405509, with OR comparisons showing no significant difference in effect between G-*ε2* and T-*ε2*, G-*ε3* and T-*ε3* or G-*ε4* and T-*ε4* following adjustment for age group, sex and smoking status. However, several slight differences between this study and that of Fritsche and colleagues are worthy of consideration. Firstly, Fritsche and colleagues compared haplotype frequencies between all AMD cases and controls while this study compared frequencies between late AMD only and controls. Secondly, the original study compared the frequency of each haplotype combination against all others combined while this study compared the frequency of each haplotype against a single reference haplotype (G-*ε3*/G-*ε3*). Thirdly, while rs405509 should sufficiently define the extended *APOE* haplotype reported previously, additional SNPs were genotyped by Fritsche and colleagues which were not replicated in this study.

The retina hosts the body's second highest level of APOE production after the liver and is likely to play an important role in the maintenance of normal retinal function [Anderson et al., 2001]. Several possible mechanisms of APOE effect on AMD have been proposed such as variability in isoform dimerization potential associated with lipid cholesterol transport or variation in receptor binding affinity [Klaver et al., 1998]. In addition, the positively charged *ε4* haplotype has been proposed to improve permeability of Bruch's membrane, for lipid transport and reducing debris accumulation associated with drusen formation [Crabb et al., 2002; Souied et al., 1998]. In older eyes, evidence supporting reduced lipoprotein transportation across Bruch's membrane was reported as a consequence of ageing, leading to drusen deposition and RPE insult [Curcio et al., 2009]. More recently evidence to support*ε4* as a potential lipoprotein transporter of the macular pigments lutein and zeaxanthin, has been suggested [Loane et al., 2010] and reduced dietary intake of these carotenoids has been associated with increased risk of AMD [Seddon et al., 1994]. As such, variation in genes that modulate retinal cholesterol levels may influence AMD risk [Connor et al., 2007].

The strengths and limitations of the current study should be considered. Our analysis is almost five times larger than published meta-analyses [Bojanowski et al., 2006; Swaroop et al., 2007; Thakkinstian et al., 2006] and, although we cannot completely exclude publication bias in our estimates, this should be limited by our inclusion of both published and unpublished studies. Access to individual level data enabled appropriate adjustment to limit potential confounding by sex, age and smoking and assessment of potential interaction between covariates. In addition, phenotypic sub-classification facilitated stratification by disease sub-phenotype identifying smoking as a major contributing factor to late AMD but

not as a risk factor for the early form of the disease. However, variation in recruitment procedures between studies and indeed study type, i.e., case control versus population, limited the ability to measure the full influence of age, sex and smoking on disease risk. Nevertheless, comparison of effect sizes between population and case control studies, showed no significant differences in the odds ratios for *ε2ε2*,*ε4* and smoking status between the two different study designs.

Recent GWAS identified novel variants in the *LIPC* and *CETP* genes associated with AMD and cholesterol level modulation, suggesting some alleles may influence cholesterol levels in the macula and in the blood in opposite directions [Chen et al., 2010; Neale et al., 2010]. Improved understanding of APOE effect on AMD disease etiology will improve accuracy of AMD risk prediction models, eventually offering some therapeutic benefit. While the complexity surrounding APOE and cholesterol modulation within the retina remains, potential benefit derived from statin therapy for the treatment of cardiovascular disease (CVD) warrants further investigation in AMD, given the overlap in risk factors for both conditions [Snow and Seddon, 1999]. While *ε4* significantly increases risk associated with CVD, atherosclerosis, Alzheimer's disease (AD) and other dementias [Ang et al., 2008], it clearly plays a protective role with respect to AMD. The mechanisms by which APOE and cholesterol levels modulate AMD risk, especially with respect to the opposing effects reported for other complex diseases such as AD and CVD, are worthy of further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the patients, their families, and the control subjects who participated in the study. This work was supported in part by grants from The Guide Dogs for the Blind Association UK (2008-5a (GS) and OR2006-02d (ATM, JRWY)); Medical Research Council (JRWY, ATM G0000067); Research and Development Office, Northern Ireland Health Personal Social Services (RRG 4.5 - GS); EVI-GENORET, an integrated project funded through the European Union Research Project (FP6 - UC); The Deutsche Forschungsgemeinschaft (WE1259/18-1 and WE1259/19-1 to BHFW); the Alcon Research Institute and the Ruth and Milton Steinbach Foundation New York (BHFW). Russo Grant from Tufts University School of Medicine (JMS); Macular Degeneration Research Fund-Tufts Medical Center (JMS); Massachusetts Lions Eye Research Fund (JMS); Research to Prevent Blindness USA (MLK, PJF); Foundation Fighting Blindness (PJF); financial support from the Department of Health through the award made by the National Institute for Health Research to Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology for a Specialist Biomedical Research Centre for Ophthalmology (JRWY, ATM); the National Health & Medical Research Council of Australia Centre for Clinical Research Excellence #529923 – Translational Clinical Research in Major Eye Diseases and a Practitioner Fellowship to RHG. The Centre for Eye Research Australia receives operational infrastructure support from the Victorian Government (PNB, RHG); Macular Disease Society (GMK and AJL); T.F.C. Frost Charity (AJL) and British Council for the Prevention of Blindness (AJL). Genetic analysis in the EUREYE study was supported by an MRC Biomarker Award G0601354 (AH and AF). Target funding (SF0940026s07) by the Estonian Ministry of Education and Science (MR).

References

Anderson DH, Ozaki S, Nealon M, Neitz J, Mullins RF, Hageman GS, Johnson LV. Local cellular sources of apolipoprotein E in the human retina and retinal pigmented epithelium: implications for the process of drusen formation. Am J Ophthalmol. 2001; 131:767–781. [PubMed: 11384575]

Ang LS, Cruz RP, Hendel A, Granville DJ. Apolipoprotein E, an important player in longevity and age-related diseases. Exp Gerontol. 2008; 43:615–622. [PubMed: 18468826]

Artiga MJ, Bullido MJ, Frank A, Sastre I, Recuero M, García MA, Lendon CL, Han SW, Morris JC, Vázquez J, Goate A, Valdivieso F. Risk for Alzheimer's disease correlates with transcriptional activity of the *APOE* gene. Hum Mol Gene. 1998; 7:1887–1892.

Augood C, Fletcher A, Bentham G, Chakravarthy U, de Jong PT, Rahu M, Seland J, Soubrane G, Tomazzoli L, Topouzis F, Vioque J, Young I. Methods for a population-based study of the prevalence of and risk factors for age-related maculopathy and macular degeneration in elderly European populations: the EUREYE study. Ophthalmic Epidemiol. 2004; 11:117–129. [PubMed: 15255027]

Baird PN, Guida E, Chu DT, Vu HT, Guymer RH. The E2 and E4 alleles of the apolipoprotein gene are associated with age-related macular degeneration. Invest Ophthalmol Vis Sci. 2004; 45:1311–1315. [PubMed: 15111582]

Baird PN, Richardson AJ, Robman LD, Dimitrov PN, Tikellis G, McCarty CA, Guymer RH. Apolipoprotein (*APOE*) gene is associated with progression of age-related macular degeneration (AMD). Hum Mutat. 2006; 27:337–342. [PubMed: 16453339]

Beatty S, Koh H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. Surv Ophthalmol. 2000; 45:115–134. [PubMed: 11033038]

Bennet AM, di Angelantonio E, Ye Z, Wensley F, Dahlin A, Ahlbom A, Keavney B, Collins R, Wiman B, de Faire U, Danesh J. Association of apolipoprotein E genotypes with lipid levels and coronary risk. JAMA. 2007; 298:1300–1311. [PubMed: 17878422]

Bergeron-Sawitzke J, Gold B, Olsh A, Schlotterbeck S, Lemon K, Visvanathan K, Allikmets R, Dean M. Multilocus analysis of age-related macular degeneration. Eur J Hum Genet. 2009; 17:1190–1199. [PubMed: 19259132]

Bojanowski CM, Shen D, Chew EY, Ning B, Csaky KG, Green WR, Chan CC, Tuo J. An apolipoprotein E variant may protect against age-related macular degeneration through cytokine regulation. Environ Mol Mutagen. 2006; 47:594–602. [PubMed: 16823865]

Campillos M, Lamas JR, Garcia MA, Bullido MJ, Valdivieso F, Vazquez J. Specific interaction of heterogeneous nuclear ribonucleoprotein A1 with the -219T allelic form modulates *APOE* promoter activity. Nucleic Acids Res. 2003; 31:3063–3070. [PubMed: 12799433]

Canter JA, Olson LM, Spencer K, Schnetz-Boutaud N, Anderson B, Hauser MA, Schmidt S, Postel EA, Agarwal A, Pericak-Vance MA, Sternberg P Jr, Haines JL. Mitochondrial DNA polymorphism A4917G is independently associated with age-related macular degeneration. PLoS ONE. 2008; 3:e2091. [PubMed: 18461138]

Centers for Disease Control and Prevention (CDC). Prevalence of visual impairment and selected eye diseases among persons aged >/= 50 years with and without diabetes- United States, 2002. MMWR Morb Mortal Wkly Rep. 2004; 53:1069–1071. [PubMed: 15549022]

Chen W, Stambolian D, Edwards AO, Branham KE, Othman M, Jakobsdottir J, Tosakulwong N, Pericak-Vance MA, Campochiaro PA, Klein ML, Tan PL, Conley YP, Kanda A, Kopplin L, Li Y, Augustaitis KJ, Karoukis AJ, Scott WK, Agarwal A, Kovach JL, Schwartz SG, Postel EA, Brooks M, Baratz KH, Brown WL, Brucker AJ, Orlin A, Brown G, Ho A, Regillo C, Donoso L, Tian L, Kaderli B, Hadley D, Hagstrom SA, Peachey NS, Klein R, Klein BE, Gotoh N, Yamashiro K, Ferris Iii F, Fagerness JA, Reynolds R, Farrer LA, Kim IK, Miller JW, Cortón M, Carracedo A, Sanchez-Salorio M, Pugh EW, Doheny KF, Brion M, Deangelis MM, Weeks DE, Zack DJ, Chew EY, Heckenlively JR, Yoshimura N, Iyengar SK, Francis PJ, Katsanis N, Seddon JM, Haines JL, Gorin MB, Abecasis GR, Swaroop A. Complications of Age-Related Macular Degeneration Prevention Trial Research Group. Genetic variants near *TIMP3* and high-density lipoprotein-associated loci influence susceptibility to age-related macular degeneration. Proc Natl Acad Sci USA. 2010; 107:7401–7406. [PubMed: 20385819]

Clemons TE, Milton RC, Klein R, Seddon JM, Ferris FL 3rd. Age-Related Eye Disease Study Research Group. Risk factors for the incidence of Advanced Age-Related Macular Degeneration in the Age-Related Eye Disease Study (AREDS) AREDS report no. 19. Ophthalmol. 2005; 112:533–539.

Conley YP, Thalamuthu A, Jakobsdottir J, Weeks DE, Mah T, Ferrell RE, Gorin MB. Candidate gene analysis suggests a role for fatty acid biosynthesis and regulation of the complement system in the etiology of age-related maculopathy. Hum Mol Genet. 2005; 14:1991–2002. [PubMed: 15930014]

Connor WE, Duell PB, Kean R, Wang Y. The prime role of HDL to transport lutein into the retina: evidence from HDL-deficient WHAM chicks having a mutant *ABCA1* transporter. Invest Ophthalmol Vis Sci. 2007; 48:4226–4231. [PubMed: 17724211]

Corbo RM, Scacchi R. Apolipoprotein E (*APOE*) allele distribution in the world. Is APOE*4 a 'thrifty' allele? Ann Hum Genet. 1999; 63:301–310. [PubMed: 10738542]

Crabb JW, Miyagi M, Gu X, Shadrach K, West KA, Sakaguchi H, Kamei M, Hasan A, Yan L, Rayborn ME, Salomon RG, Hollyfield JG. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. Proc Natl Acad Sci USA. 2002; 9:14682–14687. [PubMed: 12391305]

Curcio CA, Johnson M, Huang JD, Rudolf M. Aging, age-related macular degeneration, and the response-to-retention of apolipoprotein B-containing lipoproteins. Prog Retin Eye Res. 2009; 28:393–422. [PubMed: 19698799]

Dandekar SS, Jenkins SA, Peto T, Bird AC, Webster AR. Does smoking influence the type of age related macular degeneration causing visual impairment? Br J Ophthalmol. 2006; 90:724–727. [PubMed: 16597668]

Dewan A, Liu M, Hartman S, Zhang SS, Liu DT, Zhao C, Tam PO, Chan WM, Lam DS, Snyder M, Barnstable C, Pang CP, Hoh J. *HTRA1* promoter polymorphism in wet age-related macular degeneration. Science. 2006; 314:989–992. [PubMed: 17053108]

Dudbridge F. Likelihood-based association analysis for nuclear families and unrelated subjects with missing genotype data. Hum Hered. 2008; 66:87–98. [PubMed: 18382088]

Edwards AO, Ritter R III, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science. 2005; 308:421–424. [PubMed: 15761121]

Ennis S, Jomary C, Mullins R, Cree A, Chen X, Macleod A, Jones S, Collins A, Stone E, Lotery A. Association between the *SERPING1* gene and age-related macular degeneration: a two-stage case-control study. Lancet. 2008; 372:1828–1834. [PubMed: 18842294]

Evans J, Wormald R. Is the incidence of registrable age-related macular degeneration increasing? Br J Ophthalmol. 1996; 80:9–14. [PubMed: 8664242]

Fagerness JA, Maller JB, Neale BM, Reynolds RC, Daly MJ, Seddon JM. Variation near complement factor I is associated with risk of advanced AMD. Eur J Hum Genet. 2009; 17:100–104. [PubMed: 18685559]

Francis PJ, Hamon SC, Ott J, Weleber RG, Klein ML. Polymorphisms in *C2*, *CFB* and *C3* are associated with progression to advanced age related macular degeneration associated with visual loss. J Med Genet. 2009; 46:300–307. [PubMed: 19015224]

Fritsche LG, Loenhardt T, Janssen A, Fisher SA, Rivera A, Keilhauer CN, Weber BH. Age-related macular degeneration is associated with an unstable *ARMS2* (*LOC387715*) mRNA. Nat Genet. 2008; 40:892–896. [PubMed: 18511946]

Fritsche LG, Freitag-Wolf S, Bettecken T, Meitinger T, Keilhauer CN, Krawczak M, Weber BH. Age-related macular degeneration and functional promoter and coding variants of the apolipoprotein E gene. Hum Mutat. 2009; 30:1048–1053. [PubMed: 19384966]

Gold B, Merriam JE, Zernant J, Hancox LS, Taiber AJ, Gehrs K, Cramer K, Neel J, Bergeron J, Barile GR, Smith RT, Hageman GS, Dean M, Allikmets R. 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; 38:458–462. [PubMed: 16518403]

Haan MN, Klein R, Klein BE, Deng Y, Blythe LK, Seddon JM, Musch DC, Kuller LH, Hyman LG, Wallace RB. Hormone therapy and age-related macular degeneration: the Women's Health Initiative Sight Exam Study. Arch Ophthalmol. 2006; 124:988–992. [PubMed: 16832022]

Hadley D, Orlin A, Brown G, Brucker AJ, Ho AC, Regillo CD, Donoso LA, Tian L, Kaderli B, Stambolian D. Analysis of six genetic risk factors highly associated with AMD in the region surrounding *ARMS2* and *HTRA1* on chromosome 10q26. Invest Ophthalmol Vis Sci. 2010; 51:2191–2196. [PubMed: 19933195]

Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI, Hageman JL, Stockman HA, Borchardt JD, Gehrs KM, Smith RJ, Silvestri G, Russell SR, Klaver CC, Barbazetto I, Chang S, Yannuzzi LA, Barile GR, Merriam JC, Smith RT, Olsh AK, Bergeron J, Zernant J, Merriam JE, Gold B, Dean M, Allikmets R. A common haplotype in the complement regulatory gene factor H (*HF1/CFH*) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci USA. 2005; 102:7227–7232. [PubMed: 15870199]

Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Noureddine M, Gilbert JR, Schnetz-Boutaud N, Agarwal A, Postel EA, Pericak-Vance MA. Complement factor H variant increases the risk of age-related macular degeneration. Science. 2005; 308:419–421. [PubMed: 15761120]

Hammond BR Jr, Wooten BR, Snodderly DM. Cigarette smoking and the retinal carotenoids: implications for age-related macular degeneration. Vision Res. 1996; 18:3003–3009. [PubMed: 8917800]

Hughes AE, Orr N, Esfandiary H, Diaz-Torres M, Goodship T, Chakravarthy U. A common *CFH* haplotype, with deletion of *CFHR1* and *CFHR3*, is associated with lower risk of age-related macular degeneration. Nat Genet. 2006; 38:1173–1177. [PubMed: 16998489]

Jakobsdottir J, Conley YP, Weeks DE, Mah TS, Ferrell RE, Gorin MB. Susceptibility genes for age-related maculopathy on chromosome 10q26. Am J Hum Genet. 2005; 77:389–407. [PubMed: 16080115]

Kanda A, Chen W, Othman M, Branham KE, Brooks M, Khanna R, He S, Lyons R, Abecasis GR, Swaroop A. A variant of mitochondrial protein *LOC387715/ARMS2*, not *HTRA1*, is strongly associated with age-related macular degeneration. Proc Natl Acad Sci USA. 2007; 104:16227–16232. [PubMed: 17884985]

Klaver CC, Kliffen M, van Duijn CM, Hofman A, Cruts M, Grobbee DE, van Broeckhoven C, de Jong PT. Genetic association of apolipoprotein E with age-related macular degeneration. Am J Hum Genet. 1998; 63:200–206. [PubMed: 9634502]

Klein R, Klein B, Linton K. Prevalence of age-related maculopathy: the Beaver Dam Eye study. Ophthalmol. 1992; 99:933–943.

Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J. Complement factor H polymorphism in age-related macular degeneration. Science. 2005; 308:385–389. [PubMed: 15761122]

Klein R, Knudtson MD, Cruickshanks KJ, Klein BE. Further observations on the association between smoking and the long-term incidence and progression of age-related macular degeneration: the Beaver Dam Eye Study. Arch Ophthalmol. 2008; 126:115–121. [PubMed: 18195228]

Loane E, McKay GJ, Nolan JM, Beatty S. Apolipoprotein E genotype is associated with macular pigment optical density. Invest Ophthalmol Vis Sci. 2010; 51:2636–2643. [PubMed: 20107178]

Maller JB, Fagerness JA, Reynolds RC, Neale BM, Daly MJ, Seddon JM. Variation in complement factor 3 is associated with risk of age-related macular degeneration. Nat Genet. 2007; 39:1200–1201. [PubMed: 17767156]

McKay GJ, Silvestri G, Patterson CC, Hogg RE, Chakravarthy U, Hughes AE. Further assessment of the Complement Component 2 and Factor B region associated with Age-related Macular Degeneration. Invest Ophthalmol Vis Sci. 2009; 50:533–539. [PubMed: 18806297]

McKay GJ, Dasari S, Patterson CC, Chakravarthy U, Silvestri G. Complement component 3: an assessment of association with AMD and analysis of gene-gene and gene-environment interactions in a Northern Irish cohort. Mol Vis. 2010; 16:194–199. [PubMed: 20157618]

McKay GJ, Silvestri G, Chakravarthy U, Dasari S, Fritsche LG, Weber BH, Keilhauer CN, Klein ML, Francis PJ, Klaver CC, Vingerling JR, Ho L, de Jong PTDV, Dean M, Sawitzke J, Baird PN, Guymer RH, Stambolian D, Orlin A, Seddon JM, Peter I, Wright AF, Hayward C, Lotery AJ, Ennis S, Gorin MB, Weeks DE, Kuo CL, Hingorani AD, Sofat R, Cipriani V, Swaroop A, Othman M, Kanda A, Chen W, Abecasis GR, Yates JR, Webster AR, Moore AT, Seland JH, Rahu M, Soubrane G, Tomazzoli L, Topouzis F, Vioque J, Young IS, Fletcher AE, Patterson CC. Variations with age in Apolipoprotein E frequency in a pooled analysis of a large group of older people. Am J Epidemiol. 2011; 173:1357–1364. [PubMed: 21498624]

Minelli C, Thompson JR, Abrams KR, Thakkinstian A, Attia J. The choice of a genetic model in the meta-analysis of molecular association studies. Int J Epidemiol. 2005; 34:1319–1328. [PubMed: 16115824]

Neale BM, Fagerness J, Reynolds R, Sobrin L, Parker M, Raychaudhuri S, Tan PL, Oh EC, Merriam JE, Souied E, Bernstein PS, Li B, Frederick JM, Zhang K, Brantley MA Jr, Lee AY, Zack DJ, Campochiaro B, Campochiaro P, Ripke S, Smith RT, Barile GR, Katsanis N, Allikmets R, Daly MJ, Seddon JM. Genome-wide association study of advanced age-related macular degeneration

identifies a role of the hepatic lipase gene (*LIPC*). Proc Natl Acad Sci USA. 2010; 107:7395–7400. [PubMed: 20385826]

Park KH, Fridley BL, Ryu E, Tosakulwong N, Edwards AO. Complement Component 3 (*C3*) Haplotypes and Risk of Advanced Age-Related Macular Degeneration. Invest Ophthalmol Vis Sci. 2009; 50:3386–3393. [PubMed: 19234341]

Ramos MC, Matias S, Artiga MJ, Pozueta J, Sastre I, Valdivieso F, Bullido MJ. Neuronal specific regulatory elements in apolipoprotein E gene proximal promoter. Neuroreport. 2005; 16:1027–1030. [PubMed: 15931082]

Rivera A, Fisher SA, Fritsche LG, Keilhauer CN, Lichtner P, Meitinger T, Weber BH. Hypothetical *LOC387715* is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. Hum Mol Genet. 2005; 14:3227–3236. [PubMed: 16174643]

San Giovanni JP, Arking DE, Iyengar SK, Elashoff M, Clemons TE, Reed GF, Henning AK, Sivakumaran TA, Xu X, DeWan A, Agrón E, Rochtchina E, Sue CM, Wang JJ, Mitchell P, Hoh J, Francis PJ, Klein ML, Chew EY, Chakravarti A. Mitochondrial DNA variants of respiratory complex I that uniquely characterize haplogroup T2 are associated with increased risk of age-related macular degeneration. PLoS ONE. 2009; 4:e5508. [PubMed: 19434233]

Schmidt S, Klaver C, Saunders A, Postel E, De La Paz M, Agarwal A, Small K, Udar N, Ong J, Chalukya M, Nesburn A, Kenney C, Domurath R, Hogan M, Mah T, Conley Y, Ferrell R, Weeks D, de Jong PT, van Duijn C, Haines J, Pericak-Vance M, Gorin M. A pooled case–control study of the apolipoprotein E (*APOE*) gene in age-related maculopathy. Ophthalmic. Genet. 2002; 23:209–223.

Schmidt S, Haines JL, Postel EA, Agarwal A, Kwan SY, Gilbert JR, Pericak-Vance MA, Scott WK. Joint effects of smoking history and *APOE* genotypes in age-related macular degeneration. Mol Vis. 2005; 11:941–949. [PubMed: 16288198]

Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC, Farber MD, Gragoudas ES, Haller J, Miller DT, Yannuzzi LA, Willett W. the Eye Disease Case-Control Study Group. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. JAMA. 1994; 272:1413–1420. [PubMed: 7933422]

Siest G, Pillot T, Régis-Bailly A, Leininger-Muller B, Steinmetz J, Galteau MM, Visvikis S. Apolipoprotein E: an important gene and protein to follow in laboratory medicine. Clin Chem. 1995; 41:1068–1086. [PubMed: 7628082]

Smith W, Mitchell P, Leeder SR. Smoking and age-related maculopathy: the Blue Mountain Eye Study. Arch Ophthalmol. 1996; 114:1518–1523. [PubMed: 8953988]

Snow KK, Seddon JM. Do age-related macular degeneration and cardiovascular disease share common antecedents? Ophthalmic Epidemiol. 1999; 6:125–143. [PubMed: 10420212]

Souied EH, Benlian P, Amouyel P, Feingold J, Lagarde JP, Munnich A, Kaplan J, Coscas G, Soubrane G. The epsilon4 allele of the apolipoprotein E gene as a potential protective factor for exudative age-related macular degeneration. Am J Ophthalmol. 1998; 125:353–359. [PubMed: 9512153]

Stryker WS, Kaplan LA, Stein EA, Stampfer MJ, Sober A, Willett WC. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. Am J Epidemiol. 1988; 127:283–296. [PubMed: 3257350]

Suner IJ, Espinosa-Heidmann DG, Marin-Castano ME, Hernandez EP, Pereira-Simon S, Cousins SW. Nicotine increases size and severity of experimental choroidal neovascularization. Invest Ophthalmol Vis Sci. 2004; 45:311–317. [PubMed: 14691189]

Swaroop A, Branham KE, Chen W, Abecasis G. Genetic susceptibility to age-related macular degeneration: a paradigm for dissecting complex disease traits. Hum Mol Genet. 2007; 16(Spec No 2):R174–182. [PubMed: 17911160]

Thakkinstian A, Bowe S, McEvoy M, Smith W, Attia J. Association between apolipoprotein E polymorphisms and age-related macular degeneration: A HuGE review and meta-analysis. Am J Epidemiol. 2006; 164:813–822. [PubMed: 16916985]

van Leeuwen R, Klaver CC, Vingerling JR, Hofman A, van Duijn CM, Stricker BH, de Jong PT. Cholesterol and age-related macular degeneration: is there a link? Am J Ophthalmol. 2004; 137:750–752. [PubMed: 15059717]

Vingerling JR, Dielemans I, Hofman A, Grobbee DE, Hijmering M, Kramer CF, de Jong PT. The prevalence of age-related maculopathy in the Rotterdam Study. Ophthalmol. 1995; 102:205–210.

Vingerling JR, Hofman A, Grobbee DE, de Jong PT. Age-related macular degeneration and smoking: the Rotterdam Study. Arch Ophthalmol. 1996; 114:1193–1196. [PubMed: 8859077]

Xu L, Li Y, Zheng Y, Jonas JB. Associated factors for age-related maculopathy in the adult population in China: the Beijing Eye Study. Br J Ophthalmol. 2006; 90:1087–1090. [PubMed: 16774957]

Yang Z, Camp NJ, Sun H, Tong Z, Gibbs D, Cameron DJ, Chen H, Zhao Y, Pearson E, Li X, Chien J, Dewan A, Harmon J, Bernstein PS, Shridhar V, Zabriskie NA, Hoh J, Howes K, Zhang K. A variant of the *HTRA1* gene increases susceptibility to age-related macular degeneration. Science. 2006; 314:992–993. [PubMed: 17053109]

Yates JR, Sepp T, Matharu BK, Khan JC, Thurlby DA, Shahid H, Clayton DG, Hayward C, Morgan J, Wright AF, Armbrecht AM, Dhillon B, Deary IJ, Redmond E, Bird AC, Moore AT. Genetic Factors in AMD Study Group. Complement *C3* variant and the risk of age-related macular degeneration. N Engl J Med. 2007; 357:553–561. [PubMed: 17634448]

Zarbin MA. Current concepts in the pathogenesis of age-related macular degeneration. Arch Ophthalmol. 2004; 122:598–614. [PubMed: 15078679]

Zareparsi S, Reddick AC, Branham KE, Moore KB, Jessup L, Thoms S, Smith-Wheelock M, Yashar BM, Swaroop A. Association of apolipoprotein E alleles with susceptibility to age-related macular degeneration in a large cohort from a single center. Invest Ophthalmol Vis Sci. 2004; 45:1306–1310. [PubMed: 15111581]

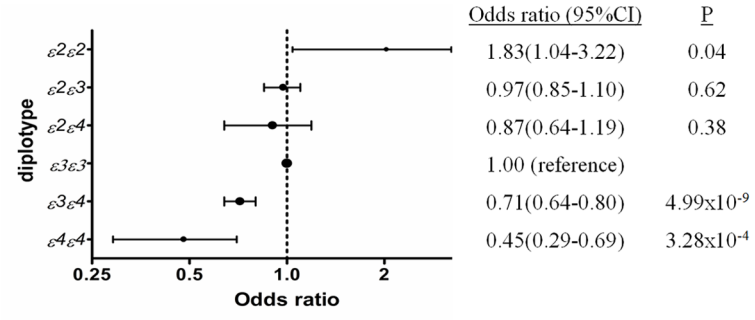

Figure 1.

Analysis of *APOE* diplotype and late AMD. Late AMD includes categories GA, NV and GANV. Odds ratios against the reference (*ε3ε3*), 95% confidence intervals and P values were adjusted for age-group and gender within each study and for smoking status (ever versus never smoker).

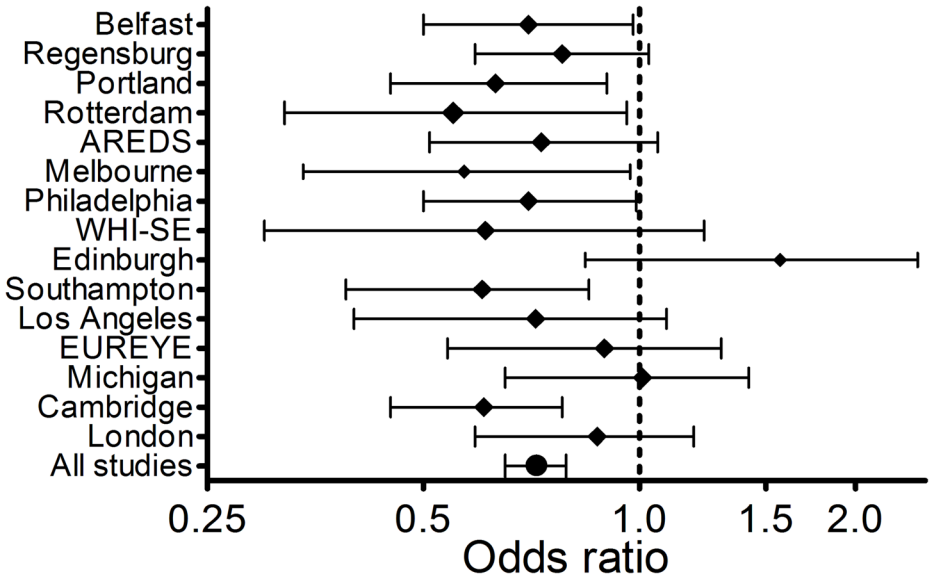

Figure 2.

Odds ratios and confidence intervals for late AMD calculated for one copy of *ε4* by study (additive model with adjustment for age-group and gender within each study and for smoking status). Meta-analyses tests for heterogeneity of *ε4* effects between studies were not significant either for late AMD (χ^2=15.0, df=14, P=0.38) or for any AMD sub-phenotype (NV:χ^2=12.0, df=14, P=0.61; GA: χ^2=9.90, df=14, P=0.77; GANV: χ^2=15.2, df=14, P=0.37; eAMD:χ^2=19.4, df=14, P=0.15).

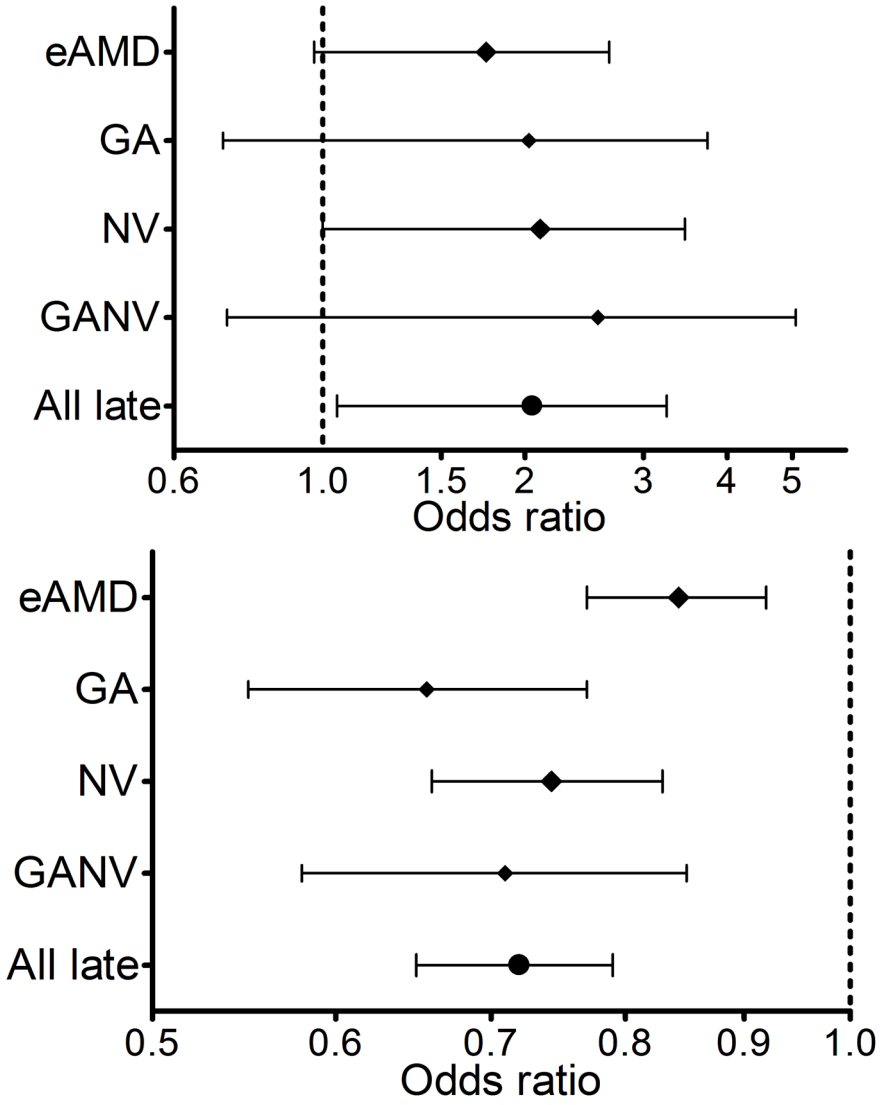

Figure 3.

Odds ratio associated with (a)*ε2* diplotype (recessive model) and (b) *ε4* haplotype and late AMD (GA, NV and GANV samples combined) were estimated after adjustment for age-group and gender within each study and smoking status. (a) *ε2* diplotype (recessive model): OR=1.83; CI: 1.04–3.23; P=0.04 (Table 2). (b) *ε4* per haplotype (additive model): OR=0.72; CI: 0.65–0.79; $P=4.41 \times 10^{-11}$ (Table 2). No significant heterogeneity in associated risk was detected between each late AMD sub-phenotype (*ε2ε2*: $\chi^2=0.35$, df=2, P=0.84; *ε4*: $\chi^2=4.33$, df=2, P=0.11) or between all late AMD and eAMD ((*ε2ε2*: $\chi^2=0.01$, df=1, P=0.91; *ε4*: $\chi^2=2.52$, df=1, P=0.11).

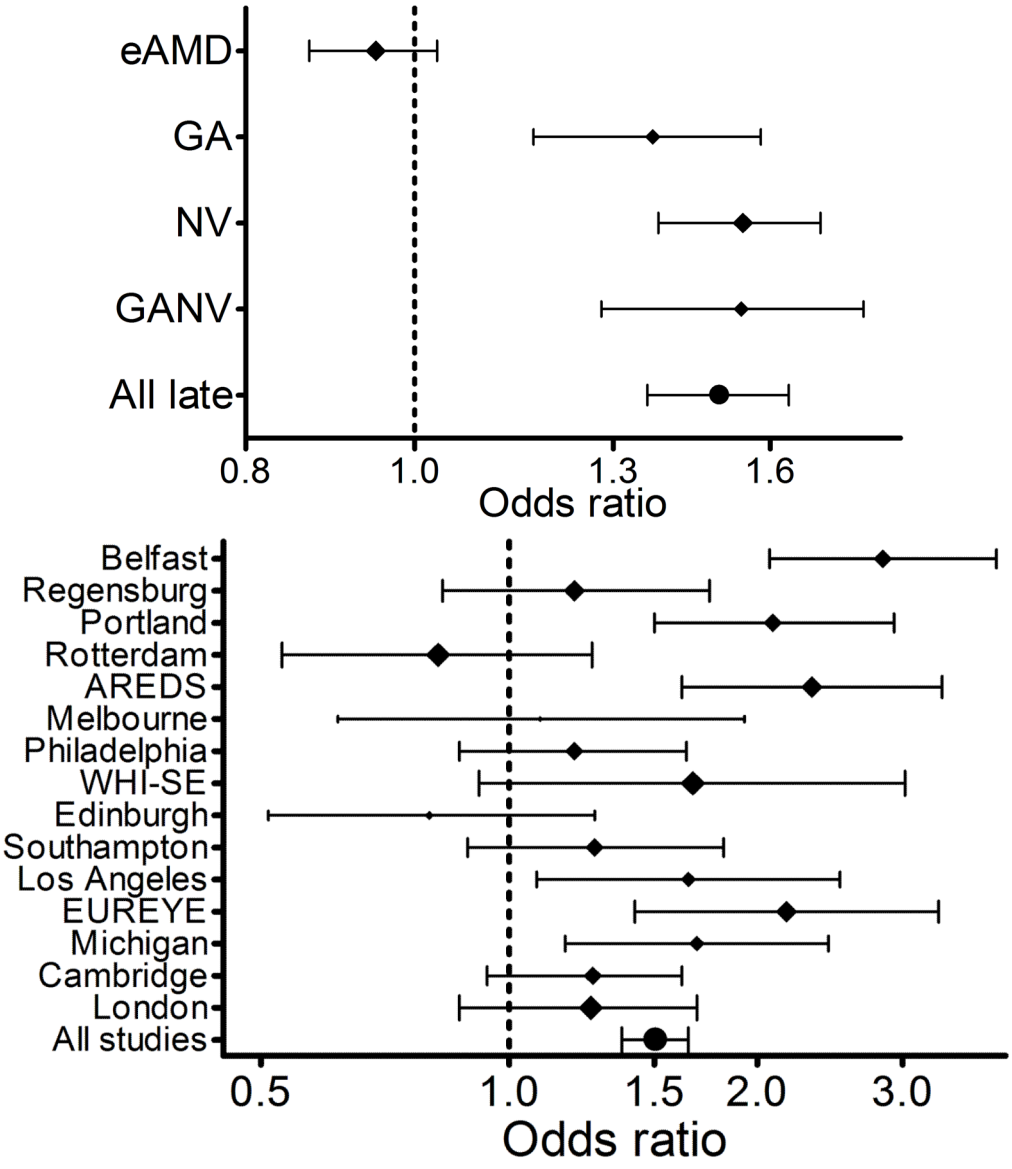

Figure 4.

Odds ratios and confidence intervals for smoking status by (a) AMD phenotype and by (b) study for late AMD only. Late AMD is composed of all GA, NV and GANV samples combined. Smoking status was categorized as ever smoker versus never smoker. ORs were estimated and adjusted for age-group and gender within each study, for smoking status and *APOE* diplotype (late AMD OR: 1.50; CI: 1.36–1.65: $P=7.92 \times 10^{-17}$). Smoking status was not significantly associated with increased risk of eAMD (OR=0.94; CI: 0.86–1.03; P=0.16). Tests for heterogeneity in smoking effect between each late AMD sub-phenotype were not significant, but a difference in the effect of smoking was detected between all late AMD and eAMD. Significant heterogeneity in smoking effect between studies was detected for late AMD, NV, but not for GA.

Table 1

Summary data on subjects from contributing studies showing sample size, disease status, average age and sex composition and smoking status

									All subjects	
Study	**Unaffected n**	**Average Age**	**Affected n**	**Average Age**	**eAMD n (%)**	**GA n (%)**	**NV n (%)**	**GANV n (%)**	**% male**	**% Never Smoker**
Belfast	436	74.8	479	77.4	29 (0.06)	31 (0.06)	419 (0.88)	0 (0.00)	39	54
Regensburg	553	76.2	724	76.7	85 (0.12)	143 (0.20)	391 (0.54)	105 (0.15)	37	87
Portland	272	73.9	646	78.1	0 (0.00)	162 (0.25)	422 (0.65)	62 (0.10)	38	46
Rotterdam	3805	67.5	554	76.5	451 (0.81)	43 (0.08)	34 (0.06)	26 (0.05)	41	37
AREDS	199	77.1	879	79.9	240 (0.27)	162 (0.18)	306 (0.35)	171 (0.19)	42	47
Melbourne	106	71.5	202	74.7	36 (0.18)	37 (0.19)	98 (0.49)	28 (0.14)	38	46
Philadelphia	379	75.6	519	80.1	153 (0.29)	38 (0.07)	263 (0.51)	65 (0.13)	43	57
WHI - SE[#]	1283	73.7	422	74.5	374 (0.89)	13 (0.03)	31 (0.07)	4 (0.01)	0	55
Edinburgh	179	73.2	325	75.8	134 (0.41)	38 (0.12)	153 (0.47)	0 (0.00)	38	47
Southampton	458	70.7	468	78.4	183 (0.39)	58 (0.12)	102 (0.22)	125 (0.27)	42	39
Los Angeles	142	72.5	639	70.0	52 (0.08)	152 (0.24)	254 (0.40)	177 (0.28)	38	45
EUREYE	1935	72.3	2254	73.8	2114 (0.94)	42 (0.02)	98 (0.04)	0 (0.00)	45	53
Michigan	253	76.6	494	80.3	62 (0.12)	117 (0.24)	195 (0.40)	120 (0.24)	39	44
Cambridge	417	75.0	840	79.0	29 (0.03)	141 (0.17)	550 (0.66)	120 (0.14)	43	39
London	206	74.9	1099	77.3	201 (0.18)	193 (0.18)	619 (0.56)	86 (0.08)	36	37
Total	10623	71.5	10544	76.5	4143 (0.39)	1370 (0.13)	3935 (0.37)	1089 (0.10)	38	50

AREDS: Age-related Eye Disease Study;

[#]WHI-SE: Women's Health Initiative Sight Exam.

Table 2

Multivariable analysis of *APOE* genotype and AMD sub-phenotype were adjusted for age-group and gender within each study and for smoking status

	Late AMD (n=6,199)			NV (n=3,821)			GA (n=1,320)			GANV (n=1,058)			eAMD (n=4,102)		
Risk Factor	**OR**	**CI**	**P value**	**OR**	**CI**	**P value**	**OR**	**CI**	**P value**	**OR**	**CI**	**P value**	**OR**	**CI**	**P value**
rec$\varepsilon 2^{\dagger}$	1.83	1.04–3.23	0.04	1.86	1.00–3.48	0.05	1.59	0.68–3.69	0.28	1.82	0.68–4.85	0.23	1.59	0.96–2.65	0.07
add$\varepsilon 4^{\ddagger}$	0.72	0.65–0.79	4.41×10^{-11}	0.74	0.66–0.83	7.88×10^{-8}	0.65	0.55–0.77	6.09×10^{-7}	0.71	0.59–0.85	2.56×10^{-4}	0.84	0.77–0.92	2.04×10^{-4}
Smoking	1.50	1.36–1.65	7.92×10^{-17}	1.54	1.38–1.72	2.80×10^{-15}	1.38	1.18–1.61	3.37×10^{-5}	1.51	1.27–1.79	3.49×10^{-6}	0.94	0.86–1.03	0.16
λ_1	0.14	0.01–0.89													
λ_2	0.49	0.27–0.89													

APOε2 associated risk assumes a recessive model. Smoking status is defined as ever versus never smoker. Late AMD includes categories GA, NV and GANV.

Age (in 5-year intervals) was treated as a categorical variable within the regression model.

Variation in recruitment policies in each study necessitated adjustment by age-group and gender within each study.

†risk associated with *ε2ε2* genotpye;

‡risk associated with each copy of *ε4*;

λ_1 = log OR(ε2ε3 vs ε3ε3)/log OR(ε2ε2 vs ε3ε3); λ_2 = log OR(ε3ε4 vs ε3ε3)/log OR(ε4ε4 vsε3ε3).

Table 3

Extended haplotypes were inferred from genotype data at rs405509 (G/T), rs429358 (T/C) and rs7412 (C/T)

	Frequency					
Haplotype	**Case**	**Control**	**Comparison**[+]	**OR**	**Confidence Interval**	**P value**
G-*ε2*	0.069	0.076	G-*ε2* v T-*ε2*	1.58	0.85–2.95	0.15
T-*ε2*	0.021	0.005		1.00		
G-*ε3*	0.441	0.426	G-*ε3* v T-*ε3*	0.99	0.88–1.11	0.85
T-*ε3*	0.371	0.343		1.00		
G-*ε4*	0.027	0.034	G-*ε4* v T-*ε4*	1.07	0.71–1.62	0.75
T-*ε4*	0.071	0.116		1.00		
G-*ε3*/G-*ε3*	0.184	0.186		1.00		
G-*ε3*/T-*ε3*	0.343	0.283	G-*ε3*/T-*ε3* v G-*ε3*/G-*ε3*	1.21	0.98–1.48	0.08
T-*ε3*/T-*ε3*	0.135	0.118	T-*ε3*/T-*ε3 v* G-*ε3*/G-*ε3*	0.95	0.74–1.22	0.68
G-*ε3*/T-*ε4*	0.083	0.122	G-*ε3*/T-*ε4 v* G-*ε3*/G-*ε3*	0.80	0.60–1.07	0.13
G-*ε3*/G-*ε2*	0.063	0.066	G-*ε3*/G-*ε2 v* G-*ε3*/G-*ε3*	1.10	0.79–1.53	0.58
G-*ε3*/G-*ε4*	0.020	0.029	G-*ε3*/G-*ε4 v* G-*ε3*/G-*ε3*	0.61	0.38–0.99	0.04
T-*ε3*/T-*ε4*	0.053	0.083	T-*ε3*/T-*ε4 v* G-*ε3*/G-*ε3*	0.77	0.55–1.07	0.12
T-*ε3*/G-*ε2*	0.069	0.058	T-*ε3*/G-*ε2 v* G-*ε3*/G-*ε3*	1.10	0.79–1.54	0.56
T-*ε4*/G-*ε2*	0.018	0.019	T-*ε4*/G-*ε2 v* G-*ε3*/G-*ε3*	1.32	0.73–2.40	0.36
T-*ε2*/T-*ε3*	0.012	0.005	T-*ε2*/T-*ε3 v* G-*ε3*/G-*ε3*	1.03	0.51–2.06	0.94
T-*ε4*/T-*ε4*	0.003	0.013	T-*ε4*/T-*ε4 v* G-*ε3*/G-*ε3*	0.27	0.08–0.85	0.03

UNPHASED (Dudbridge, 2008) was used to estimate extended haplotype frequencies for late AMD (n=1,739) and controls (n=4,725) and to obtain odds ratios (OR) with 95% confidence intervals adjusted for age-group, center and smoking status.

Diplotypes with frequency ≤1% in both cases and controls are omitted.

[+] adjusted for study, age, gender and smoking status.