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Variations in Apolipoprotein E Frequency With Age in a Pooled Analysis of a Large Group of Older People

Gareth J. McKay*, Giuliana Silvestri, Usha Chakravarthy, Shilpa Dasari, Lars G. Fritsche, Bernhard H. Weber, Claudia N. Keilhauer, Michael L. Klein, Peter J. Francis, Caroline C. Klaver, Johannes R. Vingerling, Lintje Ho, Paulus T. D. V. De Jong, Michael Dean, Julie Sawitzke, Paul N. Baird, Robyn H. Guymer, Dwight Stambolian, Anton Orlin, Johanna M. Seddon, Inga Peter, Alan F. Wright, Caroline Hayward, Andrew J. Lotery, Sarah Ennis, Michael B. Gorin, Daniel E. Weeks, Chia-Ling Kuo, Aroon D. Hingorani, Reecha Sofat, Valentina Cipriani, Anand Swaroop, Mohammad Othman, Atsuhiro Kanda, Wei Chen, Goncalo R. Abecasis, John R. Yates, Andrew R. Webster, Anthony T. Moore, Johan H. Seland, Mati Rahu, Gisele Soubrane, Laura Tomazzoli, Fotis Topouzis, Jesus Vioque, Ian S. Young, Astrid E. Fletcher, and Chris C. Patterson

* Correspondence to Dr. Gareth J. McKay, Centre for Public Health, Queen's University Belfast, Royal Victoria Hospital, Belfast BT12 6BA, Northern Ireland (e-mail: g.j.mckay@qub.ac.uk).

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Variation in the apolipoprotein E gene (*APOE*) has been reported to be associated with longevity in humans. The authors assessed the allelic distribution of *APOE* isoforms ε2, ε3, and ε4 among 10,623 participants from 15 case-control and cohort studies of age-related macular degeneration (AMD) in populations of European ancestry (study dates ranged from 1990 to 2009). The authors included only the 10,623 control subjects from these studies who were classified as having no evidence of AMD, since variation within the *APOE* gene has previously been associated with AMD. In an analysis stratified by study center, gender, and smoking status, there was a decreasing frequency of the *APOE* ε4 isoform with increasing age (χ^2 for trend = 14.9 (1 df); $P = 0.0001$), with a concomitant increase in the ε3 isoform (χ^2 for trend = 11.3 (1 df); $P = 0.001$). The association with age was strongest in ε4 homozygotes; the frequency of ε4 homozygosity decreased from 2.7% for participants aged 60 years or less to 0.8% for those over age 85 years, while the proportion of participants with the ε3/ε4 genotype decreased from 26.8% to 17.5% across the same age range. Gender had no significant effect on the isoform frequencies. This study provides strong support for an association of the *APOE* gene with human longevity.

aged; apolipoprotein E2; apolipoprotein E3; apolipoprotein E4; apolipoproteins E; longevity; meta-analysis; multicenter study

Abbreviations: AMD, age-related macular degeneration; *APOE*, apolipoprotein E gene; Arg, arginine; Cys, cysteine; LDL, low density lipoprotein; SNP, single nucleotide polymorphism.

The human apolipoprotein E gene (*APOE*; OMIM 107741), located on chromosome 19q13.2, is central to the metabolism of low density lipoprotein (LDL) cholesterol and triglycerides and has been associated with increased risk of a variety of complex and age-related disorders (1). These include coronary heart disease events (2), atherosclerosis (3), age-related macular degeneration (AMD) (4), Alzheimer's disease (5), and other dementias (6). The small, multifunctional apolipoprotein E lipid transport protein acts as a ligand for the LDL receptor and is also involved in the maintenance and repair of neuronal cell membranes in the central and peripheral nervous systems. Variation in 2 single nucleotide polymorphisms (SNPs) within the *APOE* gene, rs429358 and rs7412, results in different isoforms reported to exert opposite effects in relation to the metabolism of coronary heart disease-related blood products such as LDL cholesterol and

triglycerides (3, 7, 8). The allelic variants derived from these SNPs are commonly referred to as ε2, ε3, and ε4 and are differentiated on the basis of cysteine (Cys) and arginine (Arg) residue interchanges at positions 112 and 158 in the amino acid sequence. The 3 variants give rise to 6 biallelic genotypes (ε3/ε3, ε3/ε4, ε2/ε3, ε4/ε4, ε2/ε4, and ε2/ε2, ranked from most to least common among European populations (3)). The ε2 allele has a cysteine residue at positions 112 and 158 in the receptor-binding region of apolipoprotein E. The ε3 allele has residues Cys-112 and Arg-158, and the ε4 allele has arginine residues at both positions. These amino acid substitutions have strong physiologic consequences for protein function.

Life expectancy in Western countries has risen by 3 months per year over the past 160 years, with a net increase of approximately 40 years within this time frame, largely as a consequence of reductions in malnutrition and childhood infection (9). A number of reports on human longevity have shown that the frequency of the ε4 allele is lower in older age groups, such as octogenarians, nonagenarians, and centenarians, than in younger or middle-aged persons (10) and that absence of an ε4 allele appears to be a favorable survival factor. In populations of European origin, an elevated mortality risk has been reported for the ε3/ε4 genotype relative to the ε3/ε3 genotype, with a slightly decreased risk being associated with the ε2/ε3 genotype (11, 12). Furthermore, a gender-specific survival effect associated with ε2 has been reported, although whether this is specific to males or females is as yet unclear, with opposing associations with both genders being reported (13, 14).

The analyses undertaken in this study were subsidiary to a pooled data analysis assessing *APOE* variation in the context of AMD. The authors examined the association of *APOE* with age as a marker for longevity and assessed the potential for a gender-specific effect.

MATERIALS AND METHODS

Study population

The data originated from 15 studies carried out at 40 study centers in 11 countries: 9 in Europe (United Kingdom, Germany, Netherlands, Norway, Estonia, Italy, France, Greece, and Spain), the United States, and Australia, which had previously examined the association of *APOE* with AMD (15–28). The dates of the studies ranged from 1990 to 2009. Analysis was restricted to samples derived from participants of European descent ($n = 24{,}774$). Individual participant data, including age, gender, smoking status (ever smoker vs. never smoker), and *APOE* genotype, were available for 23,686 persons, enabling us to conduct a pooled data analysis. The Alzheimer's disease status of these participants was not available. We restricted the analysis to 10,623 participants with no evidence of any AMD, classified by means of retinal photography or clinical examination, in order to avoid confounding by disease status due to a prior association of variation within the *APOE* gene and AMD (4). Investigators from 3 studies—the Rotterdam Study, the Women's Health Initiative Sight Exam Study, and the European Eye Study—provided data derived from samples acquired through population-based surveys ($n = 7{,}023$). The remainder of the data came from case-control (association) studies.

Data on age at examination, gender, smoking status (ever vs. never), *APOE* genotype, and AMD phenotype were requested from each contributing center (Table 1). All of the studies were approved by local ethics review boards, and each participant provided written informed consent prior to recruitment. Recruitment procedures and detailed AMD grading methods for each study center have been described previously (15–28).

Statistical analysis

Both SNPs were assessed for departure from Hardy-Weinberg equilibrium by study, using a χ^2 goodness-of-fit test. Data were categorized on the basis of age into 8 groups ($\leq$60, 61–65, 66–70, 71–75, 76–80, 81–85, 86–90, and $>$90 years). Separate analyses were performed for each of the 3 *APOE* alleles in the data set. Logistic regression was used to assess the variation in *APOE* allele frequencies across age groups, with center, gender, and smoking included in the regression to adjust for possible confounding. A likelihood ratio χ^2 test was used to compare models that included and excluded a linear term for age group, thus providing a test for trend in allele frequency across age groups. This analysis also provided an odds ratio summarizing the change in odds for each allele per 5-year increase in age. Interactions between gender and age and between center and age were also tested in the logistic regression using likelihood ratio tests which compared models that included and excluded the interaction terms.

RESULTS

APOE allele frequency with age

No departure from Hardy-Weinberg equilibrium was detected for either SNP by center or within the entire data set. *APOE* allele frequencies varied between studies (data not shown), with ranges of 6.7%–10.0% (*APOE* ε2), 75.3%–82.8% (*APOE* ε3), and 7.5%–15.6% (*APOE* ε4). *APOE* genotype frequencies for the 10,623 controls are shown in Table 2. The frequency of the ε4 allele decreased from 17.6% to 8.3% (−9.3%) with increasing age, while the frequency of the ε3 allele increased from 73.3% to 83.3% (+10.0%) (Table 3, Figure 1). Following adjustment for center and smoking status to limit potential confounding, we observed a significant decrease in the frequency of ε4 with increasing age ($\chi^2 = 14.9$ (1 df); $P = 0.0001$), representing a 5% decrease in odds per 5-year increase in age (odds ratio = 0.95, 95% confidence interval: 0.92, 0.97), as well as a significantly increased frequency of ε3 ($\chi^2 = 11.3$ (1 df); $P = 0.001$), representing a 4% increase in odds per 5-year increase in age (odds ratio = 1.04, 95% confidence interval: 1.02, 1.07). The frequency of the ε2 allele showed little variation with age. The relations between allele frequency and age in males and females were compared by including an age × gender interaction in the logistic regression analysis, but none of the interactions were significant (likelihood ratio χ^2 test: ε2, $P = 0.13$; ε3, $P = 0.25$; ε4,

Table 1. Sample Size, Age Distribution, and Gender Composition Among Control Subjects From Contributing Studies in a Pooled Analysis of Variations in *APOE* Allele Frequencies With Age ($n = 10,623$), 1990–2009

First Author, Year (Reference No.)	Location of Study	No. of Subjects	Age at Recruitment, years		% Male
			Mean (SD)	Range	
McKay, 2009 (22)	Belfast, Northern Ireland	436	74.8 (6.9)	49–102	38.1
Fritsche, 2009 (15)	Regensburg, Germany	553	76.2 (5.3)	65–97	38.0
Francis, 2009 (17)	Portland, Oregon (US)	272	73.9 (6.4)	60–92	44.1
van Leeuwen, 2004 (20)	Rotterdam, the Netherlands	3,805	67.5 (8.3)	55–99	42.2
Bergeron-Sawitzke, 2009 (16)	AREDS, US	199	77.1 (4.6)	63–88	47.2
Baird, 2006 (18)	Melbourne, Australia	106	71.5 (6.5)	60–86	45.3
Hadley, 2010 (24)	Philadelphia, Pennsylvania (US)	379	75.6 (7.9)	56–96	46.4
Haan, 2006 (28)	WHI-SE, US	1,283	73.7 (4.7)	65–86	0
Yates, 2007 (23)	Edinburgh, United Kingdom	179	73.2 (7.8)	46–92	38.5
Ennis, 2008 (25)	Southampton, United Kingdom	458	70.7 (9.3)	50–91	47.8
Conley, 2005 (21)	Los Angeles, California (US)	142	72.5 (8.8)	50–91	43.7
Augood, 2004 (27)	EUREYE, Europe	1,935	72.4 (5.3)	65–95	45.2
Zareparsi, 2004 (19)	Ann Arbor, Michigan (US)	253	76.6 (5.3)	68–92	44.3
Yates, 2007 (23)	Cambridge, United Kingdom	417	75.0 (7.8)	42–96	40.0
Dandekar, 2006 (26)	London, United Kingdom	206	74.9 (7.7)	52–91	40.8
Total		10,623	71.5 (7.8)	42–102	37.7

Abbreviations: *APOE*, apolipoprotein E gene; AREDS, Age-Related Eye Disease Study; EUREYE, European Eye Study; SD, standard deviation; US, United States; WHI-SE, Women's Health Initiative Sight Exam.

$P = 0.80$). Tests of age × center interactions were also conducted and showed no evidence of heterogeneity in age effects by center (likelihood ratio χ^2 test: ε2, $P = 0.21$; ε3, $P = 0.88$; ε4, $P = 0.38$).

Although the number of persons who were homozygous for the ε4 isoform was low at 1.9% (Table 2), the age-related effect observed was most prominent in ε4 homozygotes, with a 70% reduction in frequency from 2.7% in persons aged 60 years or less to 0.8% in those over age 85 years (Table 2). A decreased frequency of 35% was also observed in ε3/ε4 heterozygotes, with a reduction from 26.8% to 17.5% recorded over the same age range (Table 2). Persons who were heterozygous for the ε2/ε4 genotype showed a nonsignificant change of 0.7% in frequency within this age range, from 3.1% to 2.4% (Table 2).

DISCUSSION

In previous studies, investigators have reported increased mortality associated with the ε4 allele of the *APOE* gene, and this has been partly attributed to the increased risk this

Table 2. Distribution of *APOE* Genotypes by Age Group in a Pooled Analysis of Variations in *APOE* Allele Frequencies With Age ($n = 10,623$), 1990–2009

Age Group, years	*APOE* Genotype												Total No.
	ε2/ε2		ε2/ε3		ε2/ε4		ε3/ε3		ε3/ε4		ε4/ε4		
	No.	%[a]	No.	%	No.	%	No.	%	No.	%	No.	%	
≤60	9	0.9	138	13.3	32	3.1	553	53.3	278	26.8	28	2.7	1,038
61–65	2	0.2	157	13.3	33	2.8	692	58.7	260	22.1	34	2.9	1,178
66–70	15	0.6	296	11.9	50	2.0	1,523	61.3	540	21.7	61	2.5	2,485
71–75	12	0.5	304	11.6	54	2.1	1,632	62.1	586	22.3	39	1.5	2,627
76–80	15	0.8	257	12.9	43	2.2	1,219	61.2	430	21.6	29	1.5	1,993
81–85	6	0.6	131	14.2	19	2.1	570	61.6	186	20.1	13	1.4	925
86–90	0	0.0	32	10.3	9	2.9	212	68.2	55	17.7	3	1.0	311
>90	0	0.0	11	16.7	0	0.0	44	66.7	11	16.7	0	0.0	66
Total	59	0.6	1,326	12.5	240	2.3	6,445	60.7	2,346	22.1	207	1.9	10,623

Abbreviation: *APOE*, apolipoprotein E gene.
[a] Row percentage.

Table 3. Variation in *APOE* Allele Frequencies With Age in a Pooled Analysis ($n = 10{,}623$), Overall and by Gender, 1990–2009

	No. of Samples	*APOE* ε2						*APOE* ε3						*APOE* ε4					
		Both Genders		Females		Males		Both Genders		Females		Males		Both Genders		Females		Males	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Age group, years																			
≤60	1,038	188	9.1	116	9.5	72	8.4	1,522	73.3	891	73.2	631	73.5	366	17.6	211	17.3	155	18.1
61–65	1,178	194	8.2	124	9.4	70	6.8	1,801	76.4	1,004	75.7	797	77.4	361	15.3	198	14.9	163	15.8
66–70	2,485	376	7.6	246	8.3	130	6.5	3,882	78.1	2,303	77.4	1,579	79.1	712	14.3	425	14.3	287	14.4
71–75	2,627	382	7.3	238	7.1	144	7.7	4,154	79.1	2,671	79.2	1,483	78.9	718	13.7	465	13.8	253	13.5
76–80	1,993	330	8.3	219	8.2	111	8.4	3,125	78.4	2,085	78.4	1,040	78.3	531	13.3	354	13.3	177	13.3
81–85	925	162	8.8	111	9.2	51	7.9	1,457	78.8	949	79.0	508	78.4	231	12.5	142	11.8	89	13.7
86–90	311	41	6.6	24	6.1	17	7.5	511	82.2	323	81.6	188	83.2	70	11.3	49	12.4	21	9.3
>90	66	11	8.3	7	8.1	4	8.7	110	83.3	73	84.9	37	80.4	11	8.3	6	7.0	5	10.9
Total	10,623	1,684	7.9	1,085	8.2	599	7.5	16,562	78.0	10,299	77.8	6,263	78.2	3,000	14.1	1,850	14.0	1,150	14.4
Trend test[a]																			
χ^2		0.03		0.20		0.06		11.3		8.84		3.29		14.9		10.2		5.43	
P value		0.86		0.65		0.80		0.001		0.003		0.07		1.1×10^{-4}		0.001		0.02	
Change per 5-year increase[b]																			
Odds ratio		1.00		0.99		1.01		1.04		1.05		1.04		0.95		0.94		0.95	
95% confidence interval		0.96, 1.03		0.95, 1.04		0.95, 1.07		1.02, 1.07		1.02, 1.08		1.00, 1.08		0.92, 0.97		0.91, 0.98		0.90, 0.99	

Abbreviation: *APOE*, apolipoprotein E gene.

[a] χ^2 tests for trend (1 df) in *APOE* allele frequency with age, with adjustment for confounding by study, gender, and smoking status, were generated by means of logistic regression performed separately for each allele.

[b] Change in the odds of having the allele per 5-year increase in age.

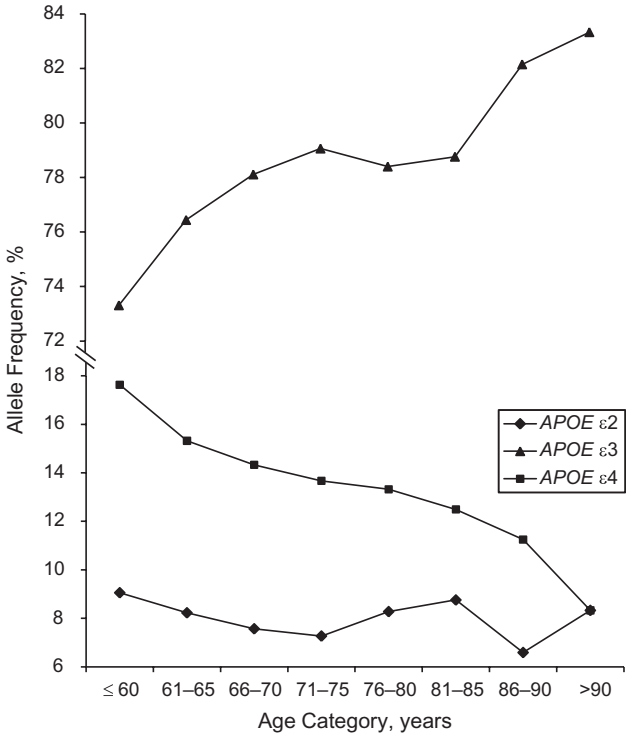

Figure 1. Allele frequency distribution of apolipoprotein E gene (*APOE*) ε2, ε3, and ε4 isoforms by age group in 10,623 subjects with European ancestry, 1990–2009.

isoform poses in relation to coronary heart disease, atherosclerosis, Alzheimer's disease, and other dementias (1–3, 5–7). Various hypotheses have been proposed surrounding the functionality of this small, multifunctional lipid transport protein, particularly through its role as a ligand for LDL cholesterol and triglycerides and its involvement in the maintenance and repair of neuronal cells. The *APOE* isoforms generated from the different alleles interact differently with the lipoprotein receptors, leading to altered cholesterol levels. High levels of LDL cholesterol are associated with *APOE* ε4, low levels with ε2, and intermediate levels with ε3 (29). However, the exact mechanisms regarding the association of *APOE* with increased mortality, and indeed its association with a variety of disease and pathologic processes conferring increased or decreased risk, have yet to be elucidated.

A study by Seripa et al. (13) suggested an association of decreased mortality with the ε2 allele in males only, particularly in relation to dementia and cardiovascular disease ($n = 1{,}710$; 757 males). Another smaller independent study (14) suggested that the reduced mortality associated with ε2 was specific to women, and thus there is some confusion with regard to the direction and magnitude of any potential gender difference. To our knowledge, our study was one of the largest to assess the relation between *APOE* and age to date, and we found no evidence to suggest that the association with age and *APOE* was different in women compared with men; therefore, our results do not suggest that differences in longevity between men and women are explained by *APOE*. Other studies with smaller sample sizes have found lower rates of mortality associated with ε2 relative to ε3 with age (12, 30), but our data did not support these findings, with a small but nonsignificant variation in the relation of ε2 with age being observed. However, with only 66 participants over age 90 years in this study, this age category was not well represented. This may explain why previous findings of an increased ε2 allele frequency in the elderly were not confirmed in this study. Furthermore, our results did not show an effect of interaction between age and AMD phenotype on *APOE* allele frequency prevalence, indicative that the observed effect occurs independently of AMD (data not shown). Note that ε4 has been previously reported to exert a protective effect against AMD (4, 15, 31, 32), and as such, an inflated frequency of this allele may be represented within the AMD-free control samples used in this study, compared with the frequency actually present in the general population.

Our findings offer further support to recently reported findings implicating the *APOE* region in longevity (33). Although the platform used in the study by Sebastiani et al. (33) did not genotype either rs429358 or rs7412 directly, an intronic SNP rs2075650, located in a gene called *TOMM40*, was used as a strong proxy for rs429358, as the 2 SNPs have previously been shown to be in strong linkage disequilibrium (34). The high level of linkage disequilibrium which exists between *TOMM40* and *APOE* makes it difficult to identify the causal variant associated with the effect observed at this locus (34). Sebastiani et al. (33) also assessed the association of this region with longevity for an interaction with gender but could find no evidence to support effect modification.

Our results show that variation in control allele frequencies needs to be carefully considered in relation to association studies of age-related conditions. Failure to do so will result in serious confounding, with an impact on unadjusted association studies that is often not appreciated; many investigators assume that frequencies are constant across the life span, and this is especially so when the incidence of the disease in question increases with age (35). It has been estimated from cross-sectional studies that the frequency distribution of the ε4 allele halves between the ages of 60 and 85 years (35), and this is supported by the current study. A decrease in the frequency of ε4 is likely to occur as a consequence of increased risk associated with this allele in relation to coronary heart disease, atherosclerosis, Alzheimer's disease, and other dementias (1). Association studies of age-related disorders investigating the effects of genes that may be influenced by longevity may yield greater variation in allele frequencies between controls alone than between cases and controls. While adjustment for age is imperative to limit confounding under these circumstances, caution is recommended in considering associations such as that for *APOE*, in case-control studies that are not well-matched for age.

The magnitude of the effect measured and the associated level of significance will be limited by sample size and the allele frequencies present within the population in question, which varies significantly in the case of *APOE*. *APOE* allele frequencies measured in this investigation (data not shown) varied by study, between 6.7% and 10.0% for ε2, 75.3% and

82.8% for ε3, and 7.5% and 15.6% for ε4. Geographic variation in *APOE* allele frequencies has been reported previously, with a resultant impact on statistical power (36–38). It was not possible for us to ascertain the geographic origin of each participant individually within this study, but adjustment for potential confounding by study bias/location was incorporated into our analyses to assess the relation between *APOE* and age. Since it was not possible to genotype markers that were informative for ancestry in these samples, we cannot rule out the possibility that population stratification could have made some contribution to our findings. Adjustment for center partly addressed some of the issues raised by Lewis and Brunner (30) with respect to population stratification and variation in genotype frequencies. Furthermore, individual smoking status data were available for subjects, although adjustment for this lifestyle risk factor failed to have any significant impact on the associations inferred.

The statistical analyses for measuring the effects of gender and age in the current study were performed on categorized data, which were arbitrarily grouped into 5-year intervals prior to undertaking the analysis. The data from this study support a 5% reduction in the odds for the ε4 allele every 5 years beyond the age of 60 years, while the associated odds for the ε3 allele increased by 4% across the same time period. Narrower and broader age intervals were assessed, with little change in terms of the significance of the effect observed, as was the case when age was treated as a continuous variable.

Limitations of our study to accurately assess the role of *APOE* in longevity pertain to the impact associated with this gene on debilitating diseases such as coronary heart disease, atherosclerosis, or age-related disorders, such as Alzheimer's disease and other dementias. Persons who are affected by such diseases may be less likely to participate in a case-control study. In addition, AMD was ruled out in our study subjects, which could also have had some effect, since *APOE* has been reported to be associated with AMD in previous studies (4, 15, 31, 32). We suspect that these effects would have been small, but ideally a prospective population-based study with a sufficient sample size and follow-up would be best placed to offer an unbiased and accurate reflection on the role of *APOE* in longevity.

The current study strongly supports the association of *APOE* alleles with human longevity by demonstrating variation in *APOE* allele frequencies in older persons and illustrates the potential confounding effect of age in association studies on *APOE*. The underlying mechanism behind the association of *APOE* with age remains to be elucidated.

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Author affiliations: Centre for Public Health, Queen's University Belfast, Belfast, Northern Ireland (Gareth J. McKay, Ian S. Young, Chris C. Patterson); Centre for Vision and Vascular Science, Queen's University Belfast, Belfast, Northern Ireland (Giuliana Silvestri, Usha Chakravarthy, Shilpa Dasari); Institute of Human Genetics, University of Regensburg, Regensburg, Germany (Lars G. Fritsche, Bernhard H. Weber); Department of Ophthalmology, University Hospital Würzburg, Würzburg, Germany (Claudia N. Keilhauer); Macular Degeneration Center, Casey Eye Institute, Oregon Health and Science University, Portland, Oregon (Michael L. Klein, Peter J. Francis); Department of Epidemiology, Erasmus Medical Centre, Rotterdam, the Netherlands (Johannes R. Vingerling, Lintje Ho); Department of Ophthalmology, Erasmus Medical Centre, Rotterdam, the Netherlands (Caroline C. Klaver); Netherlands Institute for Neuroscience, Netherlands Academy of Arts and Sciences, Amsterdam, the Netherlands (Paulus T. D. V. De Jong); Department of Ophthalmology, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands (Paulus T. D. V. De Jong); Cancer and Inflammation Program, National Cancer Institute, Frederick, Maryland (Michael Dean, Julie Sawitzke); Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, University of Melbourne, East Melbourne, Victoria, Australia (Paul N. Baird, Robyn H. Guymer); Department of Ophthalmology, University of Pennsylvania, Philadelphia, Pennsylvania (Dwight Stambolian, Anton Orlin); Department of Ophthalmology, Tufts University School of Medicine and Tufts Medical Center, Boston, Massachusetts (Johanna M. Seddon); Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, New York (Inga Peter); MRC Human Genetics Unit, Western General Hospital, Edinburgh, United Kingdom (Alan F. Wright, Caroline Hayward); Clinical Neurosciences Division, School of Medicine, University of Southampton, Southampton, United Kingdom (Andrew J. Lotery); Southampton Eye Unit, Southampton General Hospital, Southampton, United Kingdom (Andrew J. Lotery); Genetic Epidemiology and Bioinformatics Group, Human Genetics Division, University of Southampton, Southampton, United Kingdom (Sarah Ennis); Departments of Ophthalmology and Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California (Michael B. Gorin); Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania (Daniel E. Weeks, Chia-Ling Kuo); University Centre for Clinical Pharmacology, Department of Medicine, University College London, London, United Kingdom (Aroon D. Hingorani, Reecha Sofat); Department of Ophthalmology and Visual Sciences, Kellogg Eye Center, University of Michigan, Ann Arbor, Michigan (Anand Swaroop, Mohammad Othman); Neurobiology-Neurodegeneration and Repair Laboratory, National Eye Institute, Bethesda, Maryland (Anand Swaroop, Atsuhiro Kanda); Center for Statistical Genetics, Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, Michigan (Wei Chen, Goncalo R. Abecasis); Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, Cambridge, United Kingdom (John R. Yates); Institute of Ophthalmology, University College London, London, United Kingdom (John R. Yates, Andrew R. Webster, Anthony T. Moore, Valentina Cipriani); Moorfields Eye Hospital, London, United Kingdom (John R. Yates, Andrew R. Webster, Anthony T. Moore, Valentina Cipriani); Eye Department, Stavanger University Hospital, University of Bergen, Stavanger, Norway (Johan H. Seland); Department of Epidemiology and Biostatistics, National Institute

for Health Development, Tallinn, Estonia (Mati Rahu); Clinique Ophthalmologique, Universitaire de Creteil, Paris, France (Gisele Soubrane); Clinica Oculistica, Unversità degli Studi di Verona, Verona, Italy (Laura Tomazzoli); Department of Ophthalmology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece (Fotis Topouzis); Departamento Salud Publica, University Miguel Hernandez, Alicante, Spain (Jesus Vioque); Consorcio de Investigación Biomédica en Red Especializado en Epidemiología y Salud Pública (CIBERESP), Alicante, Spain (Jesus Vioque); and Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, United Kingdom (Astrid E. Fletcher).

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