

ARTICLE

Associations of apolipoprotein E gene with ischemic stroke and intracranial atherosclerosis

Shérine Abboud^{*,1,2,3}, Leena E Viiri⁴, Dieter Lütjohann⁵, Sirkka Goebeler⁴, Teemu Luoto⁴, Silvia Friedrichs⁵, Philippe Desfontaines⁶, Marie-dominique Gazagnes⁷, Patrice Laloux⁸, André Peeters⁹, Pierrette Seeldrayers¹⁰, Terho Lehtimäki³, Pekka Karhunen⁴, Massimo Pandolfo^{1,2} and Reijo Laaksonen^{2,3}

¹Department of Neurology, ULB Erasme Hospital, Brussels, Belgium; ²Laboratory of Experimental Neurology, ULB, Brussels, Belgium; ³Laboratory of Atherosclerosis Genetics, Department of Clinical Chemistry, Tampere University Hospital and the Medical School, University of Tampere, Finland; ⁴Research Unit of the Laboratory Centre, School of Medicine, Tampere University Hospital, Tampere, Finland; ⁵Department of Clinical Pharmacology, University of Bonn, Bonn, Germany; ⁶Department of Neurology, CHC Clinique de l'Espérance Montegné, Brussels, Belgium; ⁷Department of Neurology, CHU Brugmann Brussels, Brussels, Belgium; ⁸Department of Neurology, Cliniques Universitaires de Mont-Godinne, Yvoir (Mont), Belgium; ⁹Department of Neurology, Cliniques Universitaires Saint-Luc, Yvoir (Mont), Belgium; ¹⁰Department of Neurology, CHU de Charleroi, Yvoir (Mont), Belgium

The apolipoprotein E (*APOE*) $\epsilon 4$ allele is associated with elevated cholesterol and risk of atherosclerosis. However, its role in ischemic stroke (IS) remains controversial. We investigated a possible link between IS or the severity of intracranial atherosclerosis and the *APOE* promoter polymorphisms $-219G/T$ and $+113G/C$, involved in regulating *APOE* transcription. We genotyped subjects from a multicentric Belgian case–control study, including 237 middle-aged patients with IS due to small- or large-vessel atherosclerotic stroke and 326 ethnicity- and gender-matched controls and a Finnish autopsy series of 1004 non-stroke cases, who had received a quantitative score of atherosclerosis in the circle of Willis. The *APOE* $\epsilon 4 +$ genotype did not associate with IS, but was related to more severe intracranial atherosclerosis score in men (5.4 vs 4.6, $P = 0.044$). Within the most common *APOE* $\epsilon 3/\epsilon 3$ genotype group, the risk of IS associated with the G-allele of the tightly linked $-219G/T$ (OR = 6.2; 95% CI: 1.6–24.3, $P = 0.009$) and $+113G/C$ (OR = 7.1; 95% CI: 1.7–29.9, $P = 0.007$) promoter polymorphisms. There was no difference in the severity of intracranial atherosclerosis between $-219G/G$ genotype carriers and non-carriers. This study suggests a multifaceted role of apoE on the risk of cerebrovascular diseases. The *APOE* $\epsilon 4 +$ genotype did not predict the risk of IS but was associated with severity of subclinical intracranial atherosclerosis in men on the autopsy study. In contrast, the promoter variants were significant predictors of IS, suggesting that quantitative rather than qualitative variation of apoE is related to IS.

European Journal of Human Genetics (2008) 16, 955–960; doi:10.1038/ejhg.2008.27; published online 27 February 2008

Keywords: *APOE* gene; ischemic stroke; intracranial atherosclerosis

*Correspondence: Dr S Abboud, Department of Neurology, U.L.B. Erasme Hospital, 808 route de Lennik, Brussels 1070, Belgium.
Tel: +0032/2 5556408; Fax: +0032/2 5554111;
E-mail: shabboud@ulb.ac.be
Received 2 October 2007; revised 5 January 2008; accepted 22 January 2008; published online 27 February 2008

Introduction

A large number of candidate gene association studies have attempted to identify genes involved in stroke.¹ The etiology of stroke is heterogeneous, and familial predisposition contributes only moderately (OR = 1.3–1.76) to

the risk of all stroke.² One way to increase the power of such studies would be to select a predefined phenotype. Genetic influence has been found to be the strongest in ischemic stroke (IS) due to small-vessel occlusion (SVO) or large-vessel atherosclerosis (LVA), as defined by the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification. This influence was especially strong in younger (<60 years) patients.^{3–5} Thus, middle-aged SVO and LVA stroke patients seem to be a suitable study population for studies attempting to identify genes involved in the pathogenesis of IS.

Despite the association of the *APOE* ϵ 4 allele with elevated LDL cholesterol levels and slightly elevated cardiovascular risk,⁶ a recent meta-analysis found no clear link between the *APOE* ϵ 4 allele and IS.⁷ On the other hand, elevated plasma concentration of apoE is a risk factor for stroke.^{8,9} Furthermore, it has been shown that apoE concentration is strongly associated with cardiovascular mortality in old age, independently of *APOE* genotype and plasma lipids.¹⁰ Plasma level of apoE is dependent on total protein, albumin level, body mass index (BMI) and alcohol consumption. Moreover, plasma apoE is largely liver-derived and could be regulated by hepatic factors. Besides the *APOE* ϵ 2/ ϵ 3/ ϵ 4 genotype affecting the structure of apoE protein, the biological activity of apoE can also be influenced by genetic factors that modify its synthesis and quantity. It has been shown that genetic variation in the *APOE* gene promoter, the $-219G/T$ single nucleotide polymorphism (SNP) located in the regulatory region of the *APOE* gene and the closely linked $+113G/C$ SNP located in the intron 1 enhancer region, may affect the transcription of *APOE* gene.¹¹ Carriers of the $-219G/G$ -genotype had 10–20% higher apoE plasma concentrations compared to $-219T/T$ genotype.¹² Moreover, the $-219G/T$ polymorphism has been reported to associate with atherogenic lipid and lipoprotein profile,^{13,14} as well as with coronary atherosclerosis.¹⁵ However, the role of these SNPs in IS and in cerebral atherosclerosis has not been studied before.

In this study, we tested the association between the *APOE* ϵ 4, $-219G/T$ and $+113G/C$ SNPs and IS in a setting of a case–control study of 237 middle-aged patients and 326 ethnicity- and gender-matched controls. To evaluate whether any positive result may be related to predisposition to intracranial atherosclerosis, we also tested whether these genetic variants are associated with atherosclerosis of the circle of Willis in an independent Finnish autopsy series.

Material and methods

The Belgian stroke study (BSS)

BSS is a case–control study. The 237 cases had SVO or LVA stroke (TOAST classification), all occurring between 45 and 60 years of age. The patients were selected from the

databases of seven stroke units in Belgium. All patients were of central European origin (>90% were Belgians). Cardiovascular risk factors (hypertension, diabetes mellitus, hyperlipidemia (hypercholesterolemia or hypertriglyceridemia), alcohol consumption (>20 g/day), smoking (former, current and never), obesity (BMI >30)) were recorded.

The control group was composed of 326 gender- and ethnicity-matched healthy volunteers without a history of stroke and living in the same area. As the mean age of stroke occurrence is typically around 70 years of age, and because we selected only younger (<60 years) stroke patients, we explicitly selected older controls than cases to decrease the likelihood that they would later in their life get an IS. Stroke patients had higher prevalence of conventional cardiovascular risk factors than controls. Therefore, to avoid a potential selection bias due to the different cardiovascular risk factors and differences in the mean age, all our genetic results were adjusted for cardiovascular risk factors and age.

Optimal methods to identify and control for population stratification in genetic association studies are not established. A recent study showed that grandparental country of origin provided better control for stratification than the SNP-based approach.¹⁶ In this study, ethnicity was checked up to the four grandparents. The ethical committees of all participating hospitals approved the study protocol. Written informed consent was obtained from all patients before study entry.

The Finnish autopsy series

The Finnish autopsy series comprised of two cross-sectional population-based autopsy studies. In total, the two series included 1004 medico-legal Caucasians autopsy cases that had died suddenly out of hospital, or were found dead. The atherosclerosis of each of the nine branches of the circle of Willis was scored semi-quantitatively as follows: 0 = normal, 1 = slight (streaks with or without elevated fibrous lesions), 2 = moderate (fibrous lesions that cause <50% stenosis), 3 = severe (>50% stenosis with extensive atherosclerosis (fatty, fibrous and calcified lesions)), giving a range of scores from 0 to 27. The cases were from two studies: the Tampere Autopsy Study (TASTY) ($n = 604$) and the Helsinki Sudden Death Study (HSDS)¹⁷ ($n = 400$). The TASTY comprised both men (64.3%, mean age 59.7 years) and women (35.7%, mean age 68.2 years), whereas the HSDS series included only men (mean age 53.7 years). In addition to gender and age, BMI was also recorded. The Finns are particularly suitable for genetic association studies being a homogenous Caucasian population, which results from genetic isolation.¹⁸

Genotyping

In the BBS, DNA was isolated from samples of whole blood, which had been stored frozen at -20°C , with a

commercially available kit (Qiagen Inc., Valencia, CA). In the autopsy series, DNA isolation was performed either from frozen blood samples (TASTY) with the salt precipitation method or from frozen (-70°C) cardiac muscle samples (HSDS) with the standard phenol-chloroform method. Genotyping was carried out by using the 5' nuclease assay and fluorogenic allele-specific TaqMan MGB probes in the ABI Prism 7900 HT sequence detection. The DNA and PCR master mix (together $5\ \mu\text{l}$) were pipeted into the 384-well plates using Tecan Freedom EVO 100 instrument and instrument software V4.8 (Tecan Schweiz AG, Switzerland). To monitor genotyping errors, random duplicates were run in parallel with unknown samples. Allele-specific fluorescence generated from each probe during the PCR amplification was measured with the allelic discrimination analysis module. The nucleotide sequences of primers and probes used in the PCR were deduced from the public databases and synthesized in conjunction with Applied Biosystems. The SNPs evaluated in this study were *APOE* $\epsilon 2/\epsilon 3/\epsilon 4$, $-219\text{G}/\text{T}$ (rs405509) and $+113\text{G}/\text{C}$ (rs440446). Because of technical problems in the PCR of the $+113\text{G}/\text{C}$ SNP and a tight linkage disequilibrium ($D' = 0.86$ $r^2 = 0.44$; calculated by the Stata 8.0 program, STATA Corporation, TX, USA) between the $-219\text{G}/\text{T}$ and $+113\text{G}/\text{C}$ SNPs, only the $-219\text{G}/\text{T}$ SNP was analyzed from all of the autopsy cases. The mean genotyping success was $>95\%$.

Statistical analysis

Data were analyzed using the SPSS software (version 12.0, SPSS Inc., Chicago, IL, USA). The clinical data were compared between the IS cases and controls, using a binary logistic regression analysis with age as continuous covariate. To examine the effect of the *APOE* $\epsilon 4$ allele, study populations were divided into $\epsilon 4$ allele carriers ($\epsilon 3/4$ and $\epsilon 4/4$) and non-carriers ($\epsilon 2/2$, $\epsilon 2/3$ and $\epsilon 3/3$). Since the $\epsilon 2/\epsilon 4$ genotype was rare ($n = 18$) and difficult to assign in a group ($\epsilon 2$ and $\epsilon 4$ allele carriers usually have opposite effects), it was excluded from the analyses. A binary logistic regression analysis with smoking, hypertension, alcohol consumption, obesity, diabetes and hyperlipidemia as dichotomous covariates and age as continuous covariate was used to evaluate the association of the genetic variants with IS. To study the association of the promoter polymorphism with IS excluding the confounding effects of the *APOE* $\epsilon 2/\epsilon 3/\epsilon 4$ genetic variation, we performed the analysis within the most common *APOE* $\epsilon 3/\epsilon 3$ genotype group. Furthermore, analysis of covariance (ANCOVA) with age and BMI as continuous covariates was used to compare the mean atherosclerosis scores between the studied genotypes. We analyzed four SNPs that are in linkage disequilibrium and located in the same gene. Therefore, applying a multiple comparison statistics, such as Bonferroni correction, is inappropriate, because the individual SNPs are not independent.

Results

BSS series

The frequencies of the $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$ alleles were 0.07, 0.80 and 0.14, respectively ($\epsilon 2/\epsilon 4$ was left out from the calculations). Within the *APOE* $\epsilon 3/\epsilon 3$ carriers, the frequencies of the -219G and -219T alleles were 0.55 and 0.45, and the frequencies of the $+113\text{G}$ and $+113\text{C}$ alleles were 0.56 and 0.44, respectively. All genotype frequency distributions were in Hardy-Weinberg equilibrium in cases and in controls. Stroke patients had higher prevalence of conventional cardiovascular risk factors, such as hypertension, hyperlipidemia, current smoking and alcohol consumption than controls (Table 1). The *APOE* $\epsilon 4$ carrier frequency did not differ significantly between the IS patients and controls (Table 2). Within the most common *APOE* $\epsilon 3/\epsilon 3$ genotype group, both the -219G and $+113\text{G}$ allele carriers were more common among the IS cases than controls (OR = 6.2; 95% CI 1.6–24.3, $P = 0.009$ and OR = 7.1; 95% CI 1.7–29.9, $P = 0.007$, respectively) after adjustment for all recorded risk factors and age (Table 2). With a frequency of $\sim 50\%$ for the at-risk allele at an α level of 0.05, our sample was evaluated to have 95% power to detect an RR of 1.6 for heterozygote and 3.2 for homozygotes ('genetic power calculator': <http://statgen.iop.kcl.ac.uk/gpc/cc2.html>).

Finnish autopsy series

The frequencies of the $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$ alleles were 0.05, 0.79 and 0.17, respectively ($\epsilon 2/\epsilon 4$ was left out from the calculations). Within the *APOE* $\epsilon 3/\epsilon 3$ carriers, the frequencies of the -219G and -219T alleles were 0.62 and 0.38, respectively. All *APOE* allele frequencies were similar to the frequencies in the BSS series. The carriers of the *APOE* $\epsilon 4$ tended to have higher intracranial atherosclerosis score (5.4 vs 4.8, $P = 0.051$) compared to non-carriers, but this association was statistically significant only among men (5.4 vs 4.6, $P = 0.044$). Within the *APOE* $\epsilon 3/\epsilon 3$ genotype group, there was no significant difference in the circle of Willis atherosclerosis score between carriers and non-carriers of the *APOE* -219G allele. (Table 3)

Discussion

Several studies have addressed the role of *APOE* $\epsilon 4$ in stroke, but the results have been inconsistent.⁷ In our selected Belgian stroke population, no association was seen between the *APOE* $\epsilon 4$ allele and IS. However, in the Finnish autopsy study, we found out that men carrying the *APOE* $\epsilon 4$ allele had significantly higher mean intracranial atherosclerosis score compared to the $\epsilon 4$ non-carriers. Previous autopsy studies have revealed a link between the *APOE* $\epsilon 4$ allele and larger coronary and aortic atherosclerotic lesion areas in men.^{17,19} Thus, it seems that the *APOE* $\epsilon 4$ allele may have a gender-specific role in the development of atherosclerosis in different vascular beds. The $\epsilon 4$ allele is

Table 1 Clinical characteristics of Belgian stroke study population and odds ratios for ischemic stroke

Characteristic	IS	Controls	OR (95% CI)	P-value
Hypertension (%)	636	380	5.4 (2.7–10.5)	0
Diabetes (%)	164	108	1.3 (0.6–3.0)	467
Hyperlipemia (%)	582	365	2.7 (1.4–5.1)	0.002
Smoking status (%)				
Current	557	93	4.7 (2.3–9.7)	0
Former	15.1	21	1.3 (0.5–3.0)	0.571
Never	29.2	69.8	1	Ref.
Alcohol consumption > 20 g/day (%)	30.4	21.2	2.2 (1.1–4.3)	0.026
Obesity, BMI > 30 (%)	16.6	13.2	0.6 (0.3–1.4)	0.243
Male (%)	67	66		

BMI = body mass index; IS = ischemic stroke; OR = odds ratio; Ref. = reference.

The *P*-values are from binary logistic regression analysis, adjusted for age.

Table 2 Genotype frequencies (%) for Ischemic stroke cases and controls in the Belgian stroke study population

Genotype	IS (n = 237)	Controls (n = 326)	OR (95% CI)	P-value
APOE				
ϵ 4+	25.2	25.2	0.9 (0.5–1.5)	0.715
ϵ 4–	74.8	74.8	1	ref.
APOE –219 (ϵ3/ϵ3)				
G +	84.1	76.4	6.2 (1.6–24.3)	0.009
T/T	15.9	23.6	1	ref.
APOE +113 (ϵ3/ϵ3)				
G +	85	76.4	7.1 (1.7–29.9)	0.007
C/C	15	23.6	1	Ref.

CI = confidence interval; G+ = carrier of the G-allele; IS = ischemic stroke; OR = odds ratio; Ref. = reference; ϵ 4– = non-carrier of the ϵ 4 allele; ϵ 4+ = carrier of the ϵ 4 allele.

The *P*-values are from binary logistic regression analyses with age, hypertension, hyperlipidemia, diabetes, obesity, smoking and alcohol consumption as covariates.

known to be associated with high LDL cholesterol level,⁶ which is an important factor in the early development of atherosclerosis. Therefore, it can be hypothesized that the ϵ 4 allele mainly affects the initial stages of cerebral atherosclerosis. In addition, ϵ 4 also seems to have an independent role in cerebral small vessel disease, as suggested by its effect on MRI white matter hyperintensities in a study by Hogh *et al.*²⁰ These authors propose that ϵ 4 may interact with other cardiovascular risk factors, such as hypertension, in affecting lipid metabolism and cellular repair mechanism.²⁰ In fact, a previous study showed that *APOE* ϵ 4 has a decreased antioxidant activity compared to other alleles.²¹

The role of the *APOE* promoter polymorphisms –219G/T and +113G/C in IS or cerebral atherosclerosis had not been studied before. Our results suggest that G-allele carriers of both polymorphisms are at an increased risk of IS. This association was significant within the most common *APOE* ϵ 3/ ϵ 3 genotype group, indicating that it is most probably independent of the *APOE* ϵ 2/ ϵ 3/ ϵ 4 genotype. The *APOE* gene promoter polymorphism –219G/T affects the transcriptional activity of the *APOE* gene, in particular the G-allele is associated with higher transcription than the T-allele.¹¹ Consequently, G-allele carriers have significantly higher plasma concentrations of apoE.¹² Recent studies suggested that the apoE level is related to stroke.^{9,8} Our results provide the evidence that a genetic determinant of higher levels of apoE increases the risk of IS.

ApoE seems to have a multifaceted role on atherosclerosis and vascular events in humans. Our findings suggest that the association of the G-alleles of both promoter SNPs with the risk of IS would involve mechanisms other than those leading to an accelerated development of stable intracranial atherosclerosis. One hypothesis would be that increased apoE level predispose to an unstable atherosclerotic plaque. This is supported by studies on apoE –/– mice. Although these animals develop severe atherosclerosis, they rarely have spontaneous plaque rupture and thrombosis.²² Furthermore, a recent study showed that increased apoE deposits in early

Table 3 Mean atherosclerosis score in Helsinki Sudden Death Study (HSDS) and Tampere Autopsy Study (TASTY) series

	All			Men			Women		
	N	Score	P-value	N	Score	P-value*	N	Score	P-value*
ϵ 4+	289	5.4 (6.0)	0.051	222	5.4 (5.9)	0.044	67	5.6 (6.4)	0.596
ϵ 4–	626	4.8 (5.6)		492	4.6 (5.4)		134	5.7 (6.5)	
–219 (within ϵ3/ϵ3)									
G+	458	4.8 (5.6)	0.960	370	4.6 (5.3)	0.591	88	5.9 (6.5)	0.399
T/T	75	4.9 (5.7)		57	4.7 (5.4)		18	5.4 (6.7)	

ϵ 4+ = carrier of the ϵ 4 allele; ϵ 4– = non-carrier of the ϵ 4 allele; G+ = carrier of the G-allele (*APOE* –219G/G and –219G/T).

Values are presented as mean (SD).

The *P*-values are from analysis of covariance (ANCOVA) with age, gender and body mass index as covariates.

*The *P*-values are from analysis of covariance (ANCOVA) with age and body mass index as covariates.

atherosclerotic lesions distinguish symptomatic from asymptomatic patients.²³ A second mechanism may be related to the finding that apoE has proinflammatory properties.^{10,24} Elevated apoE level lead to chronic inflammation that may contribute to atherosclerosis. Another possibility is that apoE influences the risk of IS by a mechanism independent of atherosclerosis. ApoE is known to play a role in the coagulation pathway, in particular, by affecting vitamin K1 metabolism. Vitamin K1 is a chylomicron-bound essential cofactor for the synthesis of several blood coagulation factors. Chylomicron uptake and clearance are affected significantly by *APOE* genotype,²⁵ leading to marked fluctuations in plasma concentrations of vitamin K1.²⁶ Low vitamin K1 levels have been measured, especially in patients carrying the $\epsilon 4$ allele²⁶ and are associated with a poor outcome in hemorrhagic stroke patients.²⁷ Thus, possible interactions between *APOE* variants and vitamin K1 metabolism might be related to the risk of IS independently of the development of atherosclerosis.

A possible explanation of the observed difference of *APOE* effect on cerebral atherosclerosis in men and women could be related to the gender-dependent effect of apoE isoforms on immune system activation^{28,29} or to the influence of sex hormones on apoE protein production.³⁰

In summary, this study revealed a multifaceted role of *APOE* gene on IS and subclinical intracranial atherosclerosis. The *APOE* $\epsilon 4 +$ genotype was associated with the severity of subclinical intracranial atherosclerosis in men, but was not a predictor of IS. On the other hand, the promoter variants affecting apoE synthesis were significant predictors of IS, suggesting that quantitative rather than qualitative variation of apoE is related to IS.

Acknowledgements

We thank the laboratory technicians Nina Peltonen and Ana Lopes Cruz for their help. This study was funded by Erasme Funds, FNRS, Emil Aaltonen Foundation (TL), Medical Research Fund of Tampere University Hospital, the Pirkanmaa Regional Fund of the Finnish Cultural Foundation, the Finnish Foundation for Cardiovascular Research and the Yrjö Jahansson Foundation.

References

- Casas JP, Hingorani AD, Bautista LE, Sharma P: Meta-analysis of genetic studies in ischemic stroke: thirty-two genes involving approximately 18 000 cases and 58 000 controls. *Arch Neurol* 2004; **61**: 1652–1661.
- Flossmann E, Schulz UG, Rothwell PM: Systematic review of methods and results of studies of the genetic epidemiology of ischemic stroke. *Stroke* 2004; **35**: 212–227.
- Jerrard-Dunne P, Cloud G, Hassan A, Markus HS: Evaluating the genetic component of ischemic stroke subtypes: a family history study. *Stroke* 2003; **34**: 1364–1369.
- Polychronopoulos P, Gioldasis G, Ellul J *et al*: Family history of stroke in stroke types and subtypes. *J Neurol Sci* 2002; **195**: 117–122.
- Schulz UG, Flossmann E, Rothwell PM: Heritability of ischemic stroke in relation to age, vascular risk factors, and subtypes of incident stroke in population-based studies. *Stroke* 2004; **35**: 819–824.
- Bennet AM, Di Angelantonio E, Ye Z *et al*: Association of apolipoprotein E genotypes with lipid levels and coronary risk. *JAMA* 2007; **298**: 1300–1311.
- Sudlow C, Martinez Gonzalez NA, Kim J, Clark C: Does apolipoprotein E genotype influence the risk of ischemic stroke, intracerebral hemorrhage, or subarachnoid hemorrhage? Systematic review and meta-analyses of 31 studies among 5961 cases and 17 965 controls. *Stroke* 2006; **37**: 364–370.
- Slowik A, Iskra T, Turaj W, Hartwich J, Dembinska-Kiec A, Szczudlik A: LDL phenotype B and other lipid abnormalities in patients with large vessel disease and small vessel disease. *J Neurol Sci* 2003; **214**: 11–16.
- van Vliet P, Mooijaart SP, de Craen AJ, Rensen PC, van Heemst D, Westendorp RG: Plasma levels of apolipoprotein E and risk of stroke in old age. *Ann NY Acad Sci* 2007; **1100**: 140–147.
- Mooijaart SP, Berbee JF, van Heemst D *et al*: ApoE plasma levels and risk of cardiovascular mortality in old age. *PLoS Med* 2006; **3**: e176.
- Artiga MJ, Bullido MJ, Sastre I *et al*: Allelic polymorphisms in the transcriptional regulatory region of apolipoprotein E gene. *FEBS Lett* 1998; **421**: 105–108.
- Lambert JC, Brousseau T, Defosse V *et al*: Independent association of an *APOE* gene promoter polymorphism with increased risk of myocardial infarction and decreased *APOE* plasma concentrations—the ECTIM study. *Hum Mol Genet* 2000; **9**: 57–61.
- Viiri LE, Loimaala A, Nenonen A *et al*: The association of the apolipoprotein E gene promoter polymorphisms and haplotypes with serum lipid and lipoprotein concentrations. *Atherosclerosis* 2005; **179**: 161–167.
- Viiri LE, Raitakari OT, Huhtala H *et al*: Relations of *APOE* promoter polymorphisms to LDL cholesterol and markers of subclinical atherosclerosis in young adults. *J Lipid Res* 2006; **47**: 1298–1306.
- Viitanen L, Pihlajamaki J, Miettinen R *et al*: Apolipoprotein E gene promoter (–219G/T) polymorphism is associated with premature coronary heart disease. *J Mol Med* 2001; **79**: 732–737.
- Campbell CD, Ogburn EL, Lunetta KL *et al*: Demonstrating stratification in a European American population. *Nat Genet* 2005; **37**: 868–872.
- Ilveskoski E, Perola M, Lehtimäki T *et al*: Age-dependent association of apolipoprotein E genotype with coronary and aortic atherosclerosis in middle-aged men: an autopsy study. *Circulation* 1999; **100**: 608–613.
- Peltonen L, Pekkarinen P, Aaltonen J: Messages from an isolate: lessons from the Finnish gene pool. *Biol Chem Hoppe Seyler* 1995; **376**: 697–704.
- Hixson JE: Apolipoprotein E polymorphisms affect atherosclerosis in young males. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) research group. *Arterioscler Thromb* 1991; **11**: 1237–1244.
- Hogh P, Garde E, Mortensen EL, Jorgensen OS, Krabbe K, Waldemar G: The apolipoprotein E epsilon4-allele and antihypertensive treatment are associated with increased risk of cerebral MRI white matter hyperintensities. *Acta Neurol Scand* 2007; **115**: 248–253.
- Miyata M, Smith JD: Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. *Nat Genet* 1996; **14**: 55–61.
- Calara F, Silvestre M, Casanada F, Yuan N, Napoli C, Palinski W: Spontaneous plaque rupture and secondary thrombosis in apolipoprotein E-deficient and LDL receptor-deficient mice. *J Pathol* 2001; **195**: 257–263.
- Wyler von Ballmoos M, Dubler D, Mirlacher M, Cathomas G, Muser J, Biedermann BC: Increased apolipoprotein deposits in early atherosclerotic lesions distinguish symptomatic from

- asymptomatic patients. *Arterioscler Thromb Vasc Biol* 2006; **26**: 359–364.
- 24 van den Elzen P, Garg S, Leon L *et al*: Apolipoprotein-mediated pathways of lipid antigen presentation. *Nature* 2005; **437**: 906–910.
- 25 Weintraub MS, Eisenberg S, Breslow JL: Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. *J Clin Invest* 1987; **80**: 1571–1577.
- 26 Kohlmeier M, Salomon A, Saupe J, Shearer MJ: Transport of vitamin K to bone in humans. *J Nutr* 1996; **126**: 1192S–1196S.
- 27 Weir CJ, McCarron MO, Muir KW *et al*: Apolipoprotein E genotype, coagulation, and survival following acute stroke. *Neurology* 2001; **57**: 1097–1100.
- 28 Garcia-Segura LM, Azcoitia I, DonCarlos LL: Neuroprotection by estradiol. *Prog Neurobiol* 2001; **63**: 29–60.
- 29 Colton CA, Brown CM, Vitek MP: Sex steroids, APOE genotype and the innate immune system. *Neurobiol Aging* 2005; **26**: 363–372.
- 30 Phillips NR, Havel RJ, Kane JP: Sex-related differences in the concentrations of apolipoprotein E in human blood plasma and plasma lipoproteins. *J Lipid Res* 1983; **24**: 1525–1531.