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The Effect of a Gender Difference in the Apolipoprotein E Gene DNA Polymorphism on Serum Lipid Levels in a Serbian Healthy Population

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To date, no data have been available on relationship between apolipoprotein E polymorphism and lipid levels in Serbian populations. Blood samples were obtained from 591 healthy normal individuals (193 women and 398 men). A 244 bp sequence of the apolipoprotein E gene including the two polymorphic sites was amplified by polymerase chain reaction. After digestion with *Hha*I, DNA fragments were visualized by microplate array diagonal gel electrophoresis. In men, levels of both total and low-density lipoprotein cholesterol among the three apolipoprotein E genotype groups differed significantly ($p < 0.05$). The ε2 allele was associated with lower concentrations of both total and low-density lipoprotein cholesterol, where the ε4 allele had the opposite effects. No significant effects of apolipoprotein E polymorphism on serum lipid levels were observed in women. The presented data could be taken into consideration in any future disease risk evaluation in this population.

Key words: Apolipoprotein E (ApoE) gene; DNA polymorphism; Polymerase chain reaction (PCR); Gender-specific effects; Lipids; Healthy population.

Abbreviations: ApoE, apolipoprotein E; BMI, body mass index; HL, hepatic lipase; HDL, high density lipoprotein; LCAT, lecithin cholesterol acyltransferase; Lp(a), lipoprotein(a); LPL, lipoprotein lipase; LDL, low density lipoprotein; PCR, polymerase chain reaction; TG, triglyceride; VLDL, very low density lipoprotein.

Introduction

Apolipoprotein E (ApoE) plays an important role in lipid metabolism. It is one of the major protein constituents of chylomicrons, very low density lipoproteins (VLDL), their remnant particles, and high density lipoproteins (HDL). On these particles, it serves as a ligand for uptake by lipoprotein receptors (1, 2). Other functions and properties ascribed to apoE include its roles in immunoregulation, nerve regeneration, inhibition of endothelial and tumor cell proliferation, modulation of intracellular cholesterol utilization and steroidogenesis in adrenal cells, and either activation or modulation of enzymes such as hepatic lipase (HL), lipoprotein lipase (LPL), and lecithin cholesterol acyltransferase (LCAT) (3–5). ApoE plays a significant role in the pathogenesis of atherosclerosis (6). One of the more controversial and newer functions suggested for apoE is based on observations that apoE is found in amyloid plaques associated with Alzheimer's and Creutzfeldt-Jakob diseases, as well as in a variety of types of cerebral and systemic amyloidoses (7).

Circulating human apoE is a single-chain protein of 34.2 kDa, encoded by a single gene. Together with the apo CI, apo CI′ and apo CII genes, the apoE gene forms a gene cluster on the long arm of chromosome 19 (19q13.2) (8). The apoE gene is 3597 nucleotides long and consists of four exons and three introns, yielding a mRNA of 1163 nucleotides (9). ApoE is initially synthesized as a propeptide of 317 amino acid residues. After post-translational cleavage of a signal peptide of 18 amino acids, the mature protein consists of 299 amino acid residues (10, 11). ApoE is produced in various organs, including liver, brain, kidney, adrenal glands, gonads, spleen, and keratinocytes (12–17).

The human apoE gene is polymorphic, with three common alleles (ε2, ε3, ε4) coding for three isoforms (E2, E3, E4) (18). The molecular basis of apoE polymorphism is cysteine-arginine interchange (19). ApoE3 contains a single cysteine at residue 112 and an arginine at position 158; apoE2 contains cysteine residues at both positions 112 and 158, and apoE4 contains arginine residues at both positions. This polymorphism leads to the presence of six different phenotypes in the human population: three homozygous (E3/3, E2/2, and E4/4) and three heterozygous (E2/3, E2/4, and E3/4). Individuals carrying the ε2 (Arg 158 → Cys) allele display high levels of apoE and low levels of plasma cholesterol, low-density lipoprotein (LDL)-cholesterol, apoB, and lipoprotein(a) (Lp(a)), whereas ε4 (Cys 112 → Arg) allele carriers show the opposite (20). The influence of apoE on lipid levels is often suggested to have major implications for the risk of coronary artery disease, individuals with an ε4 (Cys 112 → Arg) allele are at higher risk, as compared to ε2 (Arg 158 → Cys) allele carriers (21). Apoε4 (Cys 112 → Arg) allele frequency has been described as high in Alzheimer's disease and other neurodegenerative disorders (22–24). The common ε2 (Arg 158 → Cys) isoform exhibits a markedly reduced affinity for hepatic lipoprotein receptors; homozygosity for this isoform is a prerequisite for type III hyperlipoproteinemia (25). Significant interpopulation differences exist with respect to the relative frequencies of three common apoE alleles (26). The ε3 allele is universally the most common. This has been also confirmed in the Serbian population study (27). However no data are available to date and on relationship between apoE polymor-

phism and lipid levels in this population. Thus the purpose of the present study was to investigate the impact of apoE polymorphism on serum lipid concentrations.

Materials and Methods

Samples

Blood samples were obtained from 591 healthy individuals (193 women and 398 men; age ranging from 18 to 81 years). Informed consent was obtained from each participant in the study. Personal data (age, sex, weight, height, and blood pressure) were obtained from all participants. Blood pressure measurements (mm Hg) were determined while the participants were seated. In addition a questionnaire with approximately 30 questions was designed to obtain relevant social (marital status, profession, salary, smoking, alcohol consumption, physical activity), medical (general health, cardiovascular health, physician visits) and family history (general and cardiovascular health of parents and siblings). All subjects with a personal or a family history of cardiovascular disease, diabetes and/or thyroid dysfunction were excluded. Individuals taking any drugs with lipid-lowering effects were also excluded. Descriptive statistics of concomitants and lipid variables are present in Table 1.

Blood samples were collected from participants after 12 hours of fasting and centrifuged within 3 hours of collection. Serum was transferred to tubes and used for determination of lipids. The total plasma cholesterol and triglyceride (TG) levels were determined on a Monarch Plus apparatus (Instrumentation Laboratory, Lexington, USA) using enzymatic colorimetric methods. The HDL cholesterol was determined after dextran sulfate – Mg^{2+} precipitation of VLDL and LDL; using CHOD-PAP method. The LDL cholesterol was calculated using the Friedewald formula (28) for participants with TG levels <4.5 mmol/l. All reagent kits were provided by Instrumentation Laboratory (Lexington, USA). Fasting peripheral blood samples (10ml) for DNA analysis were collected in sodium citrate-containing tubes.

DNA analysis

DNA was extracted by Triton X-100 lysis, proteinase K digestion and phenol/chloroform extraction (29). When the procedure could not be carried out within 2–4 days after blood collection, the blood was frozen at –20°C and the DNA was extracted within the following 4–8 weeks. The concentration of isolated DNA was determined by spectrophotometer (LKB-Pharmacia, Uppsala, Sweden).

A 244bp sequence of the apoE gene including the two polymorphic sites was amplified by polymerase chain reaction (PCR) in a Hybaid Omnigene thermocycler (Teddington, UK) using the oligonucleotide primer pair F4 and F6 described by Emi *et al.* (30). The assay conditions were as published previously (27).

After digestion with *Hha*I, 3 µl of each PCR product was added to 1.5 µl of formamide dye mix and then loaded into the wells of a 12g/l microplate array diagonal gel electrophoresis (MADGE gel). Gel was prestained in a solution of 10 µl ethidium bromide in 100 ml of 1xTris-borate-EDTA, pH 8.3 for 10min in an electrophoresis tank (31). The gels are supported on glass and contain 96 wells (6.28mm^3) for sample loading in an 8×12 array. Electrophoresis was at 10 V/cm at room temperature for 45min. We observed the gel with the use of an ultraviolet trans-illuminator and acquired a digital image of the gel with a CCD camera and frame grabber (GS8000 Documentation System, UVP Inc, USA).

Statistical analysis

The frequencies of ε2, ε3, and ε4 alleles were estimated by the gene counting method. To express variances of the allele frequencies, *i.e.* the sample size-dependent standard error of the estimated frequencies, the upper and lower limits of the 95% confidence intervals for the three alleles were calculated. Chi-square statistics were used to test for goodness of fit to the Hardy-Weinberg equilibrium.

Only five apoE genotypes were included in the estimation of impact of apoE polymorphism on quantitative traits. Subjects with an E2/4 genotype were not included because of the potentially opposite effects of the ε2 and ε4 alleles on serum lipid levels. The subjects were pooled into three groups (E2-, E3-, and E4-containing genotypes) to explore the allelic effect and to increase statistical power. The E2-group was composed of the subjects with E2/2 and E2/3 genotypes, the E3-group was composed of the E3/3 subjects and the E4-group was composed of the E3/4 and E4/4 subjects. A one way analysis of variance (ANOVA) was performed separately for men and women to test the null hypothesis of equality of lipid levels between ApoE genotypes (or genotype groups). We considered statistical significance at the 0.05 level.

Initially both HDL cholesterol and TG concentrations, were normalized by taking natural logarithms to ensure the distribution of the residual error terms for each dependent variable was Gaussian. All lipid values were adjusted to remove the effects of the set of significant covariates (age, body mass index (BMI) and cigarette smoking). The impact of apoE alleles on levels of blood lipids was determined separately within each group using the average excess statistics (32):

$$\alpha_i = \frac{F_{ii} \cdot \mu_{ii} + (1/2) \Sigma_{i \neq j} F_{ij} \cdot \mu_{ij}}{F_i} - \mu,$$

where α_i is the average excess of the *i*th allele, j is the other allele at the locus, μ_{ij} is the mean lipid level of the *ij*th genotype, µ is the overall group lipid mean, F_{ii} is the observed frequency of the homozygous *ii* class, F_{ij} is the observed frequency of the heterozygous *ij* class, and F_i is the allele frequency of the *i*th allele.

Tab. 1 Characteristics of a studied population of Serbia*.

Variable		Total	Men	Women
n		591	398	193
Age (years)	X±SD	39.46±13.54	38.18±12.65	42.10±14.91
Smokers	n (%)	279 (47.2%)	210 (52.8%)	69 (35.8%)
BMI (kg/m^2)	X±SD	26.32±3.57	26.32±3.57	24.29±4.09
TC (mmol/l)	X±SD	5.90±1.17	5.84±1.26	5.93±1.13
HDLC (mmol/l)	X±SD	1.38±0.29	1.50±0.31	1.33±0.26
LDLC (mmol/l)	X±SD	3.85±1.04	3.77±1.10	3.89±1.01
TG (mmol/l)	X±S	1.47±0.81	1.27±0.68	1.57±0.86

* Non-adjusted values (means ± SD) are shown. BMI = body mass index; TC = total cholesterol; HDLC = high density lipoprotein cholesterol; LDLC low density lipoprotein cholesterol; TG = triglycerides.

Results

ApoE genotype and allele frequencies

The numbers and relative frequencies of the common apoE genotypes in a sample of 591 Serbians are shown in Table 2. The most frequent allele was ε3 (relative frequency 0.742), the next most common ε2 (relative frequency 0.147), followed by ε4 (relative frequency 0.111). The apoE genotype frequencies in the Serbian healthy population studied were in Hardy-Weinberg equilibrium ($\chi^2 = 8.98$, $p = 0.11$).

Association of apoE polymorphism with serum lipid levels

Adjusted means of serum lipid levels (total cholesterol, HDL cholesterol, LDL cholesterol and TGs) according to apoE genotypes are shown in Table 3. A significant difference in both LDL cholesterol and TG levels was obtained in whole sample and in LDL cholesterol levels in men as well.

The sample of 591 healthy subjects (398 men and 193 women) was grouped according to their genotypes (see Materials and Methods). Table 4 presents the average adjusted serum lipid levels for each apoE genotype group in men and women. In men, levels of both total and LDL cholesterol among the three apoE genotype groups differed significantly ($p < 0.05$). Subjects in the apoE4 genotype group were found to have a significantly higher value of total cholesterol compared with the ones of both apoE2 ($p = 0.021$) and apoE3 ($p = 0.022$) group. Subjects in the apoE4 genotype group had significantly higher LDL cholesterol in comparison with the apoE2 genotype group ($p = 0.006$). The same effects of apoE polymorphism were not observed in women. We also observed a borderline significance for the effect of apoE polymorphism on TG level in men ($p = 0.06$). TG levels were higher in both apoE2 and apoE4 groups compared with the apoE3 group. No apparent effect was seen in women. Variation of HDL cholesterol levels among three apoE groups was not significant in both sexes.

In Table 5 we have summarized the estimates of the average excess of the three ε alleles (α) on each of the lipid traits. A general tendency is clear in the Serbian data. The ε2 allele was associated with lower both total and LDL cholesterol, where the ε4 allele had the opposite effect.

Tab. 2 Allele frequencies and prevalance of apoE genotypes in a Serbian population.

Allele		Relative frequency		
ε2		0.147		
ε3		0.742		
ε4		0.111		
Genotype	Observed		Expected	
	n	(%)	n	(%)
E2/2	22	3.70	13	2.20
E2/3	113	19.10	128.84	21.80
E2/4	17	2.90	19.50	3.30
E3/3	331	56.00	325.05	55.00
E3/4	102	17.30	97.52	16.50
E4/4	6	1.00	7.09	1.20
Whole sample	591	100	591	100

Tab. 3 Serum lipid levels (mmol/l) in relation to apoE genotypes in a healthy population of Serbia.

Genotype apoE	n	Total cholesterol (mmol/l)	HDL cholesterol (mmol/l)	LDL cholesterol (mmol/l)	Tri-glycerides (mmol/l)
Whole sample	591	5.96	1.39	3,89	1.49
2/2	22	5.71	1.46	3.55	1.43
2/3	113	5.84	1.40	3.75	1.52
2/4	17	5.88	1.38	3.62	1.93
3/3	331	5.95	1.38	3.92	1.45
3/4	102	6.17	1.40	4.07	1.53
4/4	6	6.23	1.38	3.98	1.93
	ANOVA (p)	NS	NS	<0.05	<0.05
Men	398	5.94	1.32	3.89	1.60
22	14	5.61	1.41	3.40	1.60
23	73	5.84	1.37	3.74	1.59
24	12	5.72	1.25	3.54	2.05
33	216	5.91	1.30	3.91	1.55
34	80	6.21	1.33	4.12	1.66
44	3	5.95	1.25	3.73	2.15
	ANOVA (p)	NS	NS	<0.05	NS
Women	193	6.01	1.51	3.90	1.32
22	8	5.88	1.56	3.79	1.16
23	40	5.89	1.45	3.79	1.41
24	5	6.21	1.63	3.80	1.68
33	115	6.06	1.51	3.95	1.31
34	22	5.95	1.54	3.85	1.21
44	3	6.33	1.54	4.11	1.50
	ANOVA (p)	NS	NS	NS	NS

Discussion

The apoE allele frequencies found in the present study are somewhat different from those reported in other white populations. The frequency of the ε2 allele estimated here (0.147) is the highest ever reported to our knowledge. The increase of the ε2 allele frequency is compensated for by a lowering of the ε4 frequency (0.111), whereas the ε3 allele frequency remains comparable to frequencies reported in other white populations (26).

In the present study, associations of apoE polymorphism with the lipids studied were consistent with the well-identified effects of apoE (6, 21, 33): ε4 increased both total and LDL cholesterol while ε2 decreased it.

The relation between apoE polymorphism and HDL has been reported in some studies (34, 35), but not in others (36). The connection of apoE polymorphism and

Tab. 4 Serum lipid levels (mmol/l) in relation to apoE genotype groups.

Variable	ApoE Group				
	All	E2 (E2/2, E2/3) (mmol/l)	E3 (E3/3) (mmol/l)	E4 (E3/4, E4/4) (mmol/l)	ANOVA p-value (mmol/l)
Whole sample (n)	574	135	331	108	
Total cholesterol	5.96	5.77	5.90	6.12	<0.05
HDL cholesterol	1.39	1.40	1.37	1.39	NS
LDL cholesterol	3.89	3.68	3.89	4.03	<0.05
Triglycerides	1.49	1.48	1.43	1.53	NS
Men (n)	386	87	216	83	
Total cholesterol	5.94	5.80	5.90	6.20	<0.05
HDL cholesterol	1.32	1.38	1.31	1.33	NS
LDL cholesterol	3.89	3.69	3.91	4.12	<0.05
Triglycerides	1.60	1.57	1.52	1.66	NS
Women (n)	188	48	115	25	
Total cholesterol	6.01	5.72	5.90	5.83	NS
HDL cholesterol	1.51	1.46	1.50	1.54	NS
LDL cholesterol	3.90	3.67	3.83	3.76	NS
Triglycerides	1.32	1.30	1.25	1.18	NS

NS: not significant

Tab. 5 Average excess (mmol/l) of the three common apoE alleles on the serum lipid traits in a Serbian population (whole sample).

Allele	Average excess of the allele (α)(mmol/l)			
	Total cholesterol	HDL cholesterol	LDL cholesterol	Triglycerides
ε2	–0.179	0.018	-0.222	0.007
ε3	0.004	–0.004	0.017	–0.010
ε4	0.210	0.008	0.158	0.089

HDL may be explained by these facts: apoE polymorphism affects cholesterol ester transfer protein activity; apoE is involved in extracellular efflux of cholesterol; apoE modulates LPL activity. We did not find any significant association between apoE polymorphism and HDL cholesterol level in both sexes, although there was a slightly lower average concentration in male ε4 carriers.

Whereas the effect on serum cholesterol levels is almost constant in most populations studies, there are controversial results about the effect on triglycerides, since they vary widely among and within individuals, masking a clear effect of apoE phenotype (37). Concentration of TGs appear to have more complex relations with apoE. Persons with either ε2 or ε4 alleles have higher TG levels than individuals with ε3. This U-shaped trend has been reported in many studies (36, 38, 39). The association of ε2 with higher concentration of TGs would be expected, because remnants of TG-rich lipoproteins would be cleared more slowly in these individuals. Individuals with ε4 have lower LPL activity, which could explain the higher TG concentration they have.

It has been established that males and females have different natural histories of disease, including predisposing characteristics of life-style, and have different frequency distribution of lipids and apolipoproteins, providing strong evidence that the genotype profiles should be investigated in a gender-specific manner (36, 39–44). Thus, several studies have reported similar results, finding differences that were highly significant in males, but not in females, for total and LDL-cholesterol (39, 42). Recently, Kamboh *et al.* (44) found in a study of an African black population that the apoE polymorphism is significantly associated with both total and LDL-cholesterol, but only in women. Our findings are in agreement with the proposed general effect of this polymorphism on serum cholesterol level. However, controversial results concerning gender-specific aspects of the effects in different ethnic populations could suggest that they should not be considered as fully independent of the total genetic pool of a particular population and/or specific environmental background. This statement implies there should be more detailed studying of both gene-gene and gene-environment interaction to evaluate diseases risk in the future.

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References

1. Aalto-Setala K, Benlian P, Bowyer D, Hofker M, James RW, Poledne R. European Lipoproteins Club: report of the 20th Annual Conference, Tutzing, 8–11 Sep 1997. Atherosclerosis 1998; 137:223–9.

2. St Clair RW, Beisiegel U. What do all the apolipoprotein E receptors do? Curr Opin Lipidol 1997; 8:243–5.
3. Mistry MJ, Clay MA, Kelly ME, Steiner MA, Harmony JAK. Apolipoprotein E restricts interleukin-dependent T-lymphocyte proliferation at the GI_A/GI_B boundary. Cell Immunol 1995; 160:14–23.
4. Vogel T, Guo N, Guy R, Drezlich N, Krutzsch C, Blake DA, *et al.* Apolipoprotein E: a potent inhibitor of endothelial and tumor cell proliferation. J Cell Biochem 1994; 54:299–308.
5. Ignatius MJ, Gebicke-Harter PJ, Skene JHP, Schilling JW, Weisgraber KH, Mahley RW, *et al.* Expression of apolipoprotein E during nerve degeneration and regeneration. Proc Natl Acad Sci USA 1986; 83:1125–9.
6. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arterisclerosis 1988; 8:1–21.
7. Siest G, Pillot T, Regis-Bally, A, Leininger-Muller B, Steinmetz J, Galteau MM, *et al.* Apolipoprotein E: an important gene and protein to follow in laboratory medicine. Clin Chem 1995; 41:1068–86.
8. Shaw DJ, Brook JD, Meredith AL, Harley HG, Sarfarazi M, Harper PS, *et al.* Gene mapping and chromosome 19. J Med Genet 1986; 23:2–10.
9. Paik YK, Chang DJ, Reardon CA, Davies GE, Mahley RW, Taylor JM. Nucleotide sequence and structure of the human apolipoprotein E gene. Proc Natl Acad Sci USA 1985; 82:3445–9.
10. Rall S, Weisgraber KH, Mahley RW. Human apolipoprotein E: the complete amino acid sequence. J Biol Chem 1982; 257:4171–8.
11. Brouwer DAJ, van Doormaal JJ, Muskiet FAJ. Clinical chemistry of common apolipoprotein isoforms. J Chromatography 1996; 678:23–41.
12. Basu SK, Ho YK, Brown MS, Bilheimer DW, Anderson RGW, Goldstein JL. Biochemical and genetic studies of the apolipoprotein E secreted by mouse macrophages and human monocytes. J Biol Chem 257:9788–95.
13. Blue ML, Williams DL, Zuecker S, Khan SA, Blum CB. Apolipoprotein E synthesis in human kidney, adrenal gland and liver. Proc Natl Acad Sci USA 1983; 80:283–7.
14. Driscoll MD, Shreiber JR, Shmit VM, Getz GS. Regulation of apolipoprotein E synthesis in rat ovarian granulosa cells. J Biol Chem 1985; 260:9031.
15. Beckmann MD, Olson LM, Shreiber JR. Apolipoprotein E synthesis by cultured human ovarian granulosa cells: regulation by chorionic gonadotropin and cholesterol. Fertil Steril 1991; 55:1118–25.
16. Law GL, McGuinness MP, Linder CC, Griswold MD. Expression of apolipoprotein E mRNA in the epithelium and interstitium of the testis and the pididymis. J Androl 1997; 18:32–42.
17. Rindler MJ, Traber MG, Esterman AL, Bersinger NA, Dancis J. Synthesis and secretion of apolipoprotein E by human placenta and choriocarcinoma cell lines. Placenta 1991; 12:615–24.
18. Fenjves ES, Gordon DA, Purshing LK, Williams DL, Taichman LB. Systemic distribution of apolipoprotein E secreted by grafts of epidermal keratinocytes: implications for epidermal function and gene therapy. Proc Natl Acad Sci USA 1989; 86:8803–7.
19. Weisgraber KH, Rall SC, Mahley RW. Human E apolipoprotein heterogenity (cysteine-arginine interchances in the amino acid sequence of the apoE isoforms). J Biol Chem 1981; 256:9077–83.
20. de Knijff P, Maagdenberg AMJM, Frants RR, Havekes LM. Genetic heterogeneity of apolipoprotein E and its influence on plasma lipid and lipoprotein levels. Hum Mutat 1994; 4:178–94.
21. Menzel HJ, Kladetsky RG, Assmann G. Apolipoprotein E polymorphism and coronary artery disease. Arteriosclerosis 1983; 3:310–5.
22. Hardy J. New insights into the genetics of Alzheimer's disease. Ann Med 1996; 28:255–8.
23. Rubinstein DC. The genetics of Alzheimer's disease. Prog Neurobiol 1997; 52:447–54.
24. Strittmatter WJ, Ross AD. Apolipoprotein E and Alzheimer's disease. Annu Rev Neurosci 1996; 19:53–77.
25. Mahley RW, Rall SC. Type III hyperlipoproteinemia (dysbetalipoproteinemia): the role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic basis of inherited disease, 6th ed. New York: McGraw-Hill, 1989; 6:1195.
26. Gerdes LU, Klausen IC, Sihm I, Faergman O. Apolipoprotein E polymorphism in a Danish population compared to findings in 45 other study populations around the world. Genet Epidemiol 1992; 9:155–67.
27. Stanković S, Glišić S, Alavantić D. Apolipoprotein E gene DNA polymorphism in Serbian population. Yug Med Biochem 1999; 18:9–15.
28. Friedewald WT, Levy RI, Frederickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentifuge. Clin Chem 1972; 18:499–502.
29. Kunkel LM, Smith KD, Bayer SH. Analysis of human Y-chromosome-specific reiterated DNA in chromosome variants. Proc Natl Acad Sci USA 1977; 74:1245–9.
30. Emi M, Wui LL, Robertson MA, Myers RL, Hegele RA, Williams RR, *et al.* Genotyping and sequence analysis of apolipoprotein E isoforms. Genomics 1988; 3:373-9.
31. Bolla MK, Haddad L, Humphries SE, Winder AF, Day INM. High-throughput method for determination of apolipoprotein E genotypes with use of restriction digestion analysis by microplate array diagonal gel elestrophoresis. Clin Chem 1995; 1599–604.
32. Hallman DM, Boerwinkle E, Saha N, Sandholzer C, Menzel HJ. The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. Am J Hum Genet 1991; 49:338–49.
33. Bouthillier D, Sing CF, Davignon J. Apolipoprotein E phenotyping with a single gel method: application to the study of informative matings. J Lipid Res 1983; 24:1060–9.
34. Wilson HM, Patel JC, Russel D, Skinner ER. Alterations in the concentration of an apolipoprotein E-containing subfraction of plasma high density lipoprotein in coronary heart disease. Clin Chim Acta 1993; 220:175–87.
35. Ikewaki K, Rader DJ, Zech LA, Brewer HB Jr. In vivo metabolism of apolipoprotein A-1 and E in patients with abetalipoproteinemia: implications for the roles of apolipoproteins B and E in HDL metabolism. J Lipid Res 1994; 35:1809–19.
36. Schaefer EJ, Lamon-Fava S, Johnson S, Ordovas JM, Schaefer MM, Castelli WP. Effects of gender and menopausal status on the association of apolipoprotein E phenotype with plasma lipoprotein levels. Results from Framingham Offspring Study. Arterioscler Throm 1994; 14:1105–13.
37. Austin MA. Plasma triglyceride and coronary heart disease. Arteriscl Thromb 1991; 11:2–13.
38. Kaprio J, Ferrel RE, Kottke BA, Kamboh MI, Sing CF. Effects of polymorphisms in apolipoprotein E, A-IV, and H on quantitative traits related to risk for cardiovascular disease. Arteriscler Thromb 1991; 11:1330–48.
39. Boerwinkle E, Visvikis S, Welsh D, Steinmetz J, Hanash SM, Sing CF. The use of measured genotype information in

the analysis of quantitative phenotypes in man. II. The role of apolipoprotein E polymorphism in determining levels, variability, and covariability of cholesterol, beta-lipoprotein and triglycerides in a sample of unrelated individuals. Am J Hum Genet 1987; 27:567–82.
40. Reilly SL, Ferrell RE, Kottke BA, Kamboh MI, Sing CF. The gender-specific apolipoprotein E genotype influence on the distribution of lipids and apolipoproteins in the population of Rochester, MN. I. Pleiotropic effects on means and variances. Am J Hum Genet 1991; 49:1155–66.
41. Hegele RA, Evans AJ, Tu L, Ip G, Brunt H, Connelly PW. A gene-gender interaction affecting plasma lipoproteins in a genetic isolate. Arterioscl Thromb 1994; 14:671–8.
42. Porkka KVK, Taimela S, Kontula K, Lehtimaki T, Aalto-Setala K, Akerblom HK, *et al.* Variability gene effects of DNA polymorphism at the apo B, apo AI/CIII and apo E loci on serum lipids: the cardiovascular risk in young Finns study. Clin Genet 1994; 445:113–21.
43. Payami H, Zareparsi S, Montee KR, Sexton GJ, Kaye JA, Bird TD, *et al.* Gender difference in apolipoprotein E-associated risk for familial Alzheimer disease: a possible clue to the higher incidence of Alzheimer disease in women. Am J Hum Genet 1996; 58:803–11.
44. Kamboh MI, Bunker CH, Aston CE, Nestlerode CS, McAllister AE, Ukoli FA. Genetic association of five apolipoprotein polymorphisms with serum lipoprotein-lipid levels in African Blacks. Genet Epidemiol 1999; 16:205–22.

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