

# Relations of APOE promoter polymorphisms to LDL cholesterol and markers of subclinical atherosclerosis in young adults

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**Abstract** The common apolipoprotein E (apoE) gene (APOE)  $\epsilon 2/\epsilon 3/\epsilon 4$  polymorphism explains part of serum lipid variation, and polymorphisms in the APOE promoter region have been proposed to participate in the regulation of serum lipid levels within the most common APOE  $\epsilon 3/\epsilon 3$  genotype group. We determined APOE  $-219G/T$  and  $+113G/C$  promoter genotypes and estimated APOE haplotypes in 525 participants of the Cardiovascular Risk in Young Finns Study. We studied the associations of the APOE promoter polymorphisms and their haplotypes with cross-sectional and longitudinal serum lipid and apolipoprotein concentrations as well as with flow-mediated dilatation (FMD), carotid artery compliance (CAC), and intima-media thickness (IMT) within the APOE  $\epsilon 3/\epsilon 3$  carriers. We found no significant association between the APOE promoter genotypes and serum lipids [low density lipoprotein-cholesterol (LDL-C), HDL-C, and triglycerides], apolipoproteins (apoA-I and apoB), or brachial artery FMD, CAC, or carotid IMT in either men or women. In longitudinal analyses in males, the carriers of heterozygous genotypes ( $-219G/T$  or  $+113G/C$ ) and, furthermore, carriers of the  $-219T/+113C/\epsilon 3$  haplotype had significantly higher LDL-C and total cholesterol concentrations throughout the 21 year follow-up period compared with homozygous G allele carriers or noncarriers of the  $-219T/+113C/\epsilon 3$  haplotype. Such associations were not found in females. **■** In summary, the APOE promoter polymorphisms  $-219G/T$  and  $+113G/C$  as well as their haplotype are associated with longitudinal changes in LDL-C and total cholesterol concentrations in young Finnish males but do not seem to be major determinants for FMD, CAC, or carotid IMT in

males or females.—Viiri, L. E., O. T. Raitakari, H. Huhtala, M. Kähönen, R. Rontu, M. Juonala, N. Hutri-Kähönen, J. Marniemi, J. S. A. Viikari, P. J. Karhunen, and T. Lehtimäki. **Relations of APOE promoter polymorphisms to LDL cholesterol and markers of subclinical atherosclerosis in young adults.** *J. Lipid Res.* 2006. 47: 1298–1306.

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Apolipoprotein E [apoE (protein); APOE (gene)] plays an important role in lipoprotein metabolism, which contributes to the development and progression of atherosclerosis, a disease starting already in childhood. Therefore, APOE is one of the most vigorously studied genes in relation to this disease. In addition to the effects of the commonly known APOE alleles  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$  on serum lipid levels, APOE promoter region polymorphisms also have been shown to be associated with serum lipid concentrations, especially within APOE  $\epsilon 3/\epsilon 3$  carriers (1). Srinivasan and colleagues (2) suggested in their 16 year follow-up study that the APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  polymorphism tends to influence the longitudinal change in serum low density lipoprotein-

Abbreviations: apoE, apolipoprotein E protein; APOE, apolipoprotein E gene; BMI, body mass index; CAC, carotid artery compliance; CRP, C-reactive protein; CV, coefficient of variation; FMD, flow-mediated dilatation; LDL-C, low density lipoprotein-cholesterol; IMT, intima-media thickness.

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cholesterol (LDL-C) concentrations. The relations between the APOE promoter polymorphisms and lipid levels, however, have not yet been studied in a longitudinal setting.

Ultrasound methods can be used to study early atherosclerotic changes. For example, measurements of carotid artery wall intima-media thickness (IMT), brachial artery flow-mediated dilatation (FMD), and carotid artery compliance (CAC) have been used as markers of vascular changes related to subclinical atherosclerosis (3). IMT represents a structural marker of atherosclerosis, whereas FMD is a functional marker of endothelial health; independently, they both predict cardiovascular events in populations (4, 5). CAC, on the other hand, measures the elasticity of large arteries, the decrease of which is considered to be a risk factor for cardiovascular disease. A recent meta-analysis by Elosua and coworkers (6) found no association between the APOE genotype and common carotid artery IMT in women, but in men the APOE  $\epsilon 2$  allele associated with smaller common carotid artery IMT compared with the  $\epsilon 3$  allele. The possible association of the APOE promoter polymorphisms  $-219G/T$  and  $+113G/C$  with IMT, FMD, or CAC has not been studied previously. We wanted to address this question along with studying whether the serum lipid, apolipoprotein, or C-reactive protein (CRP) concentrations differ between APOE promoter genotype groups in young Finns. The individuals were participants in the prospective multicenter Cardiovascular Risk in Young Finns Study, which was launched in 1980 to obtain data on the risk factors of coronary heart disease (7). We also wanted to investigate whether the possible differences between the genotypes/haplotypes in lipid concentrations remained constant throughout the follow-up period of 21 years from childhood to adulthood. To exclude the previously known effects of the common APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  polymorphism and to test the independent effects of the APOE promoter polymorphisms, we focused our studies on APOE  $\epsilon 3/\epsilon 3$  carriers.

### Study subjects

This study consists of a subsample of subjects from the ongoing Cardiovascular Risk in Young Finns Study, which was launched in 1980 in five university cities in Finland with medical schools and their surrounding rural communities. Details of the study design have been presented elsewhere (7). In short, the study included 3,596 randomly selected boys and girls aged 3, 6, 9, 12, 15, and 18 years. All subjects participating in 1980 were invited to follow-up studies in 1983 and 1986 and to the 21 year follow-up study in 2001. Cardiovascular risk factors, including smoking, alcohol use, diabetes, hypertension, body mass index (BMI), blood pressure values, and serum lipids were measured in 2001. Additionally, carotid artery IMT, FMD, and CAC were measured by ultrasonography in 2001. ApoE  $\epsilon 2/\epsilon 3/\epsilon 4$  phenotype analyses were carried out in 1986, and the APOE promoter polymorphisms  $-219G/T$  and  $+113G/C$  were genotyped in 2005. In total, our random subsample consists of 928 cases, the APOE genotype frequencies of which were as follows:  $\epsilon 2/\epsilon 2$ , 2 (0.2%);  $\epsilon 2/\epsilon 3$ , 47 (5.1%);  $\epsilon 2/\epsilon 4$ , 22 (2.4%);  $\epsilon 3/\epsilon 3$ , 535 (57.5%);  $\epsilon 3/\epsilon 4$ , 289 (31.1%); and  $\epsilon 4/\epsilon 4$ , 33 (3.6%). From the 535 APOE  $\epsilon 3/\epsilon 3$  carriers, we studied 525 in whom the APOE promoter genotyping was successful.

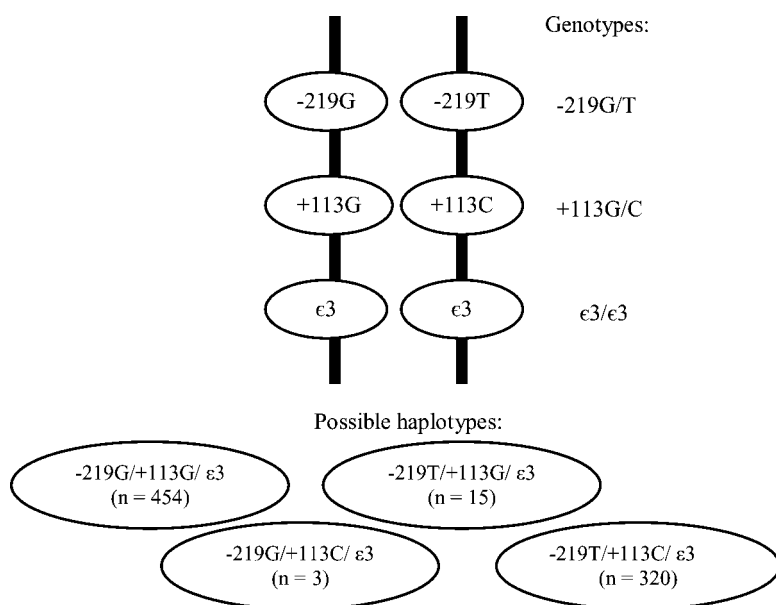
Subjects gave written informed consent in 2001, and their parents gave it in 1980. The study was approved by local ethics committees.

### Clinical characteristics

Height and weight were measured, and BMI was calculated. Blood pressure was measured with a random zero sphygmomanometer (Hawksley and Sons, Ltd., Lancin, UK). The mean of at least three measurements was used in the analysis. Smoking habits, history of diabetes, and alcohol use were ascertained as part of a self-administered questionnaire. Smokers were categorized according to daily smoking into ever or never smokers.

### Biochemical analyses

In 2001, serum lipid (apoA-I and apoB) concentrations were determined in the laboratory of the National Public Health Insti-



**Fig. 1.** Schematic representation of two chromosome strands with three single nucleotide polymorphisms [apolipoprotein E gene (APOE)  $-219G/T$ , APOE  $+113G/C$ , and APOE  $\epsilon 2/\epsilon 3/\epsilon 4$ ] forming genotypes and haplotypes. The observed frequencies of haplotype carriers in this study are presented in parentheses.

tute in Turku, Finland. Serum total cholesterol and triglycerides were determined enzymatically (Olympus System Reagent; Olympus Diagnostica GmbH, Hamburg, Germany) in a clinical chemistry analyzer (AU400; Olympus Optical, Ltd., Mishima, Japan). HDL-C was measured enzymatically from serum supernatant after precipitation of LDL and VLDL with dextran sulfate and  $MgCl_2$  (8). LDL-C was calculated by the Friedewald formula:  $LDL-C = total\ cholesterol - HDL-C - triglyceride \div 2.2$  (9). Subjects having triglyceride concentrations of  $>4\text{ mmol/l}$  ( $n = 6$  within the APOE  $\epsilon 3/\epsilon 3$  carriers) were excluded from this analysis. Serum apoA-I and apoB were analyzed immunoturbidometrically (Orion Diagnostica, Espoo, Finland). Sensitive CRP was measured using latex turbidometric immunoassay (Wako Chemicals GmbH, Neuss, Germany). The lower detection limit reported for the assay was  $0.06\text{ mg/l}$ . Serum homocysteine concentrations were measured with a microparticle enzyme immunoassay kit (Imx assay; Abbott Laboratories, Tokyo, Japan).

### Ultrasound measurements

Ultrasound studies were performed using Sequoia 512 ultrasound mainframes (Acuson, CA) with a 13.0 MHz linear array transducer, as described previously (10). In short, to measure carotid IMT, the image was focused on the posterior (far) wall of the left carotid artery. A minimum of four measurements of the common carotid far wall were taken  $\sim 10\text{ mm}$  proximal to the

bifurcation to derive mean carotid IMT values. The between-visit (two visits 3 months apart) coefficient of variation (CV) of IMT measurements was 6.4% (10).

To assess CAC indices, several 5 s image clips of the beginning of the common carotid artery were acquired. From these clips, the best-quality cardiac cycle was selected and manually analyzed to measure systolic and diastolic common carotid diameters, as described previously (11). The 3 month between-visit CV was 2.7% for diastolic carotid diameter and 16.3% for CAC.

To evaluate brachial artery FMD, the left brachial artery diameter was measured at rest and during reactive hyperemia, as described previously (12). In short, increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mm Hg for 4.5 min, followed by release. Three measurements of arterial diameter were performed at end-diastole at a fixed distance from an anatomic marker at rest as well as 40, 60, and 80 s after cuff release. The vessel diameter in scans after reactive hyperemia was expressed as a percentage relative to the resting scan value (100%). The 3 month between-visit CV was 3.2% for brachial artery diameter and 26.0% for FMD measurements (12).

### ApoE phenotyping and APOE promoter genotyping

ApoE  $\epsilon 2/\epsilon 3/\epsilon 4$  phenotyping was done as described previously (13). The APOE gene promoter  $-219G/T$  and intron  $+113G/C$

TABLE 1. Clinical and biochemical characteristics and preclinical indicators of atherosclerosis in APOE  $-219$  promoter genotype groups within APOE  $\epsilon 3/\epsilon 3$  carriers

Characteristic	Sex	$-219$			P
		G/G	G/T	T/T	
Number	F	86	151	42	0.024
	M	103	118	26	
Age (years)	F	31.6 (5.1)	31.5 (5.0)	30.9 (4.9)	0.783
	M	32.1 (4.9)	31.2 (5.2)	32.1 (4.3)	
BMI ( $kg/m^2$ )	F	24.5 (4.0)	24.4 (4.6)	24.4 (4.8)	0.966
	M	25.7 (3.4)	25.8 (3.4)	25.8 (4.4)	
Systolic blood pressure (mm Hg)	F	116.7 (12.7)	114.8 (10.8)	114.0 (9.7)	0.341
	M	128.4 (13.5)	129.0 (12.4)	130.7 (8.8)	
Diastolic blood pressure (mm Hg)	F	72.7 (8.4)	70.9 (7.7)	69.8 (6.2)	0.099
	M	75.4 (9.0)	74.8 (8.5)	76.3 (7.9)	
Daily smoking (never/ever)	F	54/30	84/66	22/19	0.382
	M	50/52	55/61	15/11	
Total cholesterol (mmol/l)	F	5.03 (0.83)	5.00 (1.02)	5.20 (0.88)	0.477
	M	5.14 (1.08)	5.29 (1.01)	5.02 (1.23)	
LDL-cholesterol (mmol/l)	F	3.11 (0.72)	3.07 (0.80)	3.33 (0.67)	0.146
	M	3.30 (0.91)	3.49 (0.91)	3.24 (1.16)	
HDL-cholesterol (mmol/l)	F	1.40 (0.29)	1.39 (0.31)	1.39 (0.33)	0.951
	M	1.18 (0.27)	1.20 (0.27)	1.16 (0.29)	
Triglycerides (mmol/l)	F	1.15 (0.55)	1.18 (0.62)	1.06 (0.45)	0.522
	M	1.44 (0.90)	1.40 (0.97)	1.45 (1.14)	
Apolipoprotein A-I (g/l)	F	1.60 (0.26)	1.58 (0.28)	1.56 (0.29)	0.689
	M	1.42 (0.21)	1.44 (0.19)	1.39 (0.20)	
Apolipoprotein B (g/l)	F	1.00 (0.23)	1.00 (0.25)	1.03 (0.21)	0.646
	M	1.11 (0.27)	1.13 (0.29)	1.09 (0.35)	
CRP (mg/l)	F	1.00 (0.41;2.42)	1.01 (0.46;2.92)	1.13 (0.26;2.49)	0.555
	M	0.65 (0.24;1.51)	0.63 (0.35;1.52)	0.56 (0.25;3.39)	
Homocysteine ( $\mu\text{mol/l}$ )	F	8.60 (7.00;10.23)	8.40 (7.40;10.40)	8.00 (5.95;9.90)	0.164
	M	9.85 (8.50;11.53)	9.80 (8.70;11.40)	10.65 (7.75;11.65)	
IMT (mm)	F	0.57 (0.09)	0.57 (0.08)	0.56 (0.09)	0.718
	M	0.59 (0.09)	0.58 (0.11)	0.59 (0.09)	
CAC (%/10 mm Hg)	F	2.36 (0.75)	2.30 (0.82)	2.31 (0.89)	0.884
	M	2.08 (0.67)	2.05 (0.64)	1.94 (0.56)	
FMD (%)	F	9.42 (3.72)	8.89 (4.52)	7.77 (4.84)	0.148
	M	7.61 (4.37)	7.46 (4.21)	8.03 (4.46)	

APOE, apolipoprotein E gene; BMI, body mass index; CAC, carotid artery compliance; CRP, C-reactive protein; F, female; FMD, flow-mediated dilatation; IMT, intima-media thickness; M, male. Values are expressed as means (SD), n values or median ( $Q^{25}$ ;  $Q^{75}$ ). Total n values differ slightly regarding BMI and FMD as a result of some missing measurements. P values are by ANOVA. Nonnormally distributed variables (triglycerides, CRP, and homocysteine) were log-transformed before analyses.

polymorphisms were genotyped using 5' nuclease assay (14) and fluorogenic TaqMan probes (Roche Molecular Systems) in the ABI Prism® 7000 Sequence Detection System (Applied Biosystems).

### Haplotype reconstruction

Haplotypes were reconstructed using the PHASE program (version 2.0.2) (15, 16). A schematic illustration of the genotypes and haplotypes used in this study is presented in Fig. 1. For purposes of statistical analyses, the study subjects were categorized into carriers and noncarriers of distinct haplotypes: -219G/+113G/ε3, -219T/+113G/ε3, and -219T/+113C/ε3. There were only three carriers of the haplotype -219G/+113C/ε3, so this haplotype was excluded from all statistical analyses.

### Statistical analyses

The APOE genotype frequencies were first tested for Hardy-Weinberg equilibrium. Then, the genotype frequencies were compared between men and women. Because the genotype frequencies differed significantly between the sexes within the APOE ε3/ε3 carriers, all further analyses were performed separately for men and women. This was true for haplotype frequencies as well. The distributions of cardiovascular risk factors and vascular parameters (measured in 2001) were compared between the APOE genotype groups using ANOVA (continuous variables) and

the Chi-square test (categorical variables). Nonnormally distributed triglyceride, CRP, and homocysteine concentrations were log-transformed before the analyses, but the results are expressed as crude.

The longitudinal lipid data were analyzed by repeated-measurement ANOVA using the APOE promoter genotypes or haplotypes as categorical factors (one at a time) and LDL-C or total cholesterol concentrations (one at a time) at different years of follow-up (1980, 1983, 1986, and 2001) as dependent repeated variables. In case of a statistically significant main effect, posthoc tests (with Bonferroni correction) were used to compare the differences between the genotype/haplotype groups. Statistical calculations were done using SPSS (version 12.0) on a personal computer.

## RESULTS

### Characteristics of the study population

The final study population consisted of 525 APOE ε3/ε3 carriers in whom the APOE promoter genotyping was successful. The frequencies of the -219G and -219T alleles were 0.62 and 0.38, respectively, and those of

TABLE 2. Clinical and biochemical characteristics and preclinical indicators of atherosclerosis in APOE +113 promoter genotype groups within APOE ε3/ε3 carriers

Characteristic	Sex	+113			P
		G/G	G/C	C/C	
Number	F	93	147	39	0.054
	M	107	112	27	
Age (years)	F	31.7 (5.1)	31.4 (4.9)	30.8 (4.9)	0.586
	M	31.9 (5.0)	31.2 (5.2)	31.9 (4.3)	
BMI (kg/m <sup>2</sup> )	F	25.0 (4.4)	24.2 (4.5)	24.0 (4.5)	0.289
	M	25.7 (3.4)	25.6 (3.3)	26.0 (4.5)	
Systolic blood pressure (mm Hg)	F	116.5 (13.0)	115.0 (10.5)	113.2 (9.6)	0.294
	M	128.3 (13.2)	129.0 (12.6)	130.3 (8.9)	
Diastolic blood pressure (mm Hg)	F	72.5 (8.4)	71.0 (7.7)	69.5 (6.3)	0.112
	M	75.2 (8.9)	74.8 (8.6)	76.0 (7.8)	
Daily smoking (never/ever)	F	57/33	83/64	21/17	0.527
	M	52/54	53/57	15/12	
Total cholesterol (mmol/l)	F	5.00 (0.85)	5.00 (1.01)	5.19 (0.91)	0.552
	M	5.13 (1.05)	5.30 (1.02)	5.02 (1.21)	
LDL-cholesterol (mmol/l)	F	3.11 (0.75)	3.07 (0.78)	3.30 (0.68)	0.246
	M	3.31 (0.90)	3.51 (0.92)	3.26 (1.14)	
HDL-cholesterol (mmol/l)	F	1.38 (0.27)	1.40 (0.32)	1.41 (0.33)	0.879
	M	1.18 (0.27)	1.19 (0.27)	1.16 (0.29)	
Triglycerides (mmol/l)	F	1.14 (0.55)	1.19 (0.62)	1.05 (0.46)	0.425
	M	1.41 (0.84)	1.41 (0.98)	1.43 (1.13)	
Apolipoprotein A-I (g/l)	F	1.58 (0.24)	1.58 (0.30)	1.58 (0.30)	0.997
	M	1.42 (0.21)	1.44 (0.19)	1.38 (0.20)	
Apolipoprotein B (g/l)	F	1.00 (0.24)	1.00 (0.25)	1.03 (0.22)	0.761
	M	1.11 (0.26)	1.14 (0.29)	1.09 (0.35)	
CRP (mg/l)	F	1.03 (0.42;2.45)	0.99 (0.45;2.93)	1.13 (0.24;2.49)	0.676
	M	0.66 (0.24;1.53)	0.60 (0.35;1.42)	0.57 (0.26;3.36)	
Homocysteine (μmol/l)	F	8.60 (7.00;10.50)	8.40 (7.40;10.13)	8.00 (6.10;9.90)	0.211
	M	9.75 (8.50;11.53)	9.85 (8.68;11.48)	10.60 (7.80;11.60)	
IMT (mm)	F	0.57 (0.09)	0.57 (0.08)	0.56 (0.09)	0.710
	M	0.59 (0.09)	0.58 (0.11)	0.58 (0.09)	
CAC (%/10 mm Hg)	F	2.32 (0.78)	2.31 (0.80)	2.36 (0.91)	0.955
	M	2.07 (0.66)	2.07 (0.65)	1.96 (0.56)	
FMD (%)	F	9.25 (3.82)	8.98 (4.48)	7.59 (4.94)	0.139
	M	7.56 (4.36)	7.43 (4.29)	8.15 (4.40)	

Values are expressed as means (SD), n values or median (Q<sup>25</sup>;Q<sup>75</sup>). Total n values differ slightly regarding BMI and FMD as a result of some missing measurements. P values are by ANOVA. Nonnormally distributed variables (triglycerides, CRP, and homocysteine) were log-transformed before analyses.



the +113G and +113C alleles were 0.63 and 0.37. The promoter genotype distributions were in Hardy-Weinberg equilibrium and similar to those seen in previous Finnish studies (1, 17, 18). Age, BMI, systolic or diastolic blood pressure, and smoking did not differ significantly between the APOE promoter genotype groups in the  $\epsilon 3/\epsilon 3$  carriers (Tables 1, 2). Also, alcohol consumption was similar in different genotype groups (data not shown). There were only four diabetic patients within the APOE  $\epsilon 3/\epsilon 3$  carriers, and there was no significant difference in their distribution into different APOE promoter genotype groups.

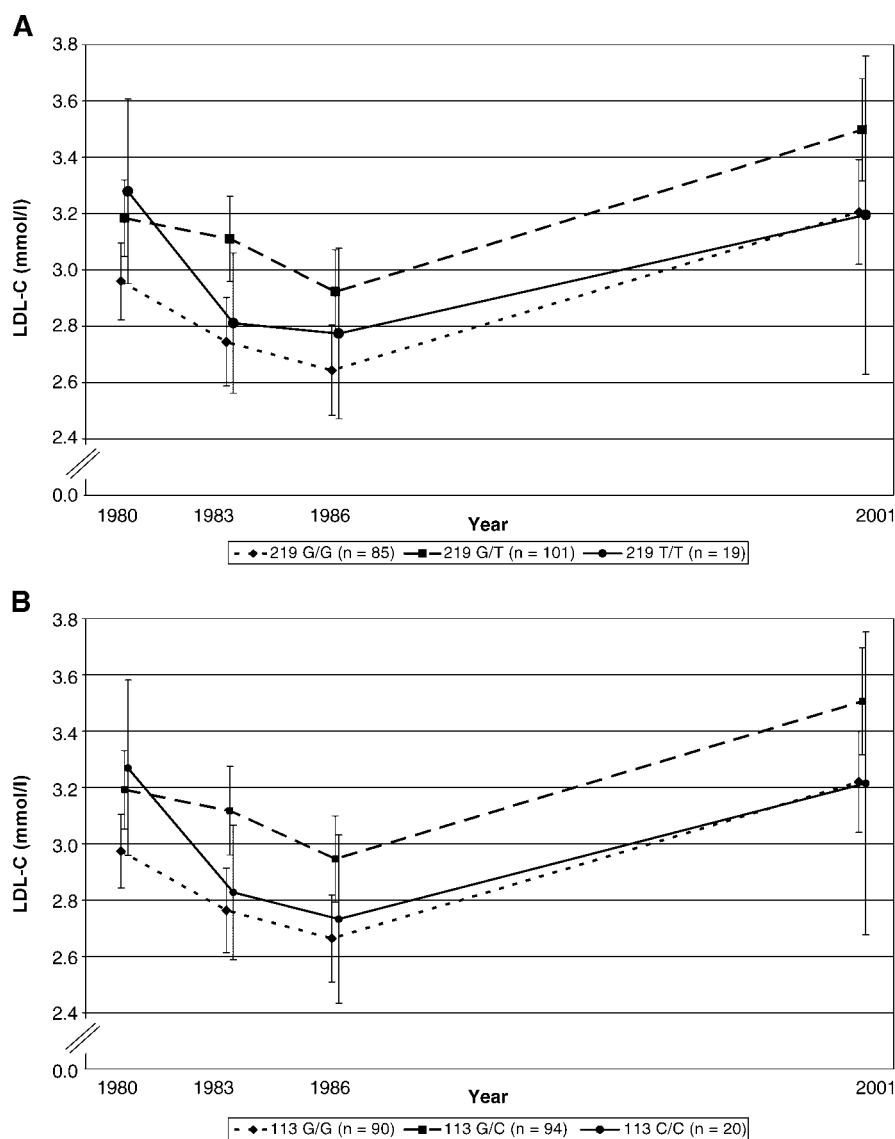
### Cross-sectional analyses

There were no major differences in serum cholesterol, triglyceride, apolipoprotein, CRP, and homocysteine con-

centrations between the APOE promoter genotype groups in men or women. In addition, IMT, CAC, and FMD values did not differ between the APOE promoter genotype groups in men or women. (Tables 1, 2).

### Longitudinal changes in serum cholesterol values

In both sexes, LDL-C and total cholesterol concentrations changed over time ( $P < 0.001$ ), but there was no interaction between time and APOE promoter genotypes, meaning that the differences between genotypes remained fairly constant through the follow-up period (from 1980 to 2001) (Fig. 2). The initial decrease in lipid curves represents the effect of puberty. In males, the APOE promoter genotypes associated significantly with the longitudinal change in LDL-C values ( $-219$ ,  $P = 0.012$ ;  $+113$ ,  $P =$

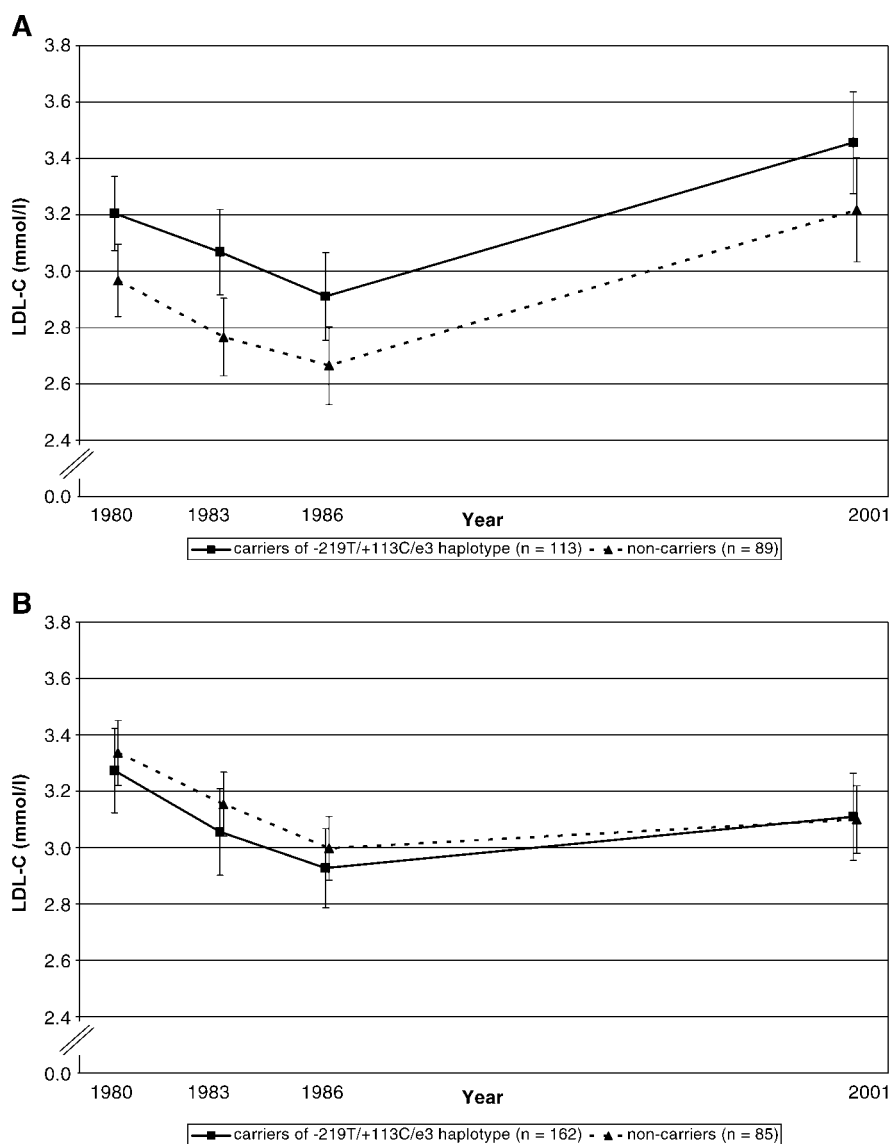


**Fig. 2.** Longitudinal change (from 1980 to 2001) of low density lipoprotein-cholesterol (LDL-C) values in males in APOE  $-219$  genotype groups (G/G, G/T, and T/T; repeated-measurement ANOVA main effect for genotype,  $P = 0.012$ ) (A) and in APOE  $+113$  genotype groups (G/G, G/C, and C/C; repeated-measurement ANOVA main effect for genotype,  $P = 0.013$ ) (B). Total cholesterol values follow the same patterns, although with higher values. Error bars represent 95% confidence intervals of the mean.

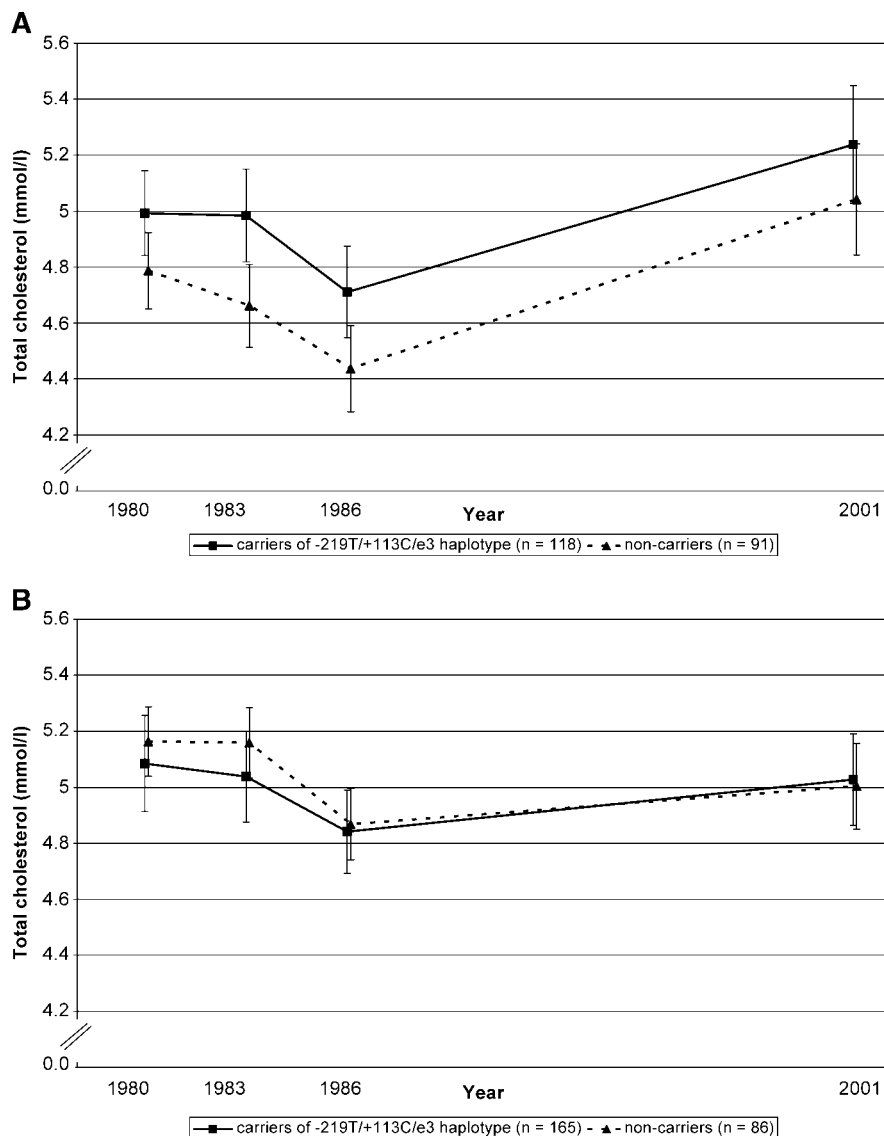
0.013). The APOE  $-219G/T$  and  $+113G/C$  genotype carriers had significantly higher LDL-C values over time compared with the  $-219G/G$  and  $+113G/G$  carriers, respectively (Fig. 2). The difference in LDL-C values between the heterozygotes and G/G homozygotes remained fairly constant ( $\sim 0.3$  mmol/l) over time ( $-219$ ,  $P = 0.009$ ;  $+113$ ,  $P = 0.010$ ). Similarly, the APOE promoter genotypes associated significantly with the longitudinal change in total cholesterol values ( $-219$ ,  $P = 0.017$ ;  $+113$ ,  $P = 0.015$ ). The APOE  $-219G/T$  and  $+113G/C$  genotype carriers had significantly higher total cholesterol values over time compared with the G/G carriers: the difference between the heterozygotes and G/G homozygotes remained fairly constant ( $\sim 0.3$  mmol/l) over time ( $-219$ ,  $P = 0.014$ ;  $+113$ ,  $P = 0.012$ ).

The longitudinal analyses also revealed that males carrying the  $-219T/+113C/\epsilon 3$  haplotype had significantly higher LDL-C concentrations throughout the follow-up period compared with noncarriers of this haplotype. Furthermore, the difference in LDL-C values between the carriers and noncarriers of the  $-219T/+113C/\epsilon 3$  haplotype remained relatively constant (on average, 0.3 mmol/l;  $P = 0.007$ ) over time (Fig. 3A). Similarly, the total cholesterol concentration was higher in  $-219T/+113C/\epsilon 3$  haplotype carriers compared with noncarriers of this haplotype, and the difference was on average 0.2 mmol/l throughout the follow-up period ( $P = 0.012$ ) (Fig. 4A).

In females, LDL-C and total cholesterol concentrations changed over time ( $P < 0.001$ ), but there were no statistically significant differences in longitudinal LDL-C or total



**Fig. 3.** Longitudinal change (from 1980 to 2001) of LDL-C values in APOE  $-219T/+113C/\epsilon 3$  haplotype carriers and noncarriers in males (repeated-measurement ANOVA main effect for haplotype,  $P = 0.007$ ) (A) and in females (repeated-measurement ANOVA main effect for haplotype,  $P = 0.492$ ) (B). The time-haplotype interaction was statistically nonsignificant in both sexes. Error bars represent 95% confidence intervals of the mean.



**Fig. 4.** Longitudinal change (from 1980 to 2001) of total cholesterol values in APOE  $-219T/+113C/\epsilon 3$  haplotype carriers and noncarriers in males (repeated-measurement ANOVA main effect for haplotype,  $P = 0.012$ ) (A) and in females (repeated-measurement ANOVA main effect for haplotype,  $P = 0.580$ ) (B). The time-haplotype interaction was statistically nonsignificant in both sexes. Error bars represent 95% confidence intervals of the mean.

cholesterol values between the APOE haplotype groups (Figs. 3B, 4B).

## DISCUSSION

In this study, males carrying the heterozygous genotypes ( $-219G/T$  or  $+113G/C$ ) or haplotype  $-219T/+113C/\epsilon 3$  had higher LDL-C and total cholesterol values throughout the 21 year follow-up period (from 1980 to 2001) compared with homozygous G allele carriers or noncarriers of the  $-219T/+113C/\epsilon 3$  haplotype. In females, such differences in longitudinal cholesterol values between genotypes or haplotypes were not seen. Nor did we find any association of APOE promoter genotypes with the early


measures of atherosclerosis in men or women in cross-sectional analyses.

We previously showed that the APOE  $-219G/+113G/\epsilon 3$  haplotype associates with higher concentrations of VLDL-C and triglycerides in middle-aged (50–59 years) Finnish men (1). Similar associations were not found in this study of younger (24–39 years) men and women; instead, associations with longitudinal change in LDL-C and total cholesterol concentrations were recognized. We also previously showed the common APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  polymorphism to be associated with LDL-C and total cholesterol values in a 6 year follow-up study (13). The association of APOE promoter genotypes and APOE haplotypes with longitudinal changes in lipid values, however, had not been studied previously. It is also noteworthy that in this

study the follow-up period is rather long (21 years) compared with that in many other studies.

The mechanisms underlying the associations of the studied APOE promoter polymorphisms and haplotypes with serum lipid concentrations are presumably diverse. The APOE  $-219G/T$  polymorphism was shown previously to affect APOE transcription, the T allele associating with lower APOE promoter activity (19, 20). Moreover, Lambert and coworkers (21) have shown that the  $-219T$  allele associates with lower plasma apoE concentration compared with the  $-219G$  allele. Therefore, the APOE  $-219G/T$  polymorphism could affect various parts of lipid metabolism, such as cholesterol absorption from the intestine, cholesterol uptake by the liver, and cholesterol synthesis, in which apoE has an important role. Functional studies of the  $+113G/C$  polymorphism are still missing, but the locus is known to locate within an enhancer region in intron 1, which suggests that it could participate in the regulation of APOE transcription (22) and hence also play a part in lipid metabolism. Our genetic association study is somewhat limited because we do not have the apoE concentrations measured. Therefore, we cannot make any definite conclusions about the effects of the APOE haplotypes on apoE plasma levels. Neither can we make any explicit conclusions about the exact mechanisms of the found associations of the APOE promoter genotypes and haplotypes with the lipid concentrations.

The relation between the APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  polymorphism and IMT has been studied extensively, some studies suggesting an association between APOE and higher IMT (23–25) and others failing to show such an association (26–28). The possible associations of the APOE  $-219G/T$  and  $+113G/C$  promoter polymorphisms with IMT, carotid elasticity, or indicators of endothelial function have not, to our knowledge, been studied previously. We found large variation in FMD (CV, 26%) and CAC (CV, 16%) measurements but small variation in IMT as well as brachial and carotid artery diameter measurements. This suggests that much of the variation of FMD and CAC relates to physiologic fluctuation in vascular function and not to measurement error. Therefore, although our study excludes major effects of the APOE promoter polymorphisms on FMD and CAC, it is possible that, as a result of the large variation in the data, weak relations may have been undetected.

In conclusion, our findings indicate that within the group of young Finnish APOE  $\epsilon 3/\epsilon 3$  carriers, the APOE promoter polymorphisms  $-219G/T$  and  $+113G/C$  and their haplotype play a part in explaining the longitudinal changes in serum cholesterol concentrations in men, but they do not seem to have an effect on the markers of subclinical atherosclerosis. 

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