DNA polymorphisms of the apolipoprotein B and A-I/C-III genes are associated with variations of serum low density lipoprotein cholesterol level in childhood

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Abstract A number of restriction fragment length polymorphisms (RFLPs) of the apolipoprotein B (apoB) and apolipoprotein A-I/C-III(apoA-I/C-III) genes have been found to be associated with serum lipoprotein levels in many adult populations. In order to examine whether these genetic polymorphisms influence serum lipoprotein levels in childhood and adolescence, we determined the apoB *Xba*I and apoA-I/C-III *Sst*I genotypes and serum lipoprotein concentrations in 307 healthy Finns aged 9 to 21 years. In the age groups of 9, 12, and 15 years, subjects homozygous for the X2 allele (the *Xba*I site present) of the apoB gene had mean serum low-density lipoprotein (LDL) cholesterol levels (3.69, 3.43, and 3.15 mmol/l, respectively) that were 12–20% higher than those in subjects homozygous for the absence of this allele (3.08, 3.02, and 2.80 mmol/l, respectively). This association was more significant in males than in females. At the age of 9 to 18 years, subjects carrying the S2 allele (*Sst*I site present) of the apoA-I/C-III gene complex had an approximately 6–15% higher mean serum LDL-cholesterol level than those homozygous for its absence. The combined genotype X2+S2+ (the simultaneous presence of the X2 allele and the S2 allele) was associated with an even more marked elevation of serum LDL-cholesterol level than either allele alone. As an example, the serum LDL cholesterol concentration was 20% higher in 9-year-old subjects with at least one X2 and one S2 allele than in those without either allele (3.55 vs. 2.97 mmol/l, $P < 0.005$). The S2 allele was found to be significantly more frequent in eastern than in western Finland, whereas no significant areal differences were seen in the occurrence of the X2 allele. In conclusion, genetic variations of the apoB and apoA-I/C-III gene loci influence serum lipoprotein concentrations already in childhood. —**Aalto-Setälä, K., J. Viikari, H. K. Åkerblom, V. Kuusela, and K. Kontula.** DNA polymorphisms of the apolipoprotein B and A-I/C-II genes are associated with variations of serum low density lipoprotein cholesterol level in childhood. *J. Lipid Res.* 1991. **32:** 1477–1487.

Supplementary key words apolipoprotein B gene • apolipoprotein A-I/C-III gene complex • serum cholesterol • serum triglycerides • adolescents

Apolipoprotein B (apoB) plays a dominant role in cholesterol homeostasis. It is required for the assembly and secretion of chylomicrons in intestine and VLDL in liver, and it also acts as the ligand for the recognition of LDL by the LDL receptor (1, 2). The apoB gene extends over 43 kb (kb, kilo base pairs) (3) and resides in chromosome 2 (4–6). Apolipoprotein A-I (apoA-I) is the major protein component of HDL (7) and also serves as a cofactor for LCAT (lecithin:cholesterol acyl transferase), an enzyme that catalyzes the conversion of cholesterol and phosphatidylcholine to cholesteryl esters and lysophosphatidylcholine (8–11). The apoA-I gene is located in chromosome 11 and is tightly linked to the apolipoprotein C-III (apoC-III) gene, located only 2.6 kb from the 3' end of the apoA-I gene (12). The function of apoC-III is not clear, but it may modulate hydrolysis of triglyceride-rich lipoproteins by lipoprotein lipase (13) and influence the hepatic uptake of chylomicron remnants (14).

Several polymorphic sites within or adjacent to the gene loci for apoB and apoA-I/C-III have been detected. The *Xba*I restriction fragment polymorphism (RFLP) of the apoB gene has been found to be associated with serum cholesterol and/or triglyceride levels in a number of adult populations (15–18). Furthermore, the presence of a polymorphic *Sst*I site within the apoA-I/C-III gene complex

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; RFLP, restriction fragment length polymorphism; kb, kilobase pairs; BMI, body mass index; CHD, coronary heart disease.

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has been proposed to be associated with elevated serum triglyceride (19–22) and cholesterol levels (23), as well as with diminished serum HDL levels (24). The mechanisms of these associations remain unknown.

We examined whether in Finland, where apoB and apoA-I/C-III RFLPs are associated with serum lipoprotein levels among adult subjects, genetic variation of these two gene loci are involved in the determination of serum lipid levels already in childhood. These data would bear two important implications. First, a lipoprotein genotype-phenotype association possibly present even at the age before and during developmental maturation would favor a primary role for lipid-regulatory genes without the need of concomitant adult hormonal milieu for their expression. Second, the demonstration of such an association would have impact on preventive measures in risk factors starting from childhood on. We also determined allelic frequencies of these RFLPs in residents of western and eastern Finland, i.e., subgroups of the same population with different prevalence of ischemic heart disease.

MATERIALS AND METHODS

Subjects

Venous blood samples were collected from 307 healthy children, adolescents, and young adults who participated in an ongoing multicentre study "Cardiovascular Risk in Young Finns" (for study design, see ref. 25). A total of 3,596 subjects aged 3, 6, 9, 12, 15, and 18 years participated in the first cross-sectional study in 1980. Equal numbers of males and females, rural and urban children, east and west Finns were required. The municipalities were chosen on the basis of their location and socioeconomic structure. The subjects were chosen at random in each municipality from the Social Insurance Institution's population register.

For the present study, 159 males and 148 females, 167 from eastern and 140 from western Finland, were randomly selected. Timing of the follow-up blood samples (1983 and 1986) and age distribution of the study population at different follow-up points is illustrated in **Table 1**. Due to the small number of subjects in the age category of 24 years, no calculations on the genotypes and lipids were performed in this group.

TABLE 1. General design of the study: timing of blood samples for lipid determinations and total number of subjects in each age category

	Number of Subjects			
Age Group	1980	1983	1986	Total in the Age Group
yr				
9	98			98
12	111	98		209
15	77	111	98	286
18	21	77	111	209
21		21	77	98
24			21	21
Total	307	307	307	

DNA analysis

Samples for the DNA analysis were taken in 1986. DNA was isolated from 10 ml of venous blood (26). DNA (5–10 μg) was digested with restriction enzymes *Xba*I and *Sst*I using conditions recommended by the manufacturer, fractionated by gel electrophoresis on 0.6% agarose, and transferred to nitrocellulose filters. The filters were prehybridized in a medium containing 6 × SSC (1 × SSC denotes 0.15 M NaCl, 0.015 M trisodium sulfate), 5 × Denhardt's solution, 50% deionized formamide, 0.2% SDS, and 100 μg/ml herring sperm DNA for 3–4 h at 42°C, and then hybridized with 1–3 × 10^6 cpm/ml of a ^{32}P-labeled apoB or apoA-I DNA probe. ApoB cDNA probe, pB23 (27), and apoA-I genomic DNA probe, pSV2 2.2 kb apoA-I, were generous gifts from Dr. Jan L. Breslow (The Rockefeller University, New York). After hybridization for 16–20 h at 42°C, the filters were washed three times with 2 × SSC–0.1% SDS at room temperature and with 0.2 × SSC–0.1% SDS at 42°C. Autoradiography of the dried filters was carried out by exposing them to Kodak XAR film for 1–5 days at −70°C.

The apoB allele resulting in the formation of an 8.6 kb *Xba*I restriction fragment (polymorphic *Xba*I site absent) is designated as X1, and that generating a 5 kb fragment (polymorphic site present) as X2. The apoA-I/C-III allele producing *Sst*I restriction fragments of 5.7 kb and 4.2 kb (polymorphic *Sst*I site absent) is designated as S1, and that resulting in the formation of fragments of 5.7 kb and 3.2 kb (polymorphic *Sst*I site present) as S2.

Lipoprotein analysis

Serum lipid and lipoprotein analyses were carried out on blood samples obtained after 12 h of fasting. Samples were stored at −20°C no more than 6 months until analyzed. Serum cholesterol (28) and triglyceride (29) levels were determined by enzymatic methods (Boehringer, Mannheim, Germany). The concentration of serum HDL-cholesterol was measured enzymatically after precipitation of LDL and VLDL with dextran sulfate and $MgCl_2$ (30). LDL-cholesterol level was calculated using the formula of Friedewald, Levy, and Fredrickson (31): LDL-cholesterol (mmol/l) = total cholesterol (mmol/l) − HDL-cholesterol (mmol/l) − triglyceride (mmol/l)/2.19.

Statistical methods

The analyses of the relationship of the apoB *Xba*I and apoA-I/C-III *Sst*I genotypes to serum lipoprotein levels

were carried out by the analysis of covariance (ANCOVA) with the relative body mass index (BMI) defined as weight (kg)/height^2(m^2) as a covariate. Serum triglyceride values were transformed logarithmically before statistical analysis. BMI was used in order to reflect the changes that occur during sexual maturation. The degree of puberty was also assessed using the Tanner scale (32). The analyses were carried out by the GLM procedure of the statistical software, SAS (Statistical Analysis System, SAS Institute, Cary, NC). Chi-square analysis was used to test allelic variation in different parts of Finland.

RESULTS

Principal characteristics concerning the developmental and lipoprotein changes of the cohort examined are summarized in **Fig. 1**. The pubertal maturation closely followed the increment of the relative body mass of the subjects and was associated with a marked decrease of serum total and LDL cholesterol levels in both sexes (Fig. 1).

Of the 307 subjects studied for their apoB genotypes, 109 (35.5%) had the genotype X1X1, 137 (44.6%) genotype X1X2, and 61 (19.9%) genotype X2X2. The apoA-I/C-III genotype S1S1 was present in 256 subjects (83.4%), genotype S1S2 in 48 (15.6%), and genotype S2S2 in 3 (1%). The frequencies of the apoB alleles did not show any significant geographical differences; in contrast, the S2 allele occurred more frequently ($P < 0.05$) in eastern than in western Finland (**Table 2**). The relative frequencies of the three apoB *Xba*I genotypes and three apoA-I/C-III *Sst*I genotypes were similar to those expected from Hardy-Weinberg equilibrium (Table 2). No statistically significant sex differences in the allelic distribution of the apoB or apoA-I/C-III genes were found.

When all lipid levels at the different age groups were taken into account and subjected to analysis of covariance with age, sex, and genotype as independent variables and BMI as a covariate, a statistically significant association was found between elevated serum total ($P < 0.001$) and LDL-cholesterol ($P < 0.002$) concentrations and the presence of the X2 allele. In specific age groups the association between the X2 allele and serum LDL cholesterol level was significant ($P < 0.05$) at the age of 15 and 18 (**Fig. 2**) in which the number of subjects investigated was the greatest (Table 1). In different age groups, subjects with the X2X2 genotype had a 4–11% higher mean serum total and a 4–20% higher mean serum LDL-cholesterol concentration than those with the genotype X1X1 (Fig. 2). Subjects with the genotype X1X2 had intermediate serum total and LDL-cholesterol concentrations except in the age categories of 18 and 21 years in which the subjects with this genotype had the lowest and highest serum LDL cholesterol levels, respectively (Fig. 2). There was no association between the apoB XbaI genotype and serum HDL cholesterol or triglyceride levels in the whole cohort or at any age (Fig. 2). The association between the X2 allele and elevated serum total and/or LDL-cholesterol level was stronger in boys than girls (**Fig. 3**). This association was statistically significant in boys aged 9–18 years (Fig. 3).

In all age groups investigated, there was a systematic trend towards elevated serum total and LDL cholesterol as well as triglyceride levels in subjects carrying the S2 allele of the apoA-I/C-III gene complex (**Fig. 4**). Analysis of covariance, with age, sex, and genotype as independent variables and BMI as a covariate, demonstrated a statistically significant association between the S2 allele and elevated serum total and LDL cholesterol levels ($P < 0.002$ and $P < 0.003$, respectively) but not with serum triglycerides, when all lipid determinations at different ages were taken into account. Considering specific age groups alone, the association between the genotype S1S2 and elevated serum total and LDL cholesterol level reached statistical significance in boys aged 15 years (**Fig. 5**), i.e., the age groups with the highest number of subjects studied (Table 1). The presence of the S2 allele was not associated with any major differences in serum HDL-cholesterol or triglyceride concentrations, whether studied with the two sexes combined (Fig. 4) or the two sexes separately (Fig. 5).

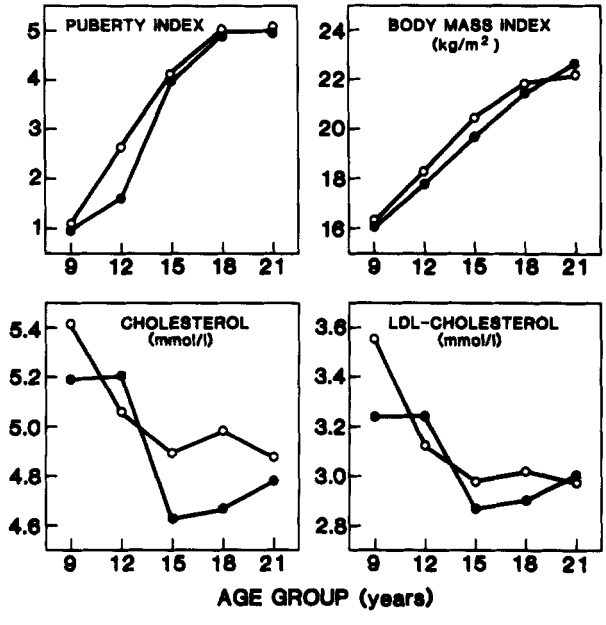

Fig. 1. General characteristics of the population sample examined: puberty (Tanner) index, body mass index, and mean serum total cholesterol and LDL cholesterol levels; open circles, females; closed circles, males.

TABLE 2. ApoB XbaI and apo-AI/C-III SstI restriction fragment length polymorphisms: genotype distributions and allelic frequencies in western and eastern Finland

	ApoB					ApoA-I/C-III				
	Genotypes			Allele Frequencies		Genotypes			Allele Frequencies	
Area of the Country	X1X1	X1X2	X2X2	X1	X2	S1S1	S1S2	S2S2	S1	S2
West (n = 140)	51	56	33	0.56	0.44	124	15	1	0.94	0.06*
East (n = 167)	58	81	28	0.59	0.41	132	33	2	0.89	0.11*

*$P<0.05$.

Inspection of scattering of the individual serum LDL choelsterol values in different age and genotype categories revealed a relatively even distribution. There were, however, three individuals with a serum LDL cholesterol concentration of 7 mmol/l or more at one or repeated examinations. All three types of the apoB *XbaI* genotypes (X1X1, X1X2, and X2X2) were represented among these three subjects and each had the apoA-I/C-III S1S1 genotype, suggesting that these three outliers did not significantly account for the genotype-related lipoprotein differences found in the present study.

Since both the apoB X2 allele and the apoA-I/C-III S2 allele were found to exert an LDL cholesterol-elevating effect, combined genotypes taking both gene loci into consideration were constructed and their effect on serum lipoprotein variation was analyzed (**Table 3**). At the ages of 9, 12, and 15 years, subjects carrying at least one X2 and one S2 allele (genotype X2+S2+) had a significantly higher serum total and LDL cholesterol concentration than those without these alleles (genotype X2-S2-) (Table 3). This association was statistically strongest at the age of 9 years when subjects with the combined genotype X2+S2+ had, on the average, a 14% higher serum total cholesterol and a 20% higher serum LDL-cholesterol concentration than those with the combined genotype X2-S2- (Table 3). A qualitatively similar but statistically insignificant association between the genotype X2+S2+ and elevated serum (LDL−cholesterol concentration level was seen in the other (18 and 21 years) age groups (Table 3). It is worth noticing, however, that the association between the combined genotype and serum total/LDL-cholesterol levels was seen in

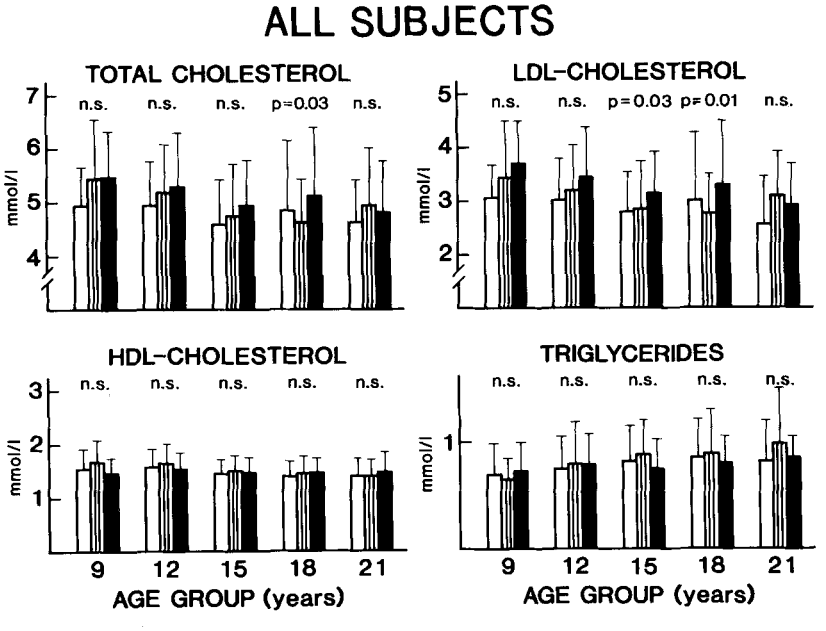

Fig. 2. The mean (± SD) serum cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride concentrations and the apoB XbaI genotypes of the whole series examined (n = 98-286 depending on the age group; see Table 1). The degree of statistical significance (analysis of covariance) is also indicated (n.s., not significant); open bars, genotype X1X1; striped bars, genotype X1X2; closed bars, genotype X2X2.

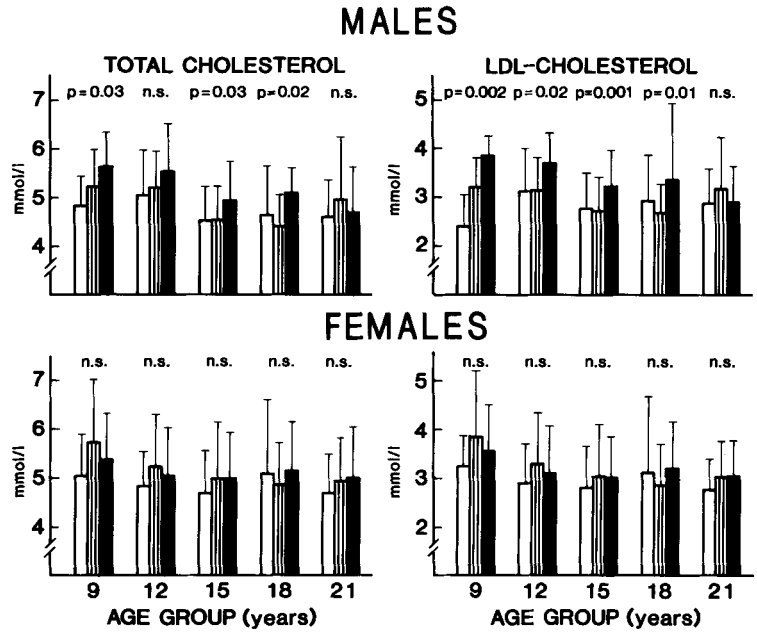

Fig. 3. The mean (± SD) serum cholesterol and LDL-cholesterol levels and the apoB XbaI genotypes, studied separately in males and females. The degree of statistical significance (analysis of covariance) is also indicated (n.s., not significant); open bars, genotype X1X1; striped bars, genotype X1X2; closed bars, genotype X2X2.

males only when the sexes were analyzed separately (Table 3). No evidence of interaction between the independent variables (age, sex, and genotype) was found. There was no significant association between the combined genotypes and serum HDL-cholesterol or triglyceride concentrations in any age category or either sex (data not shown).

Genotype-related LDL cholesterol variation seemed to persist, although slightly diminished, during successive blood samplings with 3-year intervals while the subjects got older. Thus, in the total cohort examined (n = 307) the mean serum LDL cholesterol levels in the different apoB *Xba*I genotypes (X1X1, X1X2, and X2X2) were as follows: during the first sampling, 3.05, 3.21, and 3.49

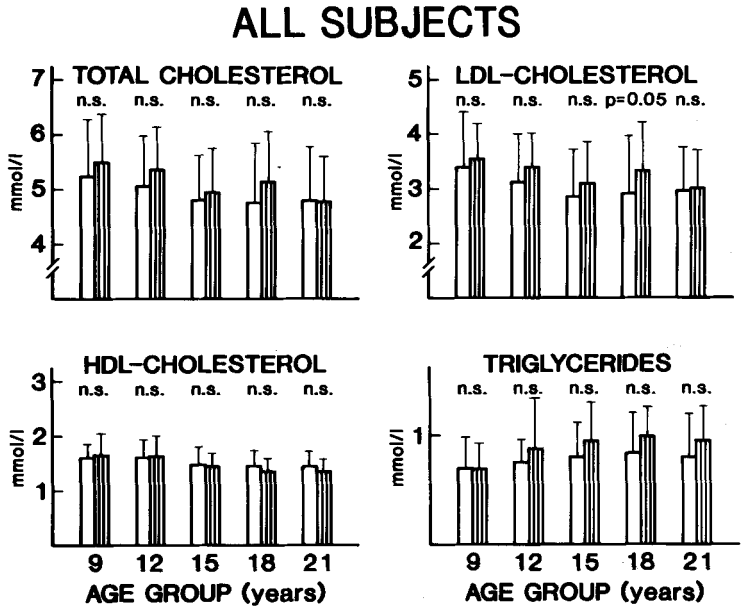

Fig. 4. The mean (± SD) serum cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride concentrations and the apoA-I/C-III SstI genotypes of the whole series examined. There were three subjects with the genotype S2S2, not included in the analysis. The degree of statistical significance (analysis of covariance) is also indicated (n.s., not significant); open bars, genotype S1S1; striped bars, genotype S1S2.

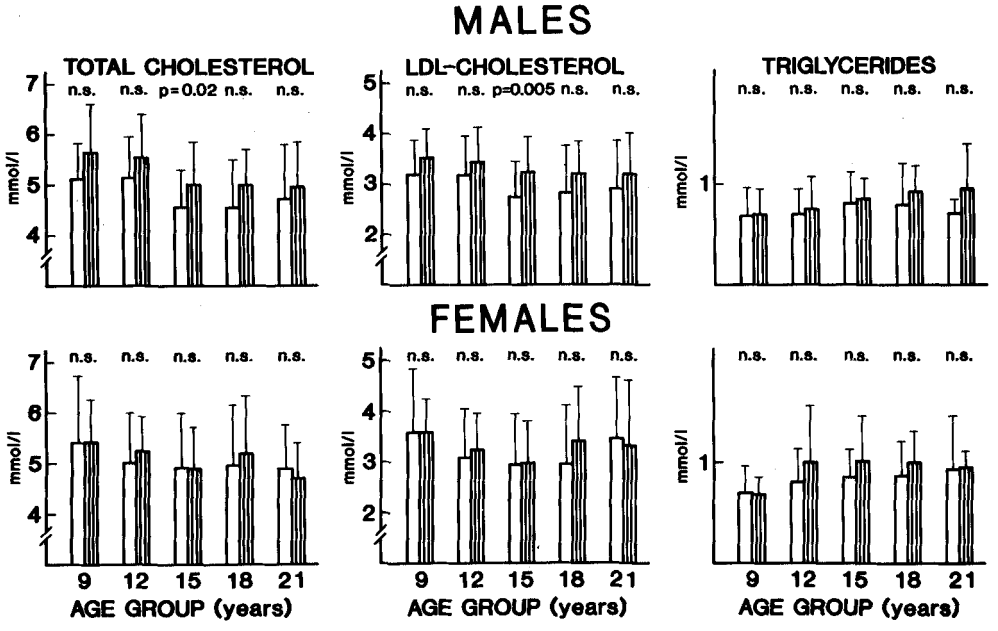

Fig. 5. The mean (± SD) serum total cholesterol, LDL-cholesterol, and triglyceride levels and the apoA-I/C-III SstI genotypes, studied separately in males and females. The degree of statistical significance (analysis of covariance) is also indicated (n.s., not significant); open bars, genotype S1S1; striped bars, genotype S1S2.

mmol/l ($P < 0.01$); during the second sampling, 2.86, 2.95, and 3.12 mmol/l (N.S.); and during the third sampling, 2.84, 2.87, and 3.17 mmol/l (N.S.), respectively. The corresponding LDL cholesterol levels in the different apoA-I/C-III genotypes (S1S1 and S1S2) were as follows: during the first sampling, 3.16 mmol/l and 3.38 mmol/l ($P < 0.05$); during the second sampling, 2.89 and 3.29 mmol/l ($P < 0.01$); and during the third sampling, 2.88 and 3.14 mmol/l (N.S.), respectively.

The association between the apoB XbaI genotype and serum LDL-cholesterol concentration was present both in western and eastern Finland; this relation was stronger in western Finland, however (**Table 4**). The association between the apoA-I/C-III S2 allele and elevated serum LDL-cholesterol concentration was likewise somewhat stronger in western than eastern Finland (Table 4). There were no significant areal differences in the mean concentrations of any of the serum lipid fractions studied.

DISCUSSION

The influence of commonly occurring genetic variation within some candidate gene loci, notably those for serum apolipoproteins, on serum lipoprotein levels has been studied virtually exclusively in adult subjects (for review, see refs. 33–35). We reasoned that a genetic influence on lipoprotein metabolism may become progressively masked with advancing age with the influence of environmental factors, such as diet, body weight, and the extent of physical activity. This prompted us to investigate whether such associations that are known to exist between a given DNA polymorphism and concentration of a serum lipoprotein fraction in adult population would become manifest as early as 9–21 years of age. It should be emphasized that this interval includes the pubertal maturation process when exogeneous factors, including psychosocial and dietary changes, may markedly influence lipid metabolism and thus complicate the establishment of any associations between genetic factors and lipid levels. We attempted to minimize any interference by puberty in lipoprotein levels by adjusting the data for BMI of the subjects. This parameter was found to correlate closely to the Tanner scale classification of the subjects (Fig. 1).

The two RFLPs, the apoB *Xba*I RFLP and apoA-I/C-III *Sst*I RFLP, were selected for the present study by virtue of their documented association with variations of serum cholesterol (18) and triglyceride (36) levels, respectively, in the adult Finnish population. Finland forms a nearly ideal venue for this type of study due to its genetically isolated position. Linguistic, cultural, and religious differences between Finland and its neighboring countries have prevented any major intercourse between the Finns and other populations; only insignificant immigration from Sweden and Russia has occurred since the settlement of the ancestor Finns more than 1500 years ago (37, 38). Studies on blood group and serum markers have demonstrated that the Finnish population is relatively

TABLE 3. Serum total and LDL cholesterol levels (mean ± SD) grouped according to the combined genotypes of the subjects

Age/Sex	Total Cholesterol (mmol/l)		LDL-Cholesterol (mmol/l)	
Category	X2-S2-	X2 + S2 +	X2-S2-	X2 + S2 +
9 Years				
All subjects	4.83 ± 0.67 (26)	5.52 ± 0.89** (14)	2.97 ± 0.56 (26)	3.55 ± 0.64*** (14)
Boys	4.85 ± 0.64 (14)	5.79 ± 0.92** (7)	2.90 ± 0.57 (14)	3.65 ± 0.52** (7)
Girls	4.82 ± 0.72 (12)	5.25 ± 0.83 (7)	3.11 ± 0.57 (12)	3.45 ± 0.76 (7)
12 Years				
All subjects	4.88 ± 0.81 (61)	5.44 ± 0.78** (22)	2.97 ± 0.77 (61)	3.41 ± 0.68* (22)
Boys	5.00 ± 0.86 (31)	5.70 ± 0.76* (11)	3.01 ± 0.83 (31)	3.59 ± 0.56 (11)
Girls	4.75 ± 0.74 (30)	5.18 ± 0.73 (11)	2.87 ± 0.70 (30)	3.22 ± 0.77 (11)
15 Years				
All subjects	4.58 ± 0.83 (83)	5.00 ± 0.87** (29)	2.76 ± 0.75 (83)	3.16 ± 0.77* (29)
Boys	4.46 ± 0.71 (43)	5.06 ± 0.86*** (14)	2.77 ± 0.65 (43)	3.28 ± 0.67*** (14)
Girls	4.70 ± 0.94 (40)	4.93 ± 0.90 (15)	2.82 ± 0.85 (40)	3.04 ± 0.86 (15)
18 Years				
All subjects	4.87 ± 1.38 (61)	5.30 ± 0.93 (19)	3.01 ± 1.36 (61)	3.47 ± 0.92 (19)
Boys	4.61 ± 1.03 (30)	5.15 ± 0.56 (8)	2.84 ± 0.98 (30)	3.31 ± 0.54 (8)
Girls	5.12 ± 1.62 (31)	5.41 ± 1.14 (11)	3.16 ± 1.64 (31)	3.59 ± 1.13 (11)
21 Years				
All subjects	4.70 ± 0.81 (28)	4.98 ± 0.93 (10)	2.82 ± 0.71 (28)	3.14 ± 0.76 (10)
Boys	4.61 ± 0.81 (15)	5.13 ± 1.00 (5)	2.80 ± 0.78 (15)	3.29 ± 0.88 (5)
Girls	4.79 ± 0.84 (13)	4.83 ± 0.94 (5)	2.83 ± 0.65 (13)	2.99 ± 0.70 (5)

The data shown are actual values. Subjects carrying at least one X2 allele and one S2 allele are designated as X2 + S2 + , while those homozygous for both the X1 and S1 alleles are designated as X2-S2-. Numbers of subjects examined are shown in parentheses below each experimental value.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ (analysis of covariance, with data adjusted for BMI).

homogeneous in genetic terms (38). However, because eastern and northern parts of the country represent younger settlements of Finland and the number of their founder individuals was probably small, deviations in gene frequencies between the southwestern and northeastern parts of the country may have arisen (37, 38).

The present data demonstrate that in young Finnish subjects there is an association between serum LDL cholesterol level and the *Xba*I polymorphism of the apoB gene. The association of the X2 allele with elevated serum lipid levels has been found in several studies on adult subjects (15-17), including our previous one performed in Finns (18). The impact of the apoB *Xba*I RFLP on serum LDL cholesterol level seems to be approximately equal in the young and adult Finns as demonstrated in **Table 5**. Thus, in the age groups with the highest number of subjects studied (i.e., 12, 15, and 18 years), serum LDL-cholesterol concentration in the genotype X2X2 was 10-14% higher than that in the genotype X1X1; this compares well with the data from adult Finns in which the corresponding figure was 12% (Table 5; ref. 18). The mechanisms behind this association remain unknown. The *Xba*I RFLP of the apoB gene does not involve a change in the amino acid sequence (39), suggesting that this single base variation may be in linkage disequilibrium with a functionally more important DNA alteration that predisposes to elevated serum LDL-cholesterol concentration. There is evidence that the fractional catabolic rate of LDL is lower in subjects with an X2X2 genotype than in those with an X1X1 genotype, suggesting that this poly-

TABLE 4. Serum LDL cholesterol concentrations (mean ± SD) in western and eastern Finland, grouped according to the apoB and apo A-I/C-III genotypes of the subjects.

Area/Age Category	ApoB Genotype			ApoA-I/C-III Genotype	
	X1X1	X1X2	X2X2	S1S1	S1S2
	mmol/l			*mmol/l*	
Western Finland					
9 years (n = 57)	2.96 ± 0.63	3.27 ± 0.49	3.65 ± 0.67*	3.17 ± 0.64	3.90 ± 0.61**
12 years (n = 102)	2.95 ± 0.88	3.07 ± 0.61	3.34 ± 0.98	2.96 ± 0.75	3.49 ± 0.63*
15 years (n = 132)	2.75 ± 0.76	2.76 ± 0.63	3.09 ± 0.75*	2.76 ± 0.67	3.13 ± 0.67
18 years (n = 83)	3.01 ± 0.95	2.70 ± 0.62	3.57 ± 1.61**	2.92 ± 0.98	3.25 ± 0.56
21 years (n = 38)	2.87 ± 0.67	3.30 ± 0.82	2.82 ± 0.67	2.99 ± 0.71	3.18 ± 1.20
Eastern Finland					
9 years (n = 41)	3.25 ± 0.56	3.70 ± 1.40	3.90 ± 1.17	3.70 ± 1.38	3.36 ± 0.56
12 years (n = 107)	3.08 ± 0.70	3.33 ± 1.00	3.53 ± 0.95	3.30 ± 0.94	3.29 ± 0.70
15 years (n = 154)	2.84 ± 0.77	2.94 ± 1.08	3.19 ± 0.83	2.94 ± 0.99	3.09 ± 0.80
18 years (n = 126)	3.01 ± 1.47	2.79 ± 0.81	3.08 ± 0.97*	2.87 ± 1.11	3.34 ± 1.00
21 years (n = 60)	2.75 ± 0.68	3.00 ± 0.92	3.00 ± 0.81	2.90 ± 0.89	2.93 ± 0.48

The data shown are actual values. Three subjects with the genotype S2S2 (one in western and two in eastern group) were excluded from the analysis.
*$P < 0.05$, **$P < 0.01$ (analysis of covariance, with data adjusted for gender and BMI).

morphism is associated with structural variation in the apoB molecule (40, 41). When portions of the apoB gene encoding the putative receptor binding regions were sequenced, no consistent DNA alterations were yet found between the X1 and X2 alleles (P. Talmud, personal communication). We cannot offer any explanation for the stronger association of the XbaI RFLP with serum LDL cholesterol level in males. It is unlikely that the expression of the cholesterol-regulating effect of the *Xba*I polymorphism would be sex steroid-dependent since our earlier study indicated this association to become apparent to the same extent in adult males and females (18).

In a previous study (36) we found that the S2 allele bearing an additional *Sst*I restriction site in the apoA-I/C-III gene complex is present in 62% of Finnish adult subjects with hypertriglyceridemia but in only 16% of healthy controls ($P < 0.001$). The association of the S2 allele with elevated serum triglyceride levels has been found in some other (19, 20, 22) but not all adult populations (21, 42). The importance of the apoA-I/C-III gene complex as a candidate gene locus for common inherited lipid disorders is further substantiated by the recent report of linkage of the *Xmn*I polymorphism of this gene cluster to familial combined hyperlipidemia (43). In the present study on young subjects, we did not find any association between the apoA-I/C-III *Sst*I genotype and serum triglyceride concentrations, but there was a significant association, in the youngest age groups and males in particular, between the presence of S2 allele and elevated serum total as well as LDL cholesterol levels (Figs. 4 and 5). Only three subjects with the genotype S2S2 were found, invalidating any conclusions on their lipid levels. Since the single base variation responsible for the *Sst*I polymorphism does not lead to changes in the amino acid sequence of either

TABLE 5. Serum LDL cholesterol concentrations (mean ± SD) in young and adult Finns according to the apoB XbaI genotypes, and the difference (in percentage) between the respective concentrations in the X2X2 and X1X1 genotypes.

Group	ApoB Genotype			Difference X2X2 minus X1X1
	X1X1	X1X2	X2X2	
	mmol/l			%
Young Finns				
9 years (n = 98)	3.08 ± 0.60	3.48 ± 1.03	3.69 ± 0.80	+ 20
12 years (n = 209)	3.02 ± 0.79	3.21 ± 0.85	3.43 ± 0.95	+ 14
15 years (n = 286)	2.80 ± 0.76	2.86 ± 0.91	3.14 ± 0.78	+ 12
18 years (n = 209)	3.01 ± 1.27	2.75 ± 0.74	3.30 ± 1.30	+ 10
21 years (n = 98)	2.80 ± 0.67	3.09 ± 0.89	2.92 ± 0.74	+ 4
Adult Finns (n = 176)	3.49 ± 1.04	3.91 ± 1.38	3.91 ± 1.10	+ 12

Data for adult Finns are from ref. 18.

apoA-I or apoC-III (44), the mechanisms of any association between this RFLP and serum lipoprotein levels remain obscure.

We cannot offer any firm explanation for the fact that the S2 allele was associated with serum triglyceride levels in our previous study on adults (18), while in the present study the same allele was most significantly associated with serum LDL cholesterol levels in children. It is of note that in each age category examined, serum triglyceride and serum LDL cholesterol levels correlated significantly with each other; as an example, the correlation coefficient in the largest age group examined (15 years) was +0.24 ($P = 0.003$). It is possible that the underlying genetic abnormality linked to the S2 allele increases VLDL that is converted to LDL more efficiently in youth than adults. It should be pointed out, however, that the estimation of serum LDL concentration in the present study was based on mathematical derivation from the total and HDL cholesterol as well as triglyceride levels (31). This approach may miss a possible association of the S2 allele with elevation of intermediate density lipoproteins (IDL), i.e., small triglyceride-rich lipoproteins. Another potential source of error that may result in an underestimation of the influence of the apoB and A-I/C-III polymorphisms on serum lipid levels lies in the day-to-day and seasonal variation of serum cholesterol levels as well as the methodological variability of serum cholesterol measurements. In fact, when all three measurements for each subject were taken into account, an analysis of covariance demonstrated a highly significant ($P < 0.005$) association between, first, the presence of the X2 allele and serum total (and LDL) cholesterol levels and, second, between the presence of the S2 allele and serum total (and LDL) cholesterol levels in the total cohort (n = 307) examined.

The combined genotype X2 + S2 + was associated with an even more marked elevation of serum total and LDL cholesterol levels than either of the two alleles (X2 or S2) alone (Table 3, Figs. 2-5). Again, this association was stronger in males than females (Table 3). It did not reach statistical significance in the two older age groups, which may be explained by the fact that the genotype combinations used (X2-S2- and X2 + S2 +) excluded the subjects with the presence of only one of these alleles (X2 or S2) rendering the sizes of the groups under comparison relatively small. Altogether, these data further strengthen the assumption that common genetic variation at both the apoA-I/C-III and apoB gene loci influences serum lipoprotein levels.

Significant geographical differences in the incidence and mortality of coronary heart disease (CHD) still exist in Finland. Thus, in certain areas of east Finland the mortality rate for CHD has been almost twice that in specific areas of west Finland (45-47). The reasons for these areal differences are not completely understood but a slightly more atherogenic serum lipid profile prevalent earlier, at least in the late 1950s, 1960s, and early 1970s (46), in eastern Finland may offer a partial explanation. Furthermore, although the present sample of about 300 children and adolescents did not reveal a statistically significant east-west difference in serum lipid levels, data from the whole cohort of the study "Cardiovascular Risk in Young Finns" (more than 3000 children) have shown that serum LDL cholesterol levels of children from eastern Finland were about 5% higher ($P < 0.001$) than those of children from western Finland in 1980; this difference slightly diminished yet remained significant during follow-up screenings in 1983 and 1986 (48). It is thus pertinent to explore whether any significant geographical differences in the occurrence of the putative hyperlipidemia-predisposing apoB and apoA-I/C-III alleles exist in Finland. While the apoB *Xba*I RFLP did not display any significant areal variation, the frequency of the S2 allele of the apoA-I/C-III gene complex was significantly higher in the east than in the west (Table 3). Whether this implies any relationship to the higher CHD mortality rate in the east awaits further studies, e.g., such ones in which the occurrence of the S2 allele in survivors of myocardial infarction from both eastern and western Finland is estimated. It is unlikely that genetic variation of another serum lipid transport protein, apolipoprotein E, could explain east-west differences in serum lipid levels or CHD mortality rate in Finland since Lehtimäki et al. (49) did not find significant regional differences in the apolipoprotein E phenotype distributions in a large population sample of Finnish children.

In conclusion, the present communication provides evidence that common genetic variation at the apoA-I/C-III and apoB gene loci influences serum lipoprotein levels from at least the age of 9 years onwards. The association between the apoB X2 allele and elevated serum LDL cholesterol level in children aged 9 to 15 years is at least equally strong as in adult Finns. This study also shows that, within a given relatively homogeneous population, areal differences in the prevalence of lipid-regulating gene alterations may exist. Whether these findings imply any clinically relevant differences in atherosclerosis risk within the population or its subpopulations must await further studies.

We thank Jan L. Breslow (The Rockefeller University, New York) for giving us the cDNA probes for apoA-I/C-III and apoB, and Steve Humphries and Gisela Lindahl (Charing Cross Sunley Research Center, London) for collaboration. The expert technical assistance of Ms. Meeri Lappalainen and Ms. Kaija Kettunen is gratefully acknowledged. This work was conducted under a contract with the Finnish Life and Pension Insurance companies, and also supported by grants from The Finnish Academy of Sciences, The Sigrid Jusélius Foundation, The Paavo Nurmi Foundation, and The Juho Vainio Foundation.

Manuscript received 24 January 1991 and in revised form 23 May 1991.

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