The Ser_{447}-*Stop* Polymorphism of Lipoprotein Lipase Is Associated With Variation in Longitudinal Serum High-Density Lipoprotein-Cholesterol Profiles: The Bogalusa Heart Study

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The Ser_{447}-*Stop* polymorphism of lipoprotein lipase (LPL) has been associated with altered high-density lipoprotein-cholesterol (HDL-C) and triglyceride (TG) levels at individual measurements, but nothing is known of its associations with lipid profiles derived from serial measurements. We used multilevel statistical models to study effects of this polymorphism on longitudinal lipid profiles in 1,006 Bogalusa Heart Study subjects examined 4 to 9 times between the ages of 4 and 38 years. $Stop_{447}$ allele frequencies in African Americans (0.053 ± 0.011) and whites (0.091 ± 0.009) differed significantly ($\chi^2 = 7.595$, 1 *df*, $P = .006$; $Stop_{447}$ homozygotes and heterozygotes combined). Overall, TG levels were lower and HDL-C levels higher in blacks than in whites of the same age and sex. Longitudinal TG profiles were lower in $Stop_{447}$ carriers at all ages. However, longitudinal HDL-C profiles differed among genotype groups with age: the $Stop_{447}$ allele was associated with higher HDL-C only in subjects above approximately 10 years of age. Genotype-specific HDL-C profiles also differed significantly among race/sex groups. Thus, we found evidence of LPL genotype effects that vary within individuals with age. Possible mechanisms, which could account for age-related changes in the effects of LPL variants, are discussed.

AS THE MAJOR ENZYME involved in the hydrolysis of triglycerides (TG) in very–low-density lipoproteins (VLDL) and chylomicrons, lipoprotein lipase (LPL) plays an important role in postprandial lipid metabolism and the formation of low-density (LDL) and high-density (HDL) lipoproteins.[1] In addition to its catalytic function, it also appears to modulate binding of lipoproteins to certain receptors.[2-8]

Several variants of the LPL gene on chromosome 8p22 have been associated with altered plasma lipid levels or with atherosclerotic disease.[9-11] One that has received particular attention is the Ser_{447}-*Stop* polymorphism, in which a C-to-G transversion in exon 9 shortens the protein by 2 amino acids.[12,13] The carboxy-terminal *Ser-Gly* dipeptide of wild-type human LPL has been found in LPL from mice, guinea pigs, and cows, suggesting that it may be functionally significant.[14] In vitro studies comparing the lipolytic activity of the full-length and truncated variants have produced mixed results,[13,15-18] although the difference in catalytic activity may be minor when the relative proportions of inactive monomeric and active dimeric LPL associated with each isoform are taken into account.[19] Studies associating this polymorphism with altered plasma lipid levels in humans have also yielded conflicting results. However, a recent meta-analysis combining results from 8 studies concluded that the $Stop_{447}$ allele was associated with small, but statistically significant decreases, in plasma TG and increases in HDL-cholesterol (HDL-C)[20], supporting the conclusions of an earlier review.[21]

While the overall evidence supports an association of the $Stop_{447}$ allele of LPL with beneficial plasma lipid profiles, the inconsistent results among studies suggest that factors, which have varied among them, may influence the effects of this allele. For example, age distributions of subjects have varied among studies,[22-24] and age may affect LPL function. Serial plasma lipid level measurements within subjects are significantly correlated,[25-28] but change with age. Genetic variants contributing to plasma lipid level differences among individuals might also contribute to differences within individuals over time. We investigated this possibility by examining the effects of the LPL Ser_{447}-*Stop* polymorphism on longitudinal serum lipid profiles in a sample of individuals examined 4 or more times during a 24-year period in the Bogalusa Heart Study and found evidence that the association of this polymorphism with longitudinal serum HDL-C profiles changes significantly with age.

MATERIALS AND METHODS

Subjects

Examination of subjects took place between 1973 and 1996. During this period, screening of school-aged children for cardiovascular disease risk factors was conducted in Bogalusa, LA, approximately every 3 years. Examinations of post-high school-aged subjects previously examined as school children were undertaken at different times between 1978 and 1996. Subjects were eligible for the present study if they had been examined at least 4 times during any of 14 separate examination cycles in Bogalusa between 1973 and 1996, with at least 1 examination occurring between 1991 and 1996, when blood samples were collected to obtain DNA. When multiple members of a family were eligible, only 1 was selected, usually the 1 who had been examined most often. To increase the sample of males, however, brothers were selected in preference to sisters who had been examined more often. A total of 1,006 subjects met the eligibility criteria. All partici-

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pants gave their informed consent at each examination. For subjects under 18 years of age, consent of a parent was obtained.

Examinations

All examinations were conducted by trained examiners following detailed protocols published previously.[29] Height was measured to the nearest 0.1 cm and weight to the nearest 0.1 kg; body mass index (BMI) was calculated as weight divided by squared height in meters (kg/m^2). In subjects examined before adulthood, sexual maturity was assessed using Tanner's classification of breast and genital maturity.[30] Subjects were instructed to fast for 12 hours before examination; compliance was assessed by interview at the examination. Blood was collected by antecubital venipuncture and allowed to clot; serum was collected after centrifugation and stored at 4°C until analysis, which usually occurred the following day.

Laboratory Analyses

From 1973 to 1986, serum total cholesterol and TG were measured chemically on a Technicon AutoAnalyzer II (Technicon Instrument, Tarrytown, NY) following Lipid Research Clinics Program protocols.[31] After 1986, these were measured by enzymatic methods[32,33] using an Abbott VP instrument (Abbott Laboratories, North Chicago, IL). Both chemical and enzymatic procedures met the performance standards of the Lipid Standardization Program of the Centers for Disease Control and Prevention, Atlanta, GA, which has monitored the accuracy and precision of lipid measurements from the Bogalusa Heart Study laboratory since 1973. Serum lipoprotein cholesterol fractions were measured by heparin-Ca^{++} precipitation followed by agar or agarose gel electrophoresis.[34] Briefly, the procedures involved (1) precipitation of apolipoprotein (apo) B-containing lipoproteins using heparin-Ca^{++} reagent; (2) measurement of HDL-C in the supernatant; (3) subtraction of HDL-C from total cholesterol to estimate VLDL + LDL cholesterol; and (4) electrophoretic separation of VLDL and LDL subfractions and use of densitometric ratios to quantitate cholesterol in each subfraction, as described.[34]

Genomic DNA was isolated as previously described.[35] Genotyping of the LPL *Ser_{447}-Stop* polymorphism was performed using the Taqman assay, in which allele-specific fluorogenic probes labeled with different reporter dyes hybridize to the target DNA in a sequence-specific manner. The allele-specific probes were 6FAM-TGCTCACCAGCCTGACTTCTTATTCAGA and VIC-TGCTCACCAGCCTCACTTCTTATTCAGA. The forward and reverse polymerase chain reaction (PCR) primer sequences were CGGTATTTGTGAAATGCCATGA and AAGCTCAGGATGCCCAGTCA, respectively. After PCR amplification, the increase in fluorescent intensity of the reporter dyes was detected by an end-point read using an ABI 7700 (Applied Biosystems, Foster City, CA). Genotype was determined by automated analysis of the fluorescent signals.

Statistical Analyses

Multilevel models were used to analyze genotype effects on the patterns of individual change in lipid levels over time.[36,37] Such models partition phenotypic variation between nested levels of analysis. The current analyses distinguished 2 levels, the first comprising variances among multiple observations within individuals, the second, variances among observations of separate individuals. Multilevel models can accommodate subjects having different numbers of unequally spaced measurements and permit specification and/or testing of the covariance structure among repeated measurements.[38]

Let h independent variables at level 1 predict phenotype measurements at time i within individual j. Then,

$$y_{ij} = \beta_{0j} + \beta_{1j}x_{1ij} + \ \ldots\ + \beta_{hj}x_{hij} + r_{ij},$$

where x_{hij} are measured independent variables, β_{hj} are parameters to be estimated, and r_{ij} is the level 1 residual error, assumed to be normally distributed. The next level of the hierarchy arises from the fact that the parameters describing the trajectory of individual measurements across time within each individual can vary among individuals. Thus, for the parameters, β_{hj}, in the above model, let

$$\beta_{0j} = \gamma_{00} + \gamma_{01}z_{1j} + \ \ldots\ + \gamma_{0m}z_{mj} + \mu_{0j}$$

$$\beta_{1j} = \gamma_{10} + \gamma_{11}z_{1j} + \ \ldots\ + \gamma_{1m}z_{mj} + \mu_{1j}$$

$$\beta_{hj} = \gamma_{h0} + \gamma_{h1}z_{1j} + \ \ldots\ + \gamma_{hm}z_{mj} + \mu_{hj},$$

where z_{mj} are level 2 explanatory variables, γ_{hm} are the associated parameters, and μ_{hj} are residuals for each β parameter. Substituting these expressions for the β parameters in the level 1 model yields the complete multilevel model.

Models were fit using several different level 1 (between-measurement) covariance structures, including first-order autoregressive, compound symmetric, and conditionally-independent structures. Estimates of parameters common to each of these models were quite stable. Use of Akaike's Information Criterion[39] to compare models indicated that conditional-independence models fit well. Examination of residual correlations, profiles, and time plots also supported the assumption of conditional independence of level 1 residuals.

Maximum likelihood estimates were obtained for all model parameters, and likelihood ratio tests were used to compare the relative fit of nested models. For testing main effects, we used a significance level of $\alpha = .05$; however, because statistical power for tests of interactions is generally low[40] and because erroneously omitting interaction effects seriously compromises model validity, we adopted a significance level of $\alpha = .10$ for tests of interactions.

Variables considered for inclusion in the models for longitudinal lipid profiles included race, sex, age, age^2, age^3, age^4, BMI, BMI^2, LPL genotype group, maturity level, and interactions among these. Polynomial terms for age were needed because lipid profiles over time are not linear. Indicator variables encoded race, sex, and genotype group, with -1 indicating whites, females, and Ser_{447} homozygotes, respectively, and +1 indicating blacks, males, and $Stop_{447}$ carriers, respectively (in the presence of multiple interactions, models with indicator variables encoded this way were more stable numerically than models with variables encoded as 0 or 1). Two indicator variables with values of either 0 or 1 encoded 3 levels of sexual maturity based on Tanner's classification.[30] Prepubertal subjects (Tanner stage 1) formed the reference group; adolescents (stages 2, 3, or 4), the second group; and adults (stage 5), the third. All subjects above age 18 were classed as adults. Age and BMI values were adjusted by subtracting the means for the first examination (age = 9.45 years; BMI = 17.649 kg/m^2), since the model intercept parameter represents the expected value of the dependent variable when all covariate values equal 0, and age and BMI values of 0 are meaningless. In the multilevel models and all statistical tests of TG levels, values were transformed by taking natural logarithms (Ln TG). Multilevel models were analyzed using Proc Mixed from the SAS program package[41] and MLwiN.[42] Allele frequencies were estimated by gene counting.

RESULTS

Of 1,006 eligible subjects, 296 (29.4%) were black, a proportion nearly identical to that in the general population of Bogalusa.[43] Proportions of males and females did not differ significantly between blacks and whites ($\chi^2 = .001$, 1 df; $P = .980$); 44.6% of black subjects and 44.5% of white subjects were males. Examination counts, overall and within race/sex groups, are shown in Table 1. Most subjects were examined

Table 1. Number of Times Subjects Examined by Race/Sex Group

No. of Examinations	Black Females	Black Males	White Females	White Males	Total
2	1	0	1	1	3
3	3	2	9	1	15
4	24	22	90	88	224
5	44	46	99	69	258
6	51	33	95	83	262
7	29	21	71	45	166
8	11	7	29	29	76
9	1	1	0	0	2
Subjects	164	132	394	316	1,006
Examinations	933	734	2,183	1,747	5,597

either 4, 5, or 6 times (22.3%, 25.7%, and 26.0%, respectively). Because of incomplete data, records of only 2 or 3 examinations were available for 18 subjects (1.8%). With those examined either 2 or 3 times combined in 1 group and those examined either 8 or 9 times in another, examination frequencies differed significantly among race/sex groups ($\chi^2 = 26.311$, 15 *df; P* = .035). The major departures from expected frequencies occurred among those examined 4 times or fewer, with more white males and fewer black males and black females than expected. Examination frequencies did not differ significantly between males and females among blacks ($\chi^2 = 3.314$, 5 df; $P = .652$) or whites ($\chi^2 = 8.888$; 5 *df; P* = .114).

Individuals entered the study at different times and at different ages; age at first examination ranged from 4.0 to 19.5 years. Over all examinations, age distributions differed significantly among race/sex groups, with slightly higher proportions of blacks in younger age groups and whites in older ones (Table 2; $\chi^2 = 39.54$, 18 *df; P* = .002). Overall, 733 examinations (13.1%) were of children less than 10 years old; 2,476 (44.2%) were of subjects 10 to 18 years old; 1,903 (34%) were of subjects 19 to 30 years old; and 485 (8.7%) were of subjects 31 to 38 years old.

Means and standard deviations for age, BMI, total cholesterol (T-C), HDL-C, LDL-cholesterol (LDL-C), and TG are shown in Table 3 for race/sex groups by examination number. Mean age differed significantly among race/sex groups at all except the seventh examination, tending to be highest for white males and lowest for black females. Significant divergence of BMI among race/sex groups emerged at the second examination and persisted thereafter, generally being highest in black females. Through 7 examinations, blacks had higher mean HDL-C and lower mean TG than whites. In blacks, HDL-C and TG levels in males and females were similar at each examination, but in whites, males showed lower mean HDL-C than females at all but the first examination, consistent with previous observations of a greater drop in HDL-C levels at puberty in white males.[44] T-C and LDL-C patterns among race/sex groups were less marked; only after the fifth examination did point estimates of mean T-C in white males exceed those in white females, and the differences were not statistically significant; for LDL-C, a similar shift occurred at the fourth examination. Among blacks, point estimates of mean T-C and LDL-C were at least slightly higher in females, except at examinations 5 (T-C) and 8 (both lipids).

LPL Ser_{447}-*Stop* genotype distributions (Table 4) were in Hardy-Weinberg equilibrium in both blacks ($\chi^2 = 0.907$, 1 df; $P = .341$) and whites ($\chi^2 = 1.467$, 1 *df; P* = .226), but differed between them ($\chi^2 = 7.595$, 1 *df*, $P = .006$, with $Stop_{447}$ homozygotes and heterozygotes grouped), the $Stop_{447}$ allele being more frequent in whites (0.0891 ± 0.0076) than in blacks (0.0525 ± 0.0092). Because $Stop_{447}$ homozygotes were rare (3 among whites, none among blacks), homozygotes were combined with heterozygotes for analyses of the polymorphism's phenotypic effects; omitting homozygotes from the analyses did not materially affect the results (data not shown).

In the multilevel analyses, LPL Ser_{447}-*Stop* genotype group was significant only for Ln TG and HDL-C profiles. Parameter estimates from the best-fitting models for these lipids are shown in Tables 5 (Ln TG) and 6 (HDL-C). With each model, the fixed effects parameters, taken together, yield expected lipid profiles for individuals having a given set of covariate values. Longitudinal multilevel models partition the variance of the dependent variable into within-subject and between-subjects components: the between-subjects (level 2) parameters estimate variances and covariances among measurements of different individuals, while the within-subjects (level 1) parameters estimate the variance among multiple measurements of the same individual. More than half of the variance in both lipids was attributable to variation within individuals over time: the proportion of variance at level 1 was 0.530 for Ln TG and 0.537 for HDL-C. For both lipids, between-subject variance was not constant, but was a function of both age and BMI.

Plots of means for race, sex, and 5-year age groups showed nonlinear longitudinal profiles for both TG (Fig 1) and HDL-C (Fig 2) and suggested that complex interactions involving race, sex, and age influence the profiles of both lipids. Race, sex,

Table 2. Distribution of Ages of Subjects When Examined by Race/Sex Group

	Black Females		Black Males		White Females		White Males		Total	
Age	No.	%	No.	%	No.	%	No.	%	No.	%
4-8	101	10.8	73	10.0	231	10.6	136	7.8	541	9.7
9-13	233	25.0	171	23.3	493	22.6	375	21.5	1,272	22.7
14-18	240	25.7	200	27.2	503	23.0	453	25.9	1,396	24.9
19-23	144	15.4	119	16.2	367	16.8	273	15.6	903	16.1
24-28	117	12.5	82	11.2	323	14.8	252	14.4	774	13.8
29-33	64	6.9	55	7.5	151	6.9	161	9.2	431	7.7
34-38	34	3.6	34	4.6	115	5.3	97	5.6	280	5.0
Total	933		734		2,183		1,747		5,597	

Table 3. Descriptive Statistics Within Race/Sex Groups by Examination

Examination No.		Black Females		Black Males		White Females		White Males	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	Age*	8.8	2.8	9.2	3.0	9.4	3.4	9.9	3.2
	BMI	17.7	4.0	17.3	3.4	17.6	3.3	17.9	3.5
	T-C*	170.3	32.1	165.0	27.5	162.7	28.1	158.7	28.3
	HDL-C*	70.3	23.2	69.9	19.1	62.5	20.5	64.7	19.2
	LDL-C*	93.0	23.6	88.2	22.8	91.5	26.4	86.3	25.0
	TG*	62.7	22.7	59.2	27.0	76.7	45.2	70.0	35.8
		n = 164		n = 132		n = 394		n = 316	
2	Age*	12.0	3.4	12.3	3.2	12.6	4.0	13.2	3.6
	BMI*	20.3	5.0	19.2	4.3	19.0	3.9	20.0	4.3
	T-C*	163.9	29.5	154.8	26.5	157.2	27.3	148.0	25.1
	HDL-C*	65.4	18.4	64.0	19.0	58.7	17.4	55.6	18.2
	LDL-C*	91.3	23.5	83.9	22.5	90.8	27.8	84.4	23.1
	TG*	62.4	26.0	59.1	22.3	74.3	36.8	72.5	35.5
		n = 164		n = 132		n = 394		n = 316	
3	Age*	15.2	4.4	15.5	4.3	16.2	4.9	16.9	4.9
	BMI*	22.6	5.9	21.5	5.2	20.8	4.4	22.2	4.8
	T-C*	167.0	31.1	161.7	30.9	165.9	31.5	160.2	31.0
	HDL-C*	58.9	18.4	58.0	17.6	56.5	17.5	50.8	18.6
	LDL-C	98.8	24.9	95.1	29.8	99.3	30.0	99.1	27.6
	TG*	67.6	32.4	65.6	34.7	81.8	42.0	83.0	42.9
		n = 163		n = 132		n = 393		n = 315	
4	Age*	19.2	5.1	20.5	5.7	20.8	5.8	21.9	6.0
	BMI*	24.8	6.5	24.1	6.3	22.7	5.2	24.5	5.0
	T-C	169.0	29.7	167.9	35.7	172.6	35.2	167.7	37.1
	HDL-C*	59.8	18.4	57.8	19.1	53.4	18.4	46.5	15.8
	LDL-C*	100.0	26.1	98.9	33.0	105.7	33.7	107.3	34.1
	TG*	69.6	29.2	83.7	67.5	95.4	49.3	100.5	60.8
		n = 160		n = 130		n = 384		n = 314	
5	Age*	22.7	5.4	24.0	6.1	23.6	4.7	24.4	5.3
	BMI*	26.9	7.7	25.4	6.3	23.6	5.4	25.7	4.9
	T-C	175.7	33.3	176.3	38.4	178.2	36.1	175.5	36.0
	HDL-C*	56.9	15.9	55.0	17.1	51.7	15.7	43.7	13.0
	LDL-C	109.7	31.2	106.9	34.4	112.0	33.4	116.1	31.2
	TG*	76.1	33.5	96.0	98.8	102.4	58.5	114.5	74.8
		n = 136		n = 108		n = 293		n = 226	
6	Age*	26.4	4.4	26.5	5.3	27.7	4.8	27.8	4.5
	BMI*	28.0	7.2	25.9	6.5	23.9	5.1	26.6	4.9
	T-C	187.0	35.2	183.4	45.9	185.2	36.1	189.2	39.1
	HDL-C*	58.3	16.1	57.8	20.5	53.2	13.9	44.8	12.9
	LDL-C	118.9	33.0	113.4	43.5	116.4	31.7	124.0	34.5
	TG*	82.2	36.6	84.8	48.1	108.3	105.8	130.8	103.7
		n = 92		n = 62		n = 195		n = 157	
7	Age	29.7	4.2	29.1	4.2	30.6	3.5	30.6	3.7
	BMI*	30.2	7.8	26.9	5.6	24.5	5.9	27.6	5.0
	T-C	189.1	33.3	179.1	37.6	187.2	30.9	198.1	44.1
	HDL-C*	55.0	16.6	57.5	20.5	53.9	13.0	44.3	9.8
	LDL-C*	122.2	30.4	108.9	33.4	115.9	29.8	130.4	34.4
	TG*	81.6	36.5	95.1	60.1	107.3	63.4	137.9	105.8
		n = 41		n = 29		n = 100		n = 74	
8	Age*	31.8	2.6	32.3	3.7	34.7	1.9	34.5	2.2
	BMI*	29.9	8.4	31.4	7.7	24.8	5.1	28.0	4.3
	T-C	194.3	33.0	195.0	72.4	197.8	33.8	210.3	46.6
	HDL-C*	57.5	17.4	44.5	15.6	54.4	12.8	41.0	12.0
	LDL-C	117.2	17.6	136.1	67.9	123.3	31.8	140.1	37.3
	TG	114.9	88.8	101.9	44.3	118.2	76.8	208.4	254.5
		n = 12		n = 8		n = 29		n = 29	

NOTE. Age given in years.

Abbreviations: BMI, body mass index (kg/m^2); T-C, total cholesterol (mg/dL); HDL-C, high-density lipoprotein-cholesterol (mg/dL); LDL-C, low-density lipoprotein-cholesterol (mg/dL); TG, triglycerides (mg/dL).

*Significant difference among race/sex groups at $\alpha = .05$, adjusted for pairwise comparisons.

Table 4. LPL *Ser_{447}-Stop* Genotypes by Race

Genotype	Blacks	Whites
C/C	265	590
C/G	31	117
G/G	0	3

NOTE. G denotes the $Stop_{447}$ allele.

BMI, BMI^2, sexual maturity (adults *v* prepubertal children and adolescents combined), age, age^2, and age^3, as well as LPL genotype group, were all significantly associated with Ln TG profiles (Table 5). However, while several significant interactions involving race, sex, BMI, and age were found, none involved LPL genotype. Although mean TG levels within genotype groups appear to diverge after age 30 in 3 of 4 race/sex groups and although the direction of the genotype/phenotype association appears to be reversed in black females over age 30 (Fig 1), these effects are not statistically significant, possibly because relatively few $Stop_{447}$ carriers over age 30 were examined, especially among blacks (Table 7). Thus, the results from the multilevel model indicate that an overall association of the $Stop_{447}$ allele with lower TG profiles is similar among race/sex groups and does not change significantly with age, as shown by the predicted profiles (dashed lines) in Fig 1. To further assess genotype effects on TG levels at different ages, we adjusted Ln TG for race, sex, BMI, and BMI^2, then compared genotypes within 5-year age groups (retaining only 1 observation/individual/age group to ensure independence among observations). The $Stop_{447}$ allele was associated with significantly lower Ln TG in all age groups (data not shown).

Table 5. Best Model for Longitudinal Ln TG Profiles

	Parameter	Estimate	SE
Fixed effects	Intercept	4.04505*	.01478
	Race	−.07911*	.01093
	Sex	−.03377*	.01138
	Adult	−.05133*	.01711
	BMI (kg/m^2)	.03980*	.00293
	BMI^2	−.00064*	.00018
	Age (yr)	−.00775*	.00311
	Age^2	.00150*	.00028
	Age^3	−.00004*	.00001
	$Stop_{447}$	−.05073*	.01291
	Race × sex	.00200	.01092
	Race × age	.00286*	.00118
	Sex × age	−.00154	.00208
	Age × race × sex	.00487*	.00119
	Sex × age^2	.00020*	.00007
	Race × BMI	−.01173*	.00192
	Sex × BMI	.00484*	.00195
	BMI × age	.00058*	.00022
	BMI × race × sex	−.00542*	.00190
Between-subjects	Intercept		.05326
	BMI		.00022
	Age		.00014
	Intercept/BMI		—
	Intercept/Age		—
	BMI/Age		—
Within-subjects	Intercept		.09951

NOTE. Variable coding: Race, −1 = white; sex, −1 = female; genotype group, −1 = Ser_{447} homozygote; 1 = $Stop_{447}$ carrier.
*$P < .05$.

Table 6. Best Model for Longitudinal HDL-C Profiles

	Parameter	Estimate	SE
Fixed Effects	Intercept	66.07129*	.87525
	Race	2.58871*	.84435
	Sex	.01105	.84510
	BMI (kg/m^2)	−1.27702*	.11704
	BMI^2	.02403*	.00427
	Age (yr)	.05098	.14798
	Age^2	−.14630*	.02370
	Age^3	.01208*	.00176
	Age^4	−.00027*	.00004
	Race × sex	−.55607	.83228
	Race × age	.17578	.11086
	Sex × age	−.10088	.09947
	Sex × race × age	.18164*	.04533
	Sex × age^2	.00643*	.00314
	Race × age^2	.01153	.01190
	Race × age^3	−.00072*	.00035
	Sex × BMI	−.32204*	.11615
	Sex × BMI^2	.00794†	.00424
	$Stop_{447}$	.31465	.85100
	$Stop_{447}$ × age	.29367*	.09367
	$Stop_{447}$ × age^2	−.00922*	.00383
	$Stop_{447}$ × race	−.19077	.83296
	$Stop_{447}$ × sex	.03238	.83141
	$Stop_{447}$ × race × sex	−.05243	.83129
	$Stop_{447}$ × age × sex	.03842	.04519
	$Stop_{447}$ × age × race	.03165	.04537
	$Stop_{447}$ × age × race × sex	.10579*	.04507
Between-subjects	Intercept	614.01440	
	BMI	0.09141	
	Age	0.07198	
	Intercept/BMI	−2.09728	
	Intercept/age	−2.42629	
	BMI/age	−0.05687	
Within-subjects	Intercept	182.00970	

NOTE. Variable coding: Race, −1 = white; sex, −1 = female; genotype group, −1 = Ser_{447} homozygote, 1 = $Stop_{447}$ carrier.
*$P < .05$; †$.05 < P < .10$.

HDL-C levels tended to decline during adolescence, especially in white males (Fig 2). The best multilevel model for HDL-C profiles (Table 6) was markedly more complex than that for Ln TG; exponential age terms through age^4 were either themselves significant or interacted significantly with race, sex, or LPL genotype. Three interactions involving LPL genotype group were significant: 2-way interactions of genotype with age and genotype with age^2 and a 4-way interaction of genotype with age, race, and sex. In all race/sex groups except white males, point estimates of mean HDL-C among those 4 to 8 years of age were lower in $Stop_{447}$ carriers than in Ser_{447} homozygotes (Fig 2). Among white males 4 to 8 years of age, mean HDL-C in $Stop_{447}$ carriers (65.68 ± 15.51 mg/dL, n = 31) was slightly, but not significantly, higher than in Ser_{447} homozygotes (61.93 ± 19.21 mg/dL, n = 104). Among white females and blacks, however, mean HDL-C in $Stop_{447}$ carriers increased sharply during adolescence, while in Ser_{447} homozy-

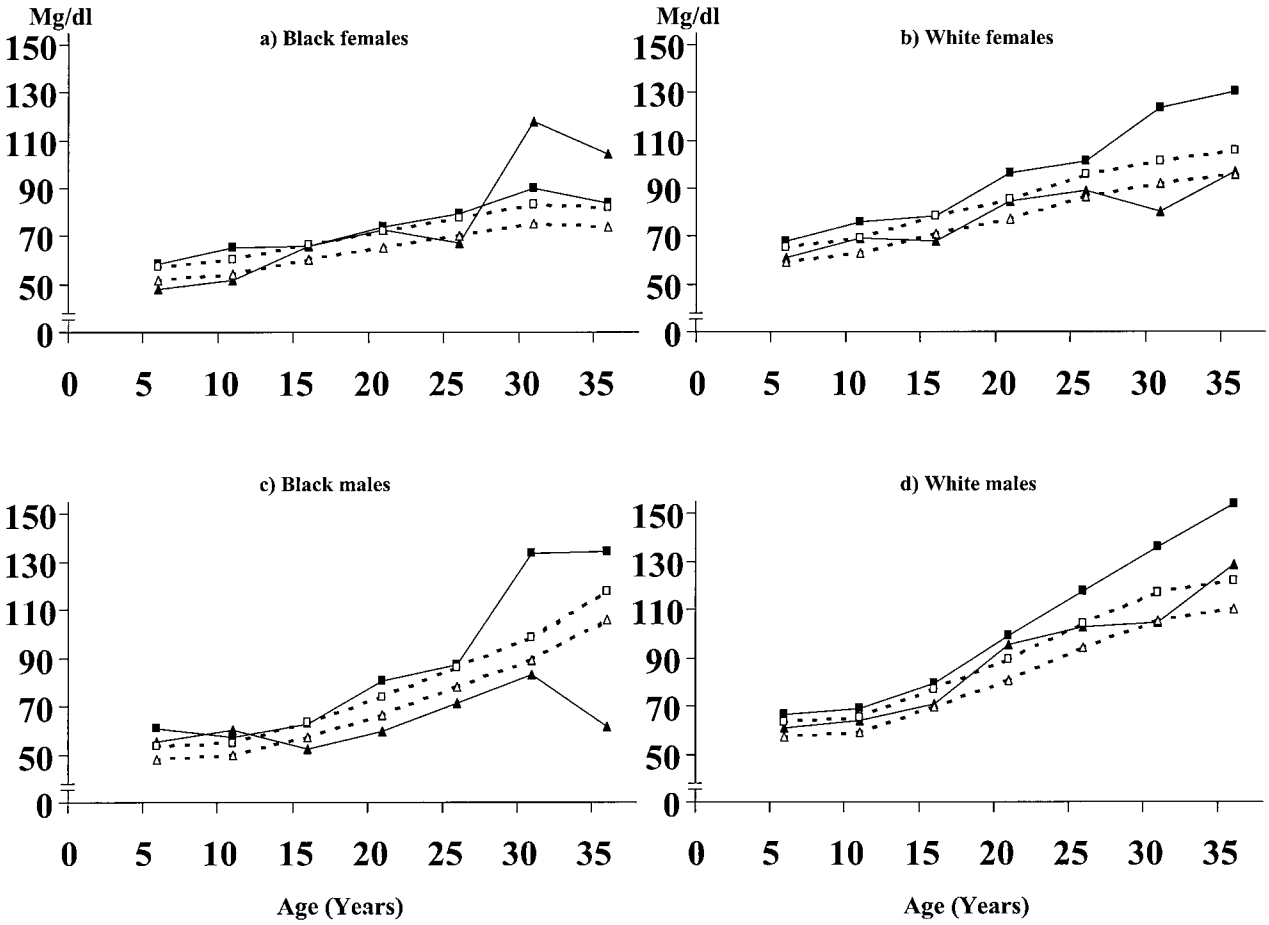

Fig 1. TG profiles for LPL genotype groups by race and sex. ■, observed mean profiles, Ser_{447} homozygotes; ▲, observed mean profiles, $Stop_{447}$ homozygotes and heterozygotes; □, predicted profiles, Ser_{447} homozygotes; △, predicted profiles, $Stop_{447}$ homozygotes and heterozygotes. Observed profiles based on means of the age groups: 4 to 8, 9 to 13, 14 to 18, 19 to 23, 24 to 28, 29 to 33, and 34 to 38 years. Predicted values based on the model shown in Table 5, setting age at the midpoint of each interval and using race/sex-specific mean BMI values within each age group.

gotes, it either declined (in both black and white females) or increased less sharply (in black males). The relatively severe decline in HDL-C levels that began during adolescence in white males affected both genotype groups; at 19 to 23 years of age, mean HDL-C was nearly identical in Ser_{447} homozygotes (44.32 ± 14.87 mg/dL, n = 196) and $Stop_{447}$ carriers (43.98 ± 15.09 mg/dL, n = 49). For white males in all other age groups, mean HDL-C in $Stop_{447}$ carriers was slightly higher, but the difference was only significant among those 24 to 28 years old (Table 8). Mean HDL-C in $Stop_{447}$ carriers was lower than in Ser_{447} homozygotes among 34- to 38-year-old black females (3 carriers) and among 29- to 33-year-old Black males (4 carriers), but the difference was not significant in either case.

As with TG, we tested genotype effects within 5-year age groups after adjusting for race, sex, BMI, and BMI^2, retaining only 1 observation/individual within each age group. The $Stop_{447}$ allele was associated with significantly higher HDL-C among those 14 to 18 ($P = .001$), 19 to 23 ($P = .022$), and 24 to 28 ($P = .002$) years of age; point estimates of mean HDL-C were higher among $Stop_{447}$ carriers in all age groups except 4- to 8-year-olds (data not shown).

Thus, while LPL Ser_{447}-*Stop* genotype affected both TG and HDL-C levels, its effect on their longitudinal profiles differed: significant genotype-by-age interactions occurred only with HDL-C. The $Stop_{447}$ allele was not associated with higher HDL-C levels in subjects under approximately 10 years of age, and overall, its subsequent association with higher HDL-C levels appeared to diminish after approximately age 30. In contrast, the association of the $Stop_{447}$ allele with lower TG levels did not change significantly with age.

DISCUSSION

The $Stop_{447}$ allele of LPL has been associated with more favorable HDL-C and/or TG profiles in many studies,[10,18,21,45,46] but not in all.[22] Our results strongly suggest that its effects on HDL-C, at least, are modulated by age, even after controlling for factors such as race, sex, and BMI. Overall, we found the $Stop_{447}$ allele to be associated with lower serum TG and higher HDL-C; however, the effects of the Ser_{447}-*Stop* polymorphism on HDL-C, but not TG, changed with age. In subjects under approximately 10 years of age, there was evi-

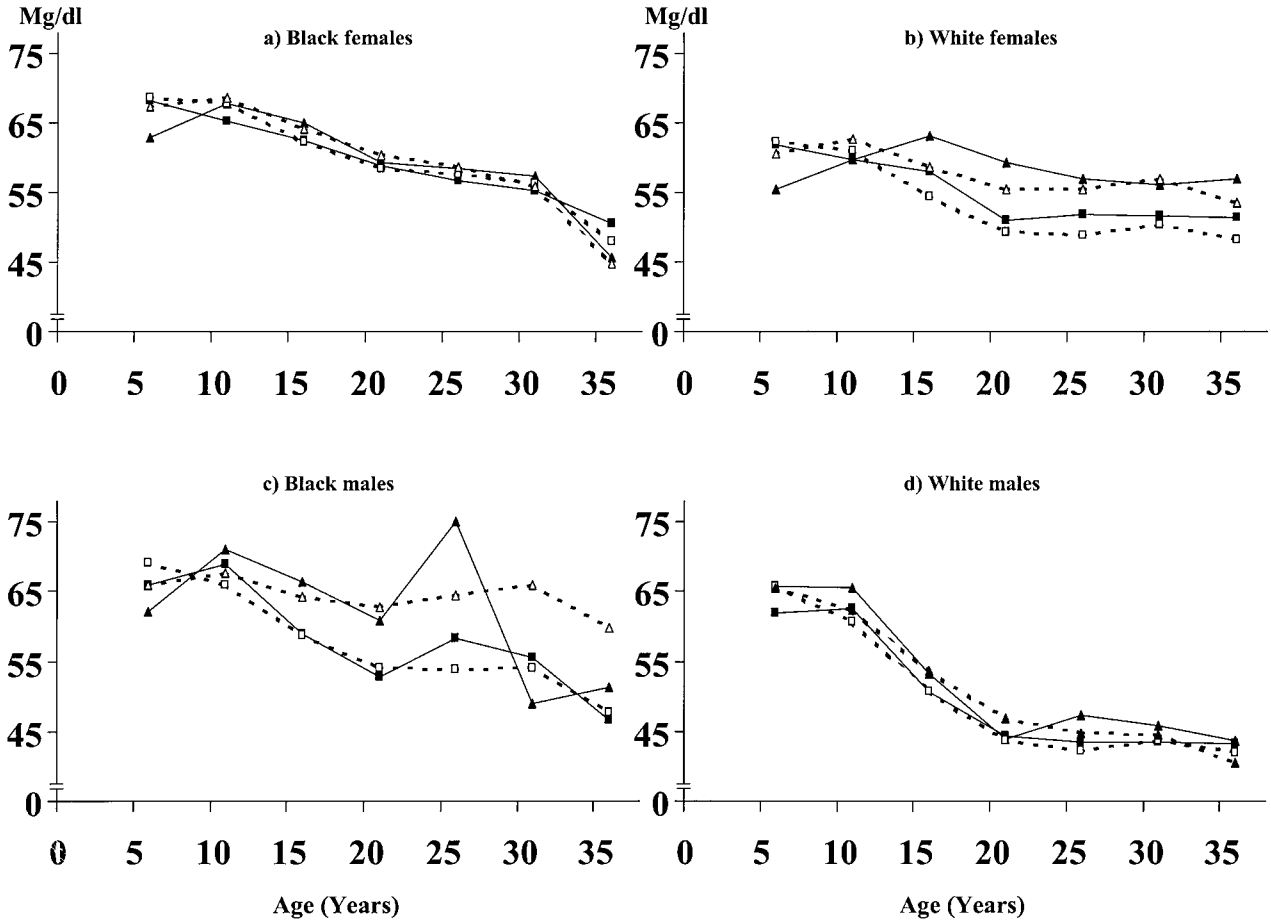

Fig 2. HDL-C profiles for LPL genotype groups by race and sex. ■, observed mean profiles, Ser_{447} homozygotes; ▲, observed mean profiles, $Stop_{447}$ homozygotes and heterozygotes; □, predicted profiles, Ser_{447} homozygotes; △, predicted profiles, $Stop_{447}$ homozygotes and heterozygotes. Observed profiles based on means of the age groups: 4 to 8, 9 to 13, 14 to 18, 19 to 23, 24 to 28, 29 to 33, and 34 to 38 years. Predicted values based on the model shown in Table 6, setting age at the midpoint of each interval and using race/sex-specific mean BMI values within each age group.

Table 7. TG Means and SD (in mg/dL) by Race, Sex, and LPL Genotype Group Within 5-Year Age Groups Including Only One Observation/Individual/Age Group

Age	Genotype Group	Black Females			Black Males			White Females			White Males		
		No.	Mean	SD	No.	Mean	SD	No.	Mean	SD	No.	Mean	SD
4-8	Ser_{447}	70	62.1	20.4*	51	62.4	30.3	161	67.2	28.3	90	67.2	27.8
	$Stop_{447}$	12	46.4	14.0	8	56.9	30.7	21	63.1	30.2	26	59.4	26.4
9-13	Ser_{447}	134	63.5	25.1†	104	54.9	18.5	286	75.1	35.1†	196	69.6	38.0
	$Stop_{447}$	16	52.8	22.5	10	53.6	21.0	42	65.4	28.3	57	63.7	27.7
14-18	Ser_{447}	135	66.5	28.2	108	64.5	24.9*	282	78.4	33.7*	207	81.4	39.2
	$Stop_{447}$	16	73.2	35.4	13	51.5	6.8	45	66.6	34.0	60	75.2	32.9
19-23	Ser_{447}	101	76.6	33.4	78	86.3	62.2†	230	97.6	44.9*	154	102.5	65.8
	$Stop_{447}$	13	74.8	48.2	11	60.3	23.9	36	82.7	55.1	41	99.6	66.1
24-28	Ser_{447}	94	78.2	34.8†	69	88.7	58.6	248	103.2	54.5	159	119.6	97.2
	$Stop_{447}$	11	64.6	36.3	5	71.0	20.1	37	90.5	47.0	53	104.9	51.0
29-33	Ser_{447}	59	90.1	51.3	50	134.4	133.4*	131	123.4	126.9*	130	136.6	101.8†
	$Stop_{447}$	4	117.8	43.2	4	83.0	11.2	20	79.9	28.4	29	104.3	42.8
34-38	Ser_{447}	31	83.7	35.4	31	133.8	90.1†	99	130.4	78.2	72	153.5	168.2
	$Stop_{447}$	3	104.3	50.3	3	61.7	10.8	16	97.0	53.1	25	128.2	83.8

NOTE. Testing difference between genotype groups: *$P < .05$; †$.05 < P < .10$.

Table 8. HDL-C Means and SD (in mg/dL) by Race, Sex, and LPL Genotype Group Within 5-Year Age Groups Including Only One Observation/Individual/Age Group

Age	Genotype Group	Black Females			Black Males			White Females			White Males		
		No.	Mean	SD	No.	Mean	SD	No.	Mean	SD	No.	Mean	SD
4-8	Ser_{447}	70	68.9	24.3	51	67.1	19.3	161	63.2	21.5	90	61.8	17.4
	$Stop_{447}$	12	63.5	17.7	8	63.2	13.2	21	55.8	23.1	26	67.3	13.9
9-13	Ser_{447}	134	68.3	22.1	104	72.0	20.0	286	60.9	18.4	196	65.4	20.9
	$Stop_{447}$	16	73.0	11.5	10	70.3	22.8	42	63.1	19.3	57	66.7	15.4
14-18	Ser_{447}	135	62.1	17.8	108	57.5	15.6*	282	56.0	17.4†	207	47.2	16.4
	$Stop_{447}$	16	63.9	18.2	13	66.0	15.1	45	63.9	15.8	60	50.8	14.4
19-23	Ser_{447}	101	58.2	16.7	78	52.2	18.4*	230	50.3	16.9†	154	43.9	14.4
	$Stop_{447}$	13	57.2	17.0	11	62.6	21.4	36	59.0	15.9	41	43.9	15.0
24-28	Ser_{447}	94	56.8	15.3	69	59.3	20.0*	248	51.5	13.2†	159	43.3	11.2†
	$Stop_{447}$	11	60.4	13.9	5	75.0	18.8	37	58.4	15.7	53	47.2	11.7
29-33	Ser_{447}	59	55.6	15.2	50	55.7	19.2	131	51.6	13.1	130	43.5	10.3
	$Stop_{447}$	4	57.2	26.7	4	49.0	7.1	20	56.0	15.6	29	45.7	11.4
34-38	Ser_{447}	31	50.6	10.7	31	46.7	17.9	99	51.4	13.4	72	43.2	10.3
	$Stop_{447}$	3	45.7	7.1	3	51.3	15.4	16	56.9	17.2	25	43.7	10.9

NOTE. Test of difference between genotype groups: *$.05 < P < .10$; †$P < .05$.

dence of lower HDL-C levels in $Stop_{447}$ carriers than in Ser_{447} homozygotes, although the differences were not statistically significant. From adolescence onward, however, HDL-C levels tended to be higher in $Stop_{447}$ carriers, although the difference between genotype groups may decline somewhat after approximately age 30.

The associations of Ser_{447} and $Stop_{447}$ alleles with HDL-C levels during and after adolescence appear to differ among race/sex groups more in magnitude than in direction. The appearance of reversed allelic associations in blacks over age 30 (Fig 2) may well be due to chance, given the small number of black $Stop_{447}$ carriers older than age 30 in our sample. In the Bogalusa population, the frequency of the $Stop_{447}$ allele was significantly lower in blacks (0.053 ± 0.011) than in whites (0.091 ± 0.009). Among blacks of Caribbean and West African descent living in London, a similar frequency of the $Stop_{447}$ allele (0.060) has been reported.[47] The frequency among Bogalusa whites was within the range reported for other Caucasian populations.[22,47-51]

Functional differences between the LPL isoforms, which might account for their effects on plasma HDL-C and TG levels at any age, have not been satisfactorily elucidated. Different studies have reported the lipolytic activity in vitro of the truncated variant to be lower than,[15,17] higher than,[16] or no different than[13,18] that of the full-length form. However, it has been suggested that there is little or no difference in their specific activities when the relative proportions of inactive monomeric and active dimeric LPL associated with each isoform are considered.[19] Activity of the truncated isoform may vary substantially with different substrates, suggesting that the mutation may affect substrate recognition.[15] Aspects of LPL biology apart from its catalytic activity may influence its effects on serum lipids, however, as suggested by the finding that some LPL haplotypes associated with higher catalytic activity in vitro are not associated with altered lipid levels in vivo.[52] In vitro, the $Stop_{447}$ allele enhances secretion of the inactive monomer[17,19]; if this also holds true in vivo, the observation that most lipoprotein-associated LPL is monomeric[53] suggests the possibility that an excess of the truncated monomers might enhance binding of TG-rich lipoproteins to endothelial surfaces or to some receptors.

Changes in serum lipid and lipoprotein levels with age have been described both in children[54,55] and in adults,[56,57] but the causes are not well understood. Hormonal changes are likely involved in some phases, as when T-C and HDL-C levels begin to decline at puberty,[54,55,58] but it is less obvious why T-C and, especially, LDL-C levels begin to increse after about age 20, particularly in men, or why peak levels occur between ages 50 to 60 years in men and 60 to 70 years in women.[56,57] That age may influence the effects of LPL on plasma lipid levels was demonstrated in 29 members of 1 pedigree, who were heterozygous for LPL deficiency and had mean adipose tissue LPL activity levels only 50% of normal. While 14 of 17 heterozygotes under age 40 had plasma TG levels below the 90th percentile for the population, 10 of 12 over age 40 had levels above this; in addition, 7 (58%) of the older carriers had HDL-C levels below the 5th percentile, compared with 4 (23.5%) of the younger ones.[59]

The relationship between the $Stop_{447}$ allele and serum HDL-C levels appeared to change around the time of puberty, suggesting that factors related to postpubertal growth and development may affect the LPL isoforms differently. In humans, growth hormone inhibits LPL activity in adipose tissue, but not in skeletal muscle, suggesting that it would have minor effects, if any, on plasma TG and HDL-C levels.[60] However, hormone replacement therapy in patients with adult growth hormone deficiency increases both production and catabolism of VLDL apo B, and lowers plasma T-C and LDL-C levels and the ratio of VLDL-C to VLDL apo B.[61] During periods of rapid growth, higher growth hormone levels might affect the function of the full-length and truncated LPL isoforms differently. Effects of growth hormone on plasma lipids may not be confined to the young; in healthy adults over age 65, growth hormone secretion has been associated negatively with T-C and LDL-C levels, positively with HDL-C levels in women, and negatively with TG levels in men.[62] In addition, the effects of synthetic growth hormone-releasing peptides reportedly vary with age.[63]

Evidence for decreasing LPL Ser_{447}-*Stop* genotype associa-

tions with HDL-C levels after age 30 in our sample was somewhat ambiguous, but suggests that age-related changes in LPL function may also occur after sexual maturation and, in women, before menopause. Even in adults, some aspects of lipid metabolism vary with age. Postheparin LPL activity is reportedly lower in older subjects.[64] Increased postprandial triglyceridemia in older subjects[64,65] may reflect slower clearance of intestinally-derived lipoproteins[66] due, in part, to decreased LPL activity.[64,67] Older age has also been associated with faster production of VLDL apo B-100[68]; lower LPL activity could exacerbate any resulting increase in serum TG levels. LDL apo B-100 has a longer plasma residence time in older subjects,[68] possibly indicating less efficient LDL receptor function or regulation with age. If LPL modulates LDL receptor-mediated uptake of LDL and VLDL,[69,70] subtle differences between the Ser_{447} and $Stop_{447}$ isoforms in their interactions with the LDL receptor might be reduced as receptor function declines with age.

How genetic variation may influence phenotypic variation not only among individuals, but within individuals over time, is only beginning to be studied. We have found evidence that the Ser_{447}-*Stop* polymorphism of LPL has effects on serum HDL-C levels that change over the period from childhood through adolescence and approaching middle age, and we have discussed mechanisms that might produce such age-related changes in allelic effects. Further studies are needed to discover and characterize genotype effects, which change with age, and to elucidate the mechanisms underlying such changes.

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REFERENCES

1. Brunzell JD: Familial lipoprotein lipase deficiency and other causes of the chylomicronemia syndrome, in Scriver CR, Beaudet AL, Sly WS, et al (eds): The Metabolic and Molecular Bases of Inherited Disease, ed 7, vol 2. New York, NY, McGraw-Hill, 1995, pp 1913-1932

2. Beisiegel U, Weber W, Bengtsson-Olivecrona G: Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. Proc Natl Acad Sci USA 88:8342-8346, 1991

3. Nykjaer A, Nielsen M, Lookene A, et al: A carboxyl-terminal fragment of lipoprotein lipase binds to the low density lipoprotein receptor-related protein and inhibits lipase-mediated uptake of lipoprotein in cells. J Biol Chem 269:31747-31755, 1994

4. Olivecrona G, Olivecrona T: Triglyceride lipases and atherosclerosis. Curr Opin Lipidol 6:291-305, 1995

5. Skottova N, Savonen R, Lookene A, et al: Lipoprotein lipase enhances removal of chylomicrons and chylomicron remnants by the perfused rat liver. J Lipid Res 36:1334-1344, 1995

6. Takahashi S, Suzuki J, Kohno M, et al: Enhancement of the binding of triglyceride-rich lipoproteins to the very low density lipoprotein receptor by apolipoprotein E and lipoprotein lipase. J Biol Chem 270:15747-15754, 1995

7. Beisiegel U: New aspects on the role of plasma lipases in lipoprotein catabolism and atherosclerosis. Atherosclerosis 124:1-8, 1996

8. Salinelli S, Lo J-Y, Mims MP, et al: Structure-function relationship of lipoprotein lipase-mediated enhancement of very low density lipoprotein binding and catabolism by the low density lipoprotein receptor. Functional importance of a properly folded surface loop covering the catalytic center. J Biol Chem 271:21906-21913, 1996

9. Thorn JA, Chamberlain JC, Alcolado JC, et al: Lipoprotein and hepatic lipase gene variants in coronary atherosclerosis. Atherosclerosis 85:55-60, 1990

10. Mattu RK, Needham EWA, Morgan R, et al: DNA variants at the LPL gene locus associate with angiographically defined severity of atherosclerosis and serum lipoprotein levels in a Welsh population. Arterioscler Thromb 14:1090-1097, 1994

11. Reymer PWA, Gagné E, Groenemeyer BE, et al: A lipoprotein lipase mutation (Asn291Ser) is associated with reduced HDL cholesterol levels in premature atherosclerosis. Nat Genet 10:28-34, 1995

12. Hata A, Robertson M, Emi M, et al: Direct detection and automated sequencing of individual alleles after electrophoretic strand separation: identification of a common nonsense mutation in exon 9 of the human lipoprotein lipase gene. Nucleic Acids Res 18:5407-5410, 1990

13. Faustinella F, Chang A, Van Biervliet JP, et al: Catalytic triad residue mutation (Asp^{156} - Gly) causing familial lipoprotein lipase deficiency. Co-inheritance with a nonsense mutation (Ser^{447} - Ter) in a Turkish family. J Biol Chem 266:14418-14424, 1991

14. Galton DJ, Mattu RK, Cavanna J: Polymorphisms of the lipoprotein lipase gene and premature atherosclerosis. J Intern Med 236:63-68, 1994 (suppl 736)

15. Kobayashi J, Nishida T, Ameis D, et al: A heterozygous mutation (the codon for Ser^{447}- a stop codon) in lipoprotein lipase contributes to a defect in lipid interface recognition in a case with type I hyperlipidemia. Biochem Biophys Res Commun 182:70-77, 1992

16. Kozaki K, Gotoda T, Kawamura M, et al: Mutational analysis of human lipoprotein lipase by carboxy-terminal truncation. J Lipid Res 34:1765-1772, 1993

17. Previato L, Guardamagna O, Dugi KA, et al: A novel missense mutation in the C-terminal domain of lipoprotein lipase (Glu^{410}-Val) leads to enzyme inactivation and familial chylomicronemia. J Lipid Res 35:1552-1560, 1994

18. Kuivenhoven JA, Groenemeyer BE, Boer JMA, et al: $Ser_{447stop}$ mutation in lipoprotein lipase is associated with elevated HDL cholesterol levels in normolipidemic males. Arterioscler Thromb Vasc Biol 17:595-599, 1997

19. Zhang H, Henderson H, Gagne SE, et al: Common sequence variants of lipoprotein lipase: Standardized studies of in vitro expression and catalytic function. Biochim Biophys Acta 1302:159-166, 1996

20. Wittrup HH, Tybjærg-Hansen A, Nordestgaard BG: Lipoprotein lipase mutations, plasma lipids and lipoproteins, and risk of ischemic heart disease: A meta-analysis. Circulation 99:2901-2907, 1999

21. Fisher RM, Humphries SE, Talmud PJ: Common variation in the lipoprotein lipase gene: Effects on plasma lipids and risk of atherosclerosis. Atherosclerosis 135:145-159, 1997

22. Peacock RE, Hamsten A, Nilsson-Ehle P, et al: Associations between lipoprotein lipase gene polymorphisms and plasma correlations of lipids, lipoproteins and lipase activities in young myocardial infarction survivors and age-matched healthy individuals from Sweden. Atherosclerosis 97:171-185, 1992

23. Groenemeijer BE, Hallman MD, Reymer PW, et al: Genetic variant showing a positive interaction with β-blocking agents with a beneficial influence on lipoprotein lipase activity, HDL cholesterol, and triglyceride levels in coronary artery disease patients: The Ser^{447}-Stop substitution in the lipoprotein lipase gene. Circulation 95:2628-2635, 1997

24. Humphries SE, Nicaud V, Margalef J, et al: Lipoprotein lipase gene variation is associated with a paternal history of premature coronary artery disease and fasting and postprandial plasma triglycerides. The European Atherosclerosis Research Study (EARS). Arterioscler Thromb Vasc Biol 18:526-534, 1998

25. Freedman DS, Shear CL, Srinivasan SR, et al: Tracking of serum lipids and lipoproteins in children over an 8-year period: The Bogalusa Heart Study. Prev Med 14:203-216, 1985

26. Lauer RM, Lee J, Clarke WR: Factors affecting the relationship between childhood and adult cholesterol levels: The Muscatine Study. Pediatrics 82:309-318, 1988

27. Webber LS, Srinivasan SR, Wattigney WA, et al: Tracking of serum lipids and lipoproteins from childhood to adulthood. The Bogalusa Heart Study. Am J Epidemiol 133:884-899, 1991

28. Guo S, Beckett L, Chumlea WC, et al: Serial analysis of plasma lipids and lipoproteins from individuals 9 - 21 y of age. Am J Clin Nutr 58:61-67, 1993

29. Berenson GS, McMahan CA, Voors AW, et al: Cardiovascular Risk Factors in Children. The Early Natural History of Atherosclerosis and Essential Hypertension. New York, NY, Oxford, 1980

30. Tanner JM: Growth at Adolescence, ed 2. Oxford, UK, Blackwell, 1962

31. Lipid Research Clinics Program: Manual of Laboratory Operations Vol I: Lipid and Lipoprotein Analysis. DHEW Publication No. (NIH) 75-628, Washington, DC, National Institutes of Health, 1974

32. Bucolo G, David H: Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 19:476-482, 1973

33. Allain CC, Poon LS, Chan CSG, et al: Enzymatic determination of total serum cholesterol. Clin Chem 20:470-475, 1974

34. Srinivasan SR, Berenson GS: Serum lipoproteins in children and methods for study, in Lewis LA, Opplt JJ (eds): CRC Handbook on Electrophoresis, vol. 3. Lipoprotein Methodology and Human Studies. Boca Raton, FL, CRC, 1983, pp 185-204

35. Anon.: Technical tips: An efficient salt-chloroform extraction of DNA from blood and tissues. Trends Genet 5:391, 1989

36. Laird NM, Ware JH: Random-effects models for longitudinal data. Biometrics 38:963-974, 1982

37. Bryk AS, Raudenbush SW: Hierarchical Linear Models. Newbury Park, CA, Sage, 1992

38. Goldstein H, Healy MJR, Rasbash J: Multilevel time series models with applications to repeated measures data. Stat Med 13:1643-1655, 1994

39. Akaike H: Information theory and an extension of the maximum likelihood principle, in Petrov BN, Czáki F (eds): 2nd International Symposium on Information Theory. Budapest, Akademiai Kiadó, 1973, pp 267-281

40. Wahlsten D: Insensitivity of the analysis of variance to heredity-environment interaction. Behav Brain Sci 13:109-161, 1990

41. SAS Institute I: SAS release 6.12, Cary, NC, 1996

42. Goldstein H, Rasbash J, Plewis I, et al: A user's guide to MLwiN. Version 1.0, London, UK, Multilevel Models Project, Institute of Education, University of London, 1998

43. U.S. Bureau of the Census: Census of Population, CP-2-1 (U.S.A.) and CP-2-20 (Louisiana), Social and Economic Characteristics. U.S. Government Printing Office, Washington, DC, 1990

44. Freedman DS, Srinivasan SR, Webber LS, et al: Black-white differences in serum lipoproteins during sexual maturation: The Bogalusa Heart Study. J Chron Dis 40:309-318, 1987

45. Stocks J, Thorn JA, Galton DJ: Lipoprotein lipase genotypes for a common premature termination codon mutation detected by PCR-mediated site-directed mutagenesis and restriction digestion. J Lipid Res 33:853-857, 1992

46. Zhang Q, Cavanna J, Winkelman BR, et al: Common genetic variants of lipoprotein lipase that relate to lipid transport in patients with premature coronary artery disease. Clin Genet 48:293-298, 1995

47. Hall S, Talmud PJ, Cook DG, et al: Frequency and allelic associations of common variants in the lipoprotein lipase gene in different ethnic groups: The Wandsworth Heart and Stroke Study. Genet Epidemiol 18:203-216, 2000

48. Chamberlain JC, Thorn JA, Oka K, et al: DNA polymorphisms at the lipoprotein lipase gene: Associations in normal and hypertriglyceridemic subjects. Atherosclerosis 79:85-91, 1989

49. Heinzmann C, Kirchgessner T, Kwiterovich PO, et al: DNA polymorphism haplotypes of the human lipoprotein lipase gene: Possible association with high density lipoprotein levels. Hum Genet 86:578-584, 1991

50. Mitchell RJ, Earl L, Bray P, et al: DNA polymorphisms at the lipoprotein lipase gene and their association with quantitative variation in plasma high-density lipoproteins and triacylglycerides. Hum Biol 66:383-397, 1994

51. Sing K, Ballantyne CM, Ferlic L, et al: Lipoprotein lipase gene mutations, plasma lipid levels, progression/regression of coronary atherosclerosis, response to therapy, and future clinical events. Lipoproteins and Coronary Atherosclerosis Study. Atherosclerosis 144:435-442, 1999

52. Hallman DM, Groenemeijer BE, Jukema JW, et al: Analysis of lipoprotein lipase haplotypes reveals associations not apparent from analysis of the constituent loci. Ann Hum Genet 63:499-510, 1999

53. Vilella E, Joven J, Fernández M, et al: Lipoprotein lipase in human plasma is mainly inactive and associated with cholesterol-rich lipoproteins. J Lipid Res 34:1555-1564, 1993

54. Srinivasan SR, Berenson GS: Serum lipids and lipoproteins in children. Pediatr Ann 21:220-223, 1992

55. Berenson GS, Wattigney WA, Bao W, et al: Rationale to study the early natural history of heart disease: The Bogalusa Heart Study. Am J Med Sci 310:S22-S28, 1995 (suppl 1)

56. Kreisberg RA, Kasim S: Cholesterol metabolism and aging. Am J Med 82:54-60, 1987

57. Johnson CL, Rifkind BM, Sempos CT, et al: Declining serum total cholesterol levels among US adults: The National Health and Nutrition Examination Surveys. JAMA 269:3002-3008, 1993

58. Srinivasan SR, Wattigney W, Webber LS, et al: Race and gender differences in serum lipoproteins of children, adolescents, and young adults— Emergence of an adverse lipoprotein pattern in white males: The Bogalusa Heart Study. Prev Med 20:671-684, 1991

59. Wilson DE, Emi M, Iverius P-H, et al: Phenotypic expression of heterozygous lipoprotein lipase deficiency in the extended pedigree of a proband homozygous for a missense mutation. J Clin Invest 86:735-750, 1990

60. Richelsen B: Effect of growth hormone on adipose tissue and skeletal muscle lipoprotein lipase activity in humans. J Endocrinol Invest 22:10-15, 1999 (suppl 5)

61. Christ ER, Cummings MH, Albany E, et al: Effects of growth hormone (GH) replacement therapy on very low density lipoprotein apolipoprotein B100 kinetics in patients with adult GH deficiency: A stable isotope study. J Clin Endocrinol Metab 84:307-316, 1999

62. O'Connor KG, Harman SM, Stevens TE, et al: Interrelationships of spontaneous growth hormone axis activity, body fat, and serum lipids in healthy elderly women and men. Metabolism 48:1424-1431, 1999

63. Arvat E, Camanni F, Ghigo E: Age-related growth hormone-releasing activity of growth hormone secretagogues in humans. Acta Paediatr Suppl 423:92-96, 1997

64. Weintraub MS, Eisenberg S, Breslow JL: Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. J Clin Invest 80:1571-1577, 1987

65. Cohn JS, McNamara JR, Cohn SD, et al: Postprandial plasma lipoprotein changes in human subjects of different ages. J Lipid Res 29:469-479, 1988

66. Krasinski SD, Cohn JS, Schaefer EJ, et al: Postprandial plasma retinyl ester response is greater in older subjects compared with younger subjects. Evidence for delayed plasma clearance of intestinal lipoproteins. J Clin Invest 85:883-892, 1990

67. Patsch JR, Prasad S, Gotto AM Jr, et al: High density lipoprotein$_2$. Relationship of the plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. J Clin Invest 80:341-347, 1987

68. Millar JS, Lichtenstein AH, Cuchel M, et al: Impact of age on the metabolism of VLDL, IDL, and LDL apolipoprotein B-100 in men. J Lipid Res 36:1155-1167, 1995

69. Mulder M, Lombardi P, Jansen H, et al: Low density lipoprotein receptor internalizes low density and very low density lipoproteins that are bound to heparan sulfate proteoglycans via lipoprotein lipase. J Biol Chem 268:9369-9375, 1993

70. Medh JD, Bowen SL, Fry GL, et al: Lipoprotein lipase binds to low density lipoprotein receptors and induces receptor-mediated catabolism of very low density lipoproteins in vitro. J Biol Chem 271:17073-17080, 1996