The β_2-Adrenergic Receptor Arg_{16}-Gly Polymorphism and Interactions Involving β_2- and β_3-Adrenergic Receptor Polymorphisms Are Associated With Variations in Longitudinal Serum Lipid Profiles: The Bogalusa Heart Study

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We examined the effects of combined genotypes of the β_2-adrenergic receptor (AR) Arg_{16}-Gly and β_3-AR Trp_{64}-Arg polymorphisms on longitudinal serum total (T-C) and low-density lipoprotein cholesterol (LDL-C) profiles in 1,198 subjects examined multiple times (6,488 observations) from 1973 to 1996 in the Bogalusa Heart Study, at ages from 4.5 to 38 years. Within 5-year age groups, T-C was significantly ($P < .05$) higher in β_2-AR Arg_{16}/Arg_{16} homozygotes than in Gly_{16} carriers among those 4 to 8 (171.4 ± 30.0 *v* 161.5 ± 27.7 mg/dL), 9 to 13 (167.7 ± 28.6 *v* 162.4 ± 27.4 mg/dL), and 14 to 18 (158.8 ± 29.6 *v* 154.7 ± 27.5 mg/dL) years of age, but not in those 19 to 23, 24 to 28, 29 to 33, or 34 to 38 years of age. The β_3-AR polymorphism was not associated with variation in either T-C or LDL-C. In multilevel polynomial growth curve models, the combination of the β_2-AR Arg_{16}/Arg_{16} genotype with either the β_3-AR Arg_{64}/Arg_{64} or Trp_{64}/Arg_{64} genotypes, denoted AA/AX, was associated with variation in longitudinal T-C ($P < .01$) and LDL-C ($P < .01$) profiles. The association between combined β_2/β_3-AR genotype and lipid profiles differed among race/sex groups, being most marked in black females, in whom the AA/AX combination was associated with higher T-C and LDL-C profiles across all ages. In White males, the AA/AX combination was most strongly associated with higher lipids in adults. In black males and white females, lipid profiles differed little between genotype groups. Our findings suggest that the β_2-AR Arg_{16}-Gly genotype influences T-C and LDL-C levels in an age-specific manner, that it may interact with β_3-AR Trp_{64}-Arg genotypes to influence longitudinal T-C and LDL-C profiles, and that the effect of combined β_2/β_3-AR genotypes on T-C and LDL-C profiles may differ among race/sex groups.

IT HAS LONG BEEN known that drugs which alter adrenergic receptor (AR) activity affect plasma lipid levels.[1] Two types of adrenergic receptors, denoted α and β, are functionally and pharmacologically distinguishable. Three known subtypes of β-adrenergic receptors (β_1, β_2, β_3) are important regulators of heart rate, vasodilation, and lipolysis, and have different pharmacological properties and different, though partly overlapping, tissue distributions.[2] The β_1-AR is expressed predominantly in cardiac muscle, and the β_2-AR, in the lungs, uterus, and both skeletal muscle and vascular smooth muscle; both are expressed in adipose tissue. The β_3-AR is expressed predominantly in adipose tissue. Given its pattern of expression, the β_3-AR is a prime candidate for a role in lipid metabolism, although its function in adult humans, who lack well-defined deposits of brown adipose tissue, has been debated.[3] In humans, the β_3-AR may function primarily in visceral adipose tissue deposits, while the β_1- and β_2-ARs are more important in regulating lipolysis in subcutaneous deposits.[4-6] Overall, the β_2-AR may be more important than the β_1-AR in regulating lipolysis in subcutaneous adipose tissue in humans,[4,7,8] but under some conditions (eg, after 4 weeks on a hypocaloric diet), the role of the β_1-AR may be enhanced.[9] Adrenergic regulation of lipolysis in human skeletal muscle is due almost entirely to the β_2-AR.[10]

Two polymorphisms of the β_2-AR in linkage disequilibrium, a Gly-Glu polymorphism at codon 27 and an Arg-Gly polymorphism at codon 16, have been associated with variation in plasma triglyceride[11-13] and total cholesterol (T-C) and/or low-density lipoprotein cholesterol (LDL-C) levels.[12,14,15] Studies relating variation in plasma lipids with a β_3-AR Trp-Arg polymorphism at codon 64 have shown widely varying results among different populations: Different studies have reported association of the Arg allele with higher T-C[16,17]; with either higher[18] or lower[19] LDL-C; and with either higher[18,20,21] or lower[22] triglycerides, while others have found no associations.[15,23,24]

Since both the β_2- and β_3-ARs are expressed in adipose tissue and can at least partially compensate for one another,[2] it is reasonable to think that interactions among variants of both might exist. A dysfunctional variant of either one might be masked by normal activity of the other, with impairment observable only when mutations are present in both. We investigated the possibility of such interactions by examining associations between combined genotypes of the β_2-AR Arg_{16}-Gly and β_3-AR Trp_{64}-Arg polymorphisms and longitudinal serum T-C and LDL-C profiles in subjects examined multiple times over a period of years in the Bogalusa Heart Study, a community-based study of risk factors for cardiovascular disease and related disorders.

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MATERIALS AND METHODS

Subjects

Subjects were examined between 1973 and 1996, when schoolchildren in Bogalusa, LA, as well as older subjects examined previously, were screened for cardiovascular disease risk factors approximately every 3 years. Subjects eligible for the present study had been examined at least 4 times between 1973 and 1996, and at least once between 1991 and 1996, when blood was collected for DNA. Participants gave informed consent at each examination; for those under 18 years of age, consent of a parent was also obtained. Study protocols were approved by institutional review boards at the institutions involved.

Examinations

Examinations were conducted by trained examiners following protocols published previously.[25] 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). Subjects were instructed to fast for 12 hours before examinations, with compliance assessed by interview. Blood was collected by antecubital venipuncture and allowed to clot; serum was collected after centrifugation and stored at 4°C until analysis, usually on the following day.

Laboratory Analyses

From 1973 to 1986, serum T-C and triglycerides were measured chemically on a Technicon AutoAnalyzer II (Technicon Instrument Corp, Tarrytown, NY), following Lipid Research Clinics Program protocols.[26] After 1986, they were measured enzymatically[27,28] using an Abbott VP instrument (Abbott Laboratories, North Chicago, IL). Both chemical and enzymatic procedures gave comparable results and met standards of the Lipid Standardization Program of the Centers for Disease Control and Prevention, Atlanta, GA. Serum lipoprotein cholesterol fractions, including LDL-C, were measured by heparin-Ca^{2+} precipitation followed by agar or agarose gel electrophoresis.[29]

Genetic Analyses

Genotyping employed TaqMan assays (Applied Biosystems, Foster City, CA). For the β_2-AR Arg_{16}-Gly polymorphism, a 121-bp fragment was amplified using 0.9 μmol/L each of the forward primer CGGCAGCGCCTTCTTG and the reverse primer GGCCAGGACGATGAGAGACA, 30 ng DNA, 5.0 mmol/L $MgCl_2$, and 1X TaqMan Universal PCR Master Mix containing AmpliTaq Gold DNA Polymerase in a 22-μL volume. An initial step of 2 minutes at 50°C and 10 minutes at 95°C, to activate the polymerase, was followed by 40 cycles of 15 seconds at 95°C and 1 minute at 62°C. A total of 0.2 μmol/L of each of the sequence-specific probes 6FAM-CTGGCACCCAATAGAAGCCATGC-TAMRA and VIC-CTGGCACCCAATGGAAGCCATGTAMRA was used in distinguishing alleles, with detection and typing performed using the ABI 7700 with Sequence Detection System software (Applied Biosystems).

For the β_3-AR Trp_{64}-Arg polymorphism, a 66-bp product was amplified using 0.9 μmol/L each of the forward primer GCAACCTGCTGGTCATCGT and the reverse primer CGAACACGTTGGTCATGGTC, following the protocol described above. A total of 0.2 μmol/L of each of the sequence-specific probes 6FAM-CCATCGCCTGGACTCCGAGAC-TAMRA and VIC-CATCGCCCGGACTCCGAGA-TAMRA was used in distinguishing alleles, with detection and typing as described above.

Statistical Analyses

Multilevel models, which partition phenotypic variation among nested levels of analysis, were used to analyze genotype effects on changes in serum lipids over time within individuals. These models can accommodate subjects having different numbers of unequally spaced measurements.[30] The levels in the current analyses comprised observations within individuals nested within families.

Let *h* independent variables predict measurements at time *i* within individual *j* from family *k*. Then,

$$y_{ijk} = \beta_{0jk} + \beta_{1jk}\, x_{1ijk} + \ldots + \beta_{hjk}\, x_{hijk} + r_{ijk}\,, \qquad (1)$$

where x_{hijk} are measured independent variables, β_{hjk} are parameters to be estimated, and r_{ijk} is the level 1 residual error, assumed to be normally distributed. Parameters describing the trajectory of measurements across time *within* each individual can vary *among* individuals. Thus, for the parameters, β_{hjk}, in the model, let

$$\beta_{0jk} = \gamma_{00} + \gamma_{01}\, z_{1jk} + \ldots + \gamma_{0m}\, z_{mjk} + \mu_{0jk} \qquad (2)$$

$$\beta_{1jk} = \gamma_{10} + \gamma_{11}\, z_{1jk} + \ldots + \gamma_{1m}\, z_{mjk} + \mu_{1jk} \qquad (3)$$

$$\vdots$$

$$\beta_{hjk} = \gamma_{h0} + \gamma_{h1}\, z_{1jk} + \ldots + \gamma_{hm}\, z_{mjk} + \mu_{hjk}\,, \qquad (4)$$

where z_{mjk} are level 2 explanatory variables, γ_{hm} are the associated parameters, and μ_{hjk} are residuals for each β parameter. Substituting for the β parameters in the level 1 model yields the level 2 model. This process can be extended to additional levels.

To find the best-fitting model for each response variable, a model was first fit that included all possible interactions involving race, sex, BMI, genotype group, and age terms through age cubed. Only records with complete data for these variables were included. Interactions were tested individually in a fixed order, using likelihood ratio tests; −2 times the difference in the model log likelihoods before and after removal of a term asymptotically follows a χ^2 distribution with 1 *df*. When all interactions of a given order involving an age term had been tested singly, the N nonsignificant terms were removed and a likelihood ratio test with N *df* conducted. Different structures for between-measurement covariance matrices were tested, including first-order autoregressive and compound symmetric structures, but different covariance structures had little impact on the fixed effects, which were our primary interest. We report results for models with unrestricted covariances at both family and individual levels.

Because there were too few β_3-AR Arg_{64}/Arg_{64} homozygotes for separate analyses, we combined Arg_{64}/Arg_{64} and Trp_{64}/Arg_{64} genotypes in 1 group, designated AX. To determine appropriate coding for the combined β_2/β_3-AR genotypes, we tested multilevel models containing either 6 (AA/AX, AA/TT, AG/AX, AG/TT, GG/AX, and GG/TT), 4 (AA/AX, AA/TT, GX/AX, and GX/TT), or 2 (AA/AX and Other) genotype groups. With 4 groups, GX denotes combined Gly_{16}/Gly_{16} and Arg_{16}/Gly_{16} genotypes. For T-C and LDL-C, models containing 4 groups fit as well as those with 6 (T-C: $\chi^2 = 1.0$, 2 *df*, $P = .61$; LDL-C: $\chi^2 = 1.4$, 2 *df*, $P = .50$), and those containing 2 groups fit as well as those with 4 (T-C: $\chi^2 = 3.7$, 2 *df*, $P = .16$; LDL-C: $\chi^2 = 1.1$, 2 *df*, $P = .58$). In the models with 4 or 6 groups, the only significant term for any of the groups was that for the AA/AX group. To simplify presentation of the analyses of combined β_2-AR and β_3-AR genotypes, we show results contrasting the AA/AX group with all other genotypes combined. Numbers of individuals and observations by combined β_2/β_3-AR genotype are shown in Table 1 for each race/sex group. Multilevel models were analyzed using SAS Proc Mixed.[31]

RESULTS

The sample included 1,198 individuals: 223 black females, 141 black males, 488 white females, and 346 white males. While 992 families were represented, 79.7% of black families and 83.5% of white families included only 1 member. Includ-

Table 1. Numbers of Observations and Individuals for Combined β_2/β_3- AR Genotype Groups, by Race and Sex

β_2/β_3-AR Group	Black Females		Black Males		White Females		White Males		Total	
	Obs	n	Obs	n	Obs	n	Obs	n	Obs	n
AA/AX	28	5	49	10	44	9	39	7	160	31
Other	1,165	210	691	126	2,580	472	1,772	335	6,208	1,143
Total	1,193	215	740	136	2,624	481	1,811	342	6,368	1,174

NOTE. Genotype groups as described in text.
Abbreviations: Obs, number of observations; n, number of individuals.

ing siblings increased sample size by 20.8%, but required nesting of individuals within families to be included in the multilevel models. Complete data from 6,488 examinations were available. The mean number of examinations per subject was 5.42 (±1.27), and did not differ significantly among race/sex groups.

Genotype counts for each locus and for both combined are shown in Table 2. In estimating allele frequencies, only 1 member of each family was used. Genotype frequencies at each locus met Hardy-Weinberg expectations in both blacks and whites (data not shown), but differed significantly between blacks and whites at both loci. In blacks, frequencies of the β_2-AR Arg_{16} and Gly_{16} alleles were nearly equal (0.49 ± 0.02 and 0.51 ± 0.02, respectively), while in whites, the frequency of the Arg_{16} allele (0.36 ± 0.01) was significantly lower ($P <$ .001). The frequency of the β_3-AR Arg_{64} allele was significantly higher in blacks than in whites (0.11 ± 0.01 *v*. 0.08 ± 0.01; $P = .004$).

Because the model for T-C with the Arg_{16}/Gly_{16} and Gly_{16}/Gly_{16} genotypes combined in one group (G/X) fit as well as the model with all 3 genotypes ($P = .254$), while the model with the Arg_{16}/Arg_{16} and Arg_{16}/Gly_{16} genotypes combined did not ($P = .043$), we used the Arg_{16}/Arg_{16} (A/A) and G/X groups in further analyses. In multilevel models with only polynomial age terms and genotype as predictors, the β_2-AR polymorphism was significantly associated with variation in T-C profiles ($P =$ .021). There was no significant main effect of β_2-AR genotype on LDL-C profiles ($P = .162$), but with interactions included, a significant age-by-genotype interaction appeared ($P = .033$). To further explore β_2-AR genotype associations, we adjusted serum lipid levels for race, sex, and BMI, then fit models within 5-year age strata, with genotype as the only predictor. Associations of β_2-AR genotype with T-C were significant in the 4- to 8-, 9- to 13-, and 14- to 18-year-old groups (Table 3). For T-C, the A/A genotype was associated with higher T-C levels in the 3 youngest age groups; the difference between genotype groups decreased with age, becoming nonsignificant in adults. After age 34, the mean T-C level was lower in the A/A group than in the G/X group, but the difference was not significant. The relationship between β_2-AR genotype and LDL-C showed a similar pattern of change across age groups. Together, these results demonstrate an age-by-genotype interaction affecting the relationship between β_2-AR Arg_{16}-Gly genotypes and serum T-C and LDL-C levels. In contrast, the β_3-AR polymorphism showed no significant associations with T-C or LDL-C either longitudinally or within age strata (data not shown).

Based on 77 observations in the AA/AX group for blacks, and 83 for whites, the effect of the combined β_2/β_3-AR genotypes was significant in multilevel models containing only age terms and genotype groups as predictors, with the AA/AX group being associated with significantly higher levels of both

Table 2. β_2-AR Arg_{16}-Gly and β_3-AR Trp_{64}-Arg Genotype Counts, by Race

	Genotype		
	Arg/Arg	Arg/Gly	Gly/Gly
β_2-AR Arg_{16}-Gly*			
Blacks	71	142	78
Whites	86	335	280
	Trp/Trp	Trp/Arg	Arg/Arg
β_3-AR Trp_{64}-Arg†			
Blacks	233	52	6
Whites	591	109	1

	Combined β_2-AR/β_3-AR Groups					
	AA/AX	AA/TT	AG/AX	AG/TT	GG/AX	GG/TT
Both loci*‡						
Blacks	15	69	38	134	19	76
Whites	16	89	68	320	40	290

Blacks *v* whites: *$P <$ .0001, †$P = .0040$.

‡β_3-AR Trp/Arg and Arg/Arg genotypes combined. A = Arg; G = Gly; T = Trp; X = TA or AA.

Table 3. T-C and LDL-C, Adjusted for Race, Sex, and BMI, by Age and β_2-AR Arg_{16}-Gly Genotype Group

Age (yr)	β_2-AR Genotype	n	T-C		LDL-C	
			Mean	SD	Mean	SD
4-8	A/A	108	171.4	30.0*	98.1	27.7‡
	G/X	530	161.5	27.7	92.4	23.5
9-13	A/A	242	167.7	28.6†	94.7	26.2
	G/X	1,219	162.4	27.4	91.2	24.0
14-18	A/A	260	158.8	29.6†	91.0	25.4‡
	G/X	1,325	154.7	27.5	87.9	23.9
19-23	A/A	151	171.1	29.4	106.4	26.1
	G/X	833	170.7	32.3	107.6	29.5
24-28	A/A	132	182.5	33.1	112.9	28.2
	G/X	743	180.2	35.4	114.6	32.8
29-33	A/A	80	190.7	36.5	124.2	34.6
	G/X	427	189.9	36.0	121.2	30.7
34-38	A/A	46	190.3	31.4	120.3	25.2‡
	G/X	272	199.7	36.8	129.2	31.4

NOTE. Genotypes: G/X = A/G or G/G. Lipids in mg/dL; n = number of observations.

Difference between genotype groups: *$P <$ .01; †$P <$ .05; ‡$.05 < P <$ .10.

Table 4. Best-Fitting Multilevel Mixed Models for LDL-C, by Sex

	Females		Males	
Fixed Effects	Estimate	SE	Estimate	SE
Intercept	103.0600	3.3055*	90.0647	2.6937*
Race	7.4257	3.0881*	2.1133	2.5842
BMI	2.9421	0.7855*	2.4343	0.7954*
BMI^2	−0.0715	0.0113*	−0.0843	0.0151*
Age	−1.7678	0.8284*	−2.2472	0.5197*
Age^2	0.1505	0.1442	0.1982	0.0340*
Age^3	−0.0017	0.0060	−0.0031	0.0009*
β_2/β_3-AR	−11.4216	3.2923*	−3.9019	2.6600
Race × β_2/β_3-AR	−7.3569	3.0532*	−0.7769	2.5489
Age × race	0.5074	0.2442*	0.0946	0.2096
Age × β_2/β_3-AR	0.4336	0.8188	0.3271	0.5019
Age × BMI	−0.3662	0.2038†	0.2399	0.1146*
Age^2 × race	−0.0741	0.0275*	−0.0177	0.0098†
Age^2 × β_2/β_3-AR	−0.0105	0.1433	−0.0274	0.0211
Age^2 × BMI	0.0354	0.0144*	0.0013	0.0057
Age^3 × Race	0.0015	0.0009†	—	—
Age^3 × β_2/β_3-AR	−0.0013	0.0060	—	—
Age^3 × BMI	−0.0009	0.0003*	−0.0004	0.0001*
BMI × race	−0.3381	0.2480	−1.0966	0.4290*
BMI × β_2/β_3-AR	−1.4647	0.7772†	0.2949	0.7827
Race × BMI × β_2/β_3-AR	—	—	0.9939	0.3690*
Age × BMI × β_2/β_3-AR	0.4732	0.2022*	−0.2320	0.1077*
Age × BMI × Race	0.0845	0.0500†	−0.0422	0.0264
Age^2 × BMI × β_2/β_3-AR	−0.0322	0.0143*	0.0107	0.0046*
Age^2 × BMI × Race	−0.0072	0.0042†	0.0025	0.0010*
Age^3 × BMI × β_2/β_3-AR	0.0007	0.0003*	—	—
Age^3 × BMI × race	0.0002	0.0001*	—	—
	Females		Males	
Random Effects	Covariance Component	SE	Covariance Component	SE
Among families				
Intercept	99.6100	51.1962*	230.0400	50.1721*
Within individuals				
Intercept	248.9100	52.1034*	96.0474	44.0782*
Age	1.4988	0.5458*	1.2017	0.4816*
Age^2	0.0015	0.0009†	0.0018	0.0009*
Intercept × age	4.9895	2.6662†	7.1394	2.4603*
Intercept × age^2	−0.2739	0.1259*	−0.1259	0.1244
Age × age^2	−0.0372	0.0221†	−0.0258	0.0207
Residual	295.8000	8.8570*	213.9300	7.9946*

NOTE. Variable coding. Race: −1 = white, +1 = black; β_2/β_3-AR: −1 = AA/AX, +1 = Other. Age in years; BMI in kg/m^2; parameter estimates in mg/dL.

Test for significant difference from 0: *$P < .05$, †$.05 < P < .10$.

T-C ($P = .008$) and LDL-C ($P = .008$). Inasmuch as T-C and LDL-C are highly correlated and gave similar results, we will focus on the results for LDL-C in the discussion that follows.

The best-fitting multilevel model for LDL-C in the full sample contained 11 interaction terms involving sex, suggesting that separate analyses by sex would be more appropriate. In the best-fitting model for females (Table 4), there were significant interactions of β_2/β_3-AR genotype and BMI with age^3 ($P = .028$), age^2 ($P = .025$), and age ($P = .019$), and of genotype with race ($P = .016$). For males, there were significant interactions of genotype and BMI with age^2 ($P = .019$), age ($P = .031$), and race ($P = .007$). The net effect of the interactions involving genotype is best shown by the predicted curves in Fig 1, where predicted values at ages 7, 11, 16, 21, 26, and 33 years were calculated by multiplying the appropriate coefficients by the parameter estimates for the fixed effects shown in Table 4, then summing over all terms. Mean age at enrollment (9.0 years for females, 9.5 years for males) was subtracted from each age value, so that the intercept estimates in the table represent expected LDL-C levels at entry into the study. BMI values were similarly adjusted by subtracting either 17.48 kg/m^2 (for females) or 17.60 kg/m^2 (for males). Also shown in Fig 1 are profiles derived from the means for each race/sex/genotype group within the age groups of 4 to 8, 9 to 13, 14 to 18, 19 to 23, 24 to 28, and 29 to 38 years, showing that, overall, observed and predicted profiles were reasonably

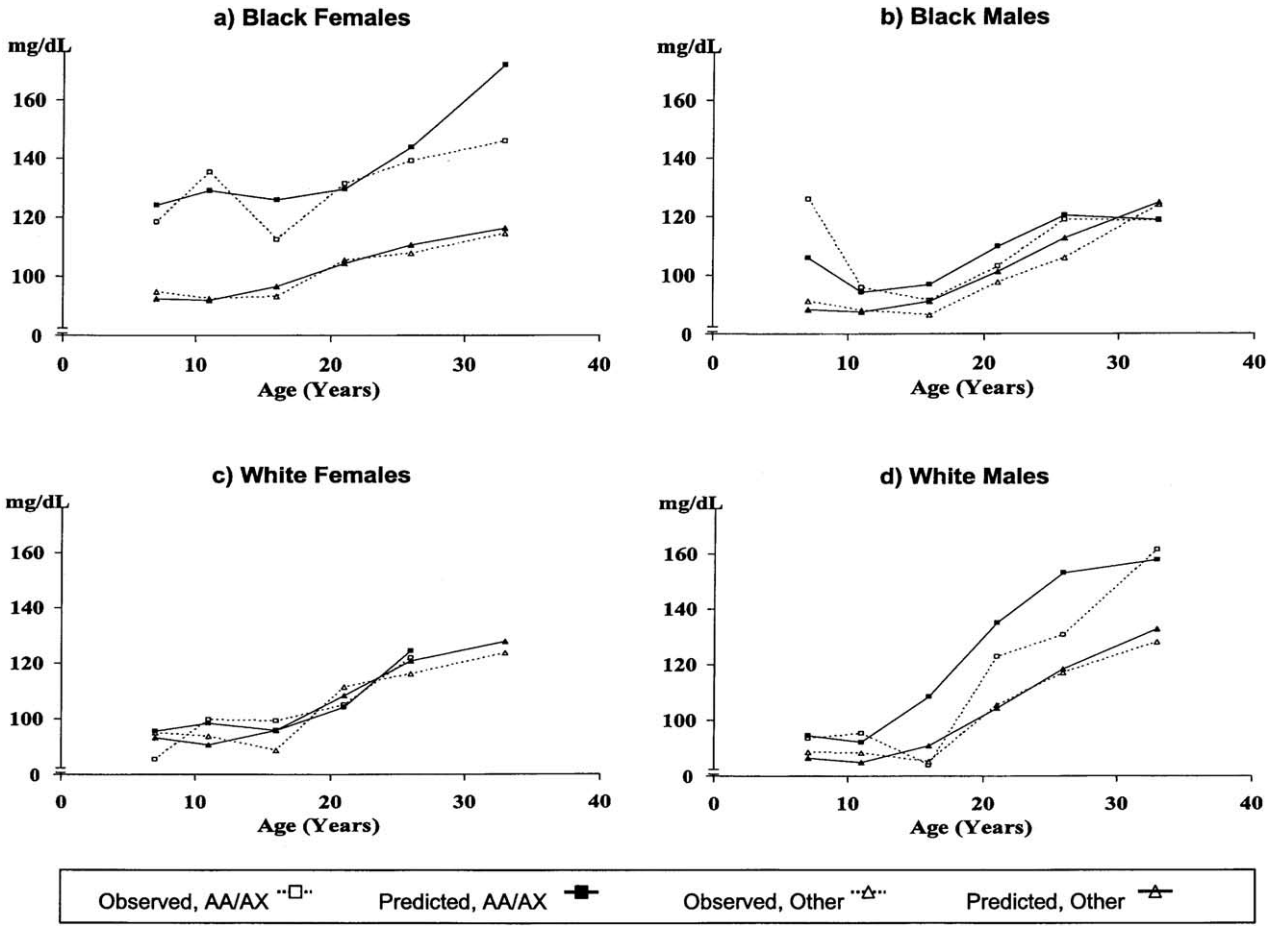

Fig 1. Observed and predicted LDL-C profiles, by race, sex, and combined genotypes of the β_2-AR codon 16 and β_3-AR codon 64 polymorphisms. (β_2/β_3-AR genotype groups: AA/AX = $Arg_{16}/Arg_{16}//Arg_{64}/Arg_{64}$ or $Arg_{16}/Arg_{16}//Trp_{64}/Arg_{64}$; Other = all other genotype combinations).

concordant. Profiles differed between genotype groups most markedly in black females, in whom LDL-C levels were higher in the AA/AX group at all ages (Fig 1a). In white males, the point estimates of mean LDL-C levels were higher in the AA/AX group except among those 14 to 18 years of age (Fig 1d); the model, however, did not predict that LDL-C values in the 2 genotype groups in white males would converge during adolescence. LDL-C profiles differed less between genotype groups in black males (Fig 1b) and, especially, white females (Fig 1c). Overall, the models suggest that the combination of the β_2-AR Arg_{16}/Arg_{16} genotype with either the Arg_{64}/Arg_{64} or Trp_{64}/Arg_{64} β_3-AR genotype is strongly associated with higher LDL-C levels in black females between approximately 5 to 38 years of age. A similar association appears in white males, though the discrepancy between observed and predicted LDL-C values for adolescents in the AA/AX group (Fig 1d) suggests that the association in white males may exist mainly in adults.

To ensure that the apparent genotype effects on LDL-C profiles were not confounded by the changes in BMI that typically occur with age, we repeated the multilevel analyses after adjusting for BMI. For females, the interaction of genotype group with race ($P = .020$) and the main effect for genotype group ($P = .005$) remained significant; for males, the interaction of genotype with race and age remained significant ($P = .020$).

DISCUSSION

Together, the results of the longitudinal and age-stratified analyses indicated that (1) the β_2-AR Arg_{16}/Arg_{16} genotype is associated with higher T-C and LDL-C levels in children and adolescents, but not in adults up to 38 years of age; and (2) the effects of combined β_2-AR Arg_{16}-Gly and β_3-AR Trp_{64}-Arg genotypes on T-C and LDL-C levels differ among race/sex groups, do not remain constant with age, and are not attributable to changes in BMI with age. LDL-C profiles by β_2/β_3-AR genotype group differed between males and females in both blacks and whites, most markedly in black females, who showed consistent differences by genotype across all ages. After the age of 8 years, black females had the highest mean BMI levels of any race/sex group in our sample; however, in both multilevel and age-stratified analyses, β_2/β_3-AR genotype effects on LDL-C persisted after adjustment for BMI.

Whenever apparent differences in metabolic parameters among groups are found, it is necessary to consider whether a biologically plausible basis for such differences may exist. Several previous studies have reported different effects of β_2-AR or β_3-AR genotypes in males and females. Effects of the β_3-AR Arg_{64} allele on obesity, weight gain, and risk of type 2 diabetes may be observable both in Trp_{64}/Arg_{64} heterozygotes and Arg_{64}/Arg_{64} homozygotes in women, but only in Arg_{64}/Arg_{64} homozygotes in men.[32] In a Spanish population, the Arg_{64} allele was associated with higher cholesterol levels only in men; it was also associated with higher BMI in men, but was associated with higher BMI in women only in those homozygous for a *HindIII* restriction site polymorphism of lipoprotein lipase.[16] In a Japanese-American sample, the Arg_{64} allele was associated with increased upper body obesity in men but not women.[17]

That the existence of differences among race/sex groups in longitudinal lipid profiles is both biologically plausible and could be related to activity of the β_2- and β_3-adrenergic receptors in adipose tissue is suggested by several lines of evidence. Males and females as young as 5 to 7 years of age may show differences in body fat mass.[33] Differences between males and females in visceral and subcutaneous fat distribution develop with age and persist into adulthood.[34-36] In women, increases in plasma T-C, LDL-C, and triglyceride levels with age may be strongly related to increases in visceral adipose tissue.[37] Body composition may also differ by race or ethnic group; the same BMI value may be associated with different body fat percentages and distribution patterns in blacks and whites.[38-42] Adrenergic regulation of lipolysis differs among adipose tissue depots in different regions of the body, and may be affected by such factors as age, sex, and obesity. Adrenergic stimulation of lipolysis may be greater in abdominal than in gluteal subcutaneous adipose tissue in adults,[43,44] but not in prepubertal children.[45] In subcutaneous adipose tissue, obese women showed a greater increase in lipolysis in response to adrenergic stimulation than did men.[46] However, visceral adipose tissue showed higher β_3-AR and lower β_2-AR sensitivity in males, with increased adrenergic-stimulated lipolysis.[47] Thus, differences in adrenergic-stimulated lipolysis in different adipose tissue deposits, together with the differences in adipose tissue distribution patterns between African Americans and whites, between males and females, and among children, adolescents, and adults could provide a mechanism leading to the differences among race, sex, and age groups in the associations of β-AR genotypes with serum lipids we observed in our sample.

Our results for the β_2-AR Arg_{16}-Gly polymorphism differ from those reported for a French-Canadian sample, in which this polymorphism was associated with significant differences in T-C and, in men only, LDL-C, with the highest mean levels in both cases occurring in Gly_{16}/Gly_{16} homozygotes.[14] In our sample, Gly_{16}/Gly_{16} homozygotes had the lowest mean levels of both lipids overall. Also in contrast to our findings, no significant interactions between the β_2-AR Arg_{16}-Gly and β_3-AR Trp_{64}-Arg polymorphisms were associated with lipid levels in the Quebec Family Study.[14] However, the association between the Gly_{16} allele and serum lipids may change with age. We observed significant associations of the β_2-AR Arg_{16}-Gly polymorphism with T-C levels only in those under 19 years of age (Table 3). In the oldest age group (34 to 38 years), mean T-C and LDL-C levels were higher in Gly_{16} carriers than in Arg_{16} homozygotes, in line with the findings in the Quebec Family Study, although the difference did not reach statistical significance. Age-related changes in β_2-AR Arg_{16}-Gly genotype effects on lipids potentially could affect interactions between the β_2-AR Arg_{16}-Gly and β_3-AR Trp_{64}-Arg polymorphisms. Thus, the very different age distributions in our sample and the French-Canadian sample may at least partly account for the contrasting results: the French-Canadian sample was much older, with a mean age of ~42.6 years[14]; in our sample, the maximum age at any examination was 38 years, and only 10% of observations were in those over 30 years of age.

The β_2-AR Arg_{16}/Arg_{16} genotype was associated with higher T-C and LDL-C levels only in children and adolescents, suggesting that changes in genotype effect with age could be related to the hormonal changes that occur with the onset of puberty. Lipid profiles by β_2/β_3-AR genotype showed no consistent patterns of change with age in the different race/sex groups, however, so factors that could contribute to age-related differences in combined β_2/β_3-AR genotype effects among race/sex groups are less readily apparent. Precise measures of body fat composition would be needed to determine whether age-related changes in the effects of β_2- or β_2/β_3-AR genotypes on serum lipids could be related to changes in patterns of body fat distribution with age that may differ among race/sex groups.

Further study will be needed to verify and characterize the association of β_2-AR and combined β_2/β_3-AR genotypes with age-related changes in serum lipid profiles. The genetic basis of many complex diseases is likely to involve closely related genes that can interact with one another or with other factors to affect disease-related metabolic parameters. The β_2- and β_3-adrenergic receptors, closely related genes with partially overlapping functions, may illustrate such interactions in the context of lipid metabolism, in which the existence of age-related changes is well established. Better understanding the factors which contribute to age-related changes in serum lipids will increase our understanding of the pathogenesis of atherosclerotic disease and may eventually suggest ways to intervene in the disease process at an early stage.

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