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APOA1/C3/A4 gene cluster variability and lipid levels in Brazilian children

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Abstract

Genetic studies have suggested that polymorphisms of genes coding for apolipoproteins are significant determinants of serum lipoprotein and lipid levels in adults. However, only a few studies have investigated the association of these polymorphisms in children. Therefore, in the present investigation we studied the distribution of *APOA1* -75 G>A, +83 C>T, *APOC3* -482 C>T, -455 T>C and 3238 C>G, and *APOA4* Q360H and T347S polymorphisms and their influence on plasma lipoprotein levels in children from a Brazilian northeastern admixed population. The seven polymorphic sites were genotyped in 414 children aged 5 to 15 years (mean 8.9 ± 2.9). The genotypes of the seven polymorphic sites were assessed by PCR-RFLP methods. The frequencies of the less common alleles were, in general, intermediate among parental populations, as expected. Strong linkage disequilibrium was detected between polymorphisms at the *APOA1*, *APOC3* and *APOA4* loci in this admixed population sample. Overall the genotype effects seen in adults were weaker or absent in children. The *APOC3*/-455 and *APOA4* T347S variants showed significant effects on HDL cholesterol in girls (P = 0.033 and P = 0.016, respectively). Significantly higher plasma total (P = 0.003) and LDL cholesterol (P = 0.004) levels were observed in boys who were carriers of the 3238G allele at the *APOC3*/3238 C>G site. These results disclosed an overall absence of associations between these polymorphisms and lipids in children. This finding is not unexpected because expression of the effect of these polymorphisms might depend on the interaction with environmental variables both internal and external to the individual.

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The genes coding for apolipoproteins A-I (apoA-I protein; *APOA1* gene), C-III (*APOC3*) and A-IV (*APOA4*) lie within a 17-kb DNA segment on the long arm of chromosome 11 (1). apoA-I is synthesized primarily in the liver and to a lesser extent in the small intestine. It is the major apolipoprotein component of high-density lipoproteins (HDL) and plays a key role in reverse cholesterol transport from peripheral tissues to the liver. It is also the obligatory activator of lecithin-cholesterol acyltransferase (2). The liver and intestine also synthesize apoC-III, although the precise function of apoC-III is not fully understood. There is increasing evidence associating this apolipoprotein with the ca-

tabolism of triglyceride-rich lipoproteins (TGRL). Animal studies have shown that apoC-III acts as an inhibitor of the lipoprotein lipase-mediated hydrolysis of TGRL (3). The precise physiological role of apoA-IV is also not completely understood, but there is evidence suggesting that the primary role of apoA-IV is in intestinal lipid absorption (4). Several functions for apoA-IV have been proposed, including activation of lecithin-cholesterol acyltransferase, modulation of lipoprotein lipase activity, regulation of cholesterol ester transfer between HDL and LDLs, and facilitation of cholesterol efflux from peripheral cells. More recently, *in vivo* experiments have provided evidence that this protein may be involved in inhibition of food intake following the ingestion of fat, thus modulating body weight gain (4).

Several polymorphic sites have been identified in the *APOA1-C3-A4* gene cluster, and have been associated with lipid levels (5-7), and coronary artery disease (8) with conflicting results (9). Genetic factors are considered to be important determinants of plasma lipoprotein in adults; however, the role of genetics in determining plasma lipoproteins in children and adolescents is not clear. Moreover, except for few sporadic studies, the distribution of apolipoprotein polymorphisms has been mainly studied in European and European-derived populations. It is well known that differences among populations in the relative frequency of susceptibility genotypes or environmental exposure will contribute to differences in the utility of a genotype for predicting a trait within a particular population. Our objective, therefore, was to test for associations between polymorphisms of the *APOA1-C3-A4* gene cluster and lipid levels in a sample of Brazilian children of mixed ethnicity free of adult diseases, taking no medications, and socioeconomically homogeneous.

The investigation conformed with the principles outlined in the Declaration of Helsinki and was approved by the Hospital Ethics Committee. Parents provided written informed consent and probands provided verbal consent to participate.

The study population resulted from an intense process of admixture since the XVI century among Europeans, Africans, and Amerindians (10). The sample consisted of 414 healthy children aged 5 to 15 years (mean 8.9 ± 2.9) selected at a pediatric hospital (Instituto Materno-Infantil de Pernambuco) in Recife, the capital of the Brazilian Northeastern State of Pernambuco. All children as well as their parents were born in the State of Pernambuco. A questionnaire was completed during an interview with the parents, which included details on medical history, drug intake, lifestyle variables such as physical activity, and demographic data. Exclusion criteria were secondary hyperlipidemia due to renal, liver or thyroid disease, diabetes and a parental history of diabetes or coronary artery disease.

The weight and height of subjects were measured in the morning after a 12-h fast. Height was measured to the nearest centimeter using a rigid stadiometer and weight was measured to the nearest 0.1 kg using a calibrated electronic scale. Body mass index (BMI, kg/m^2) was calculated.

Two blood samples were collected after 12 h of fasting. One sample was used to measure total serum cholesterol (TC), HDL cholesterol, triglycerides (TRI), and glucose concentrations using standard enzymatic methods (Roche Diagnostics, Basel, Switzerland) and an automated Hitachi spectrophotometer (Tokyo, Japan). LDL cholesterol was calculated by the Friedwald formula [TC - (TRI/5 + HDL)]. These analyses were performed on the same day of blood collection.

The second sample was frozen and sent to Porto Alegre for genotyping. Genomic DNA was isolated by standard procedures. The genotyping protocols for the seven polymorphisms investigated in this study have been described. These include *APOA1* -75 G>A, +83 C>T (5), *APOC3* -482 C>T, -455 T>C and 3238 C>G (6,7), and *APOA4*

Q360H and T347S (11).

Allele frequencies were estimated by gene counting. Allele distribution and Hardy-Weinberg equilibrium were tested by χ^2 tests. Haplotypes were derived using the Multiple Locus Haplotype Analysis program (12). Linkage disequilibrium between the sites was estimated using the Arlequin version 2.000 software (13). All continuous variables, except triglycerides, were normally distributed. All lipid values were analyzed separately by gender, and adjusted for age and BMI by linear regression analyses. Differences in mean lipid levels between genotypes were compared by the Student *t*-test, one-way ANOVA or Kruskal-Wallis non-parametric test. Statistical analyses were performed using the SPSS 8.0 statistical package.

The distribution of genotype and allele frequencies for the seven polymorphic sites is shown in Table 1. The observed genotype frequency distributions did not show statistically significant differences compared to those expected under Hardy-Weinberg equilibrium.

The frequency of the A allele of the -75 G>A polymorphism in this sample (18%) was within the same range as those reported for European or European-derived populations (11-22%) (5,14). This frequency was somewhat lower in Africans (10%) (9). The distribution of the +83 C>T variant has been much less investigated than the -75 site. Thus far, the frequency of the less common allele has been reported to be about 4% in European or European-derived populations (5). However, the frequency of this allele was 10-fold higher (40%) in an African population (9). As expected for an admixed sample, this frequency was intermediate (11%) between Africans and Europeans. It should be pointed out, however, that Amerindians have not been tested for these markers. Strong linkage disequilibrium between the two *APOA1* sites was detected (D' = 1.00, χ^2 = 22.6, P = 0.00001). The most common haplotype was -75G/+83C (71%).

A high degree of ethnic variation has been described for the *APOC3* -455 T>C, -482 C>T, and 3238 C>G polymorphisms (15,16). The less common allele frequencies observed in the present study (Table 1) were similar to those reported for Hispanic children (15) which are higher than those described for Europeans (7,15). Due to strong linkage disequilibrium, four haplotypes (-482C/-455T/3238C; -482T/-455C/3238C; 482T/-455C/3238G; 482C/-455C/3238C) accounted for 97% of the variability related to these *APOC3* polymorphisms.

As observed in other populations, the *APOA4* 360Q/Q genotype was the most frequent (94%). The 360H allele frequency (3%) observed in this admixed sample (Table 1) is very similar to that observed in African-

Table 1. Allele and genotype frequencies of the *APOA1, APOC3* and *APOA4* gene polymorphisms in children from a mixed Brazilian Northeastern population.

	Genotype		Allele
	N	%	
APOA1			
-75 G>A			A (18.1%)
G/G	277	66.9	
G/A	124	30.0	
A/A	13	3.1	
+83 C>T			T (11.1%)
C/C	329	79.5	
C/T	78	18.8	
T/T	7	1.7	
APOC3			
-482 C>T			T (42.3%)
C/C	145	35.0	
C/T	188	45.4	
T/T	81	19.6	
-455 T>C			C (46.9%)
T/T	127	30.7	
T/C	185	44.7	
C/C	102	24.6	
3238 C>G			S2 (13.8%)
C/C	312	75.4	
C/G	90	21.7	
G/G	12	2.9	
APOA4			
Q360H			H (2.8%)
Q/Q	391	94.4	
Q/H	23	5.6	
T347S			S (14.1%)
T/T	309	74.6	
T/S	94	22.6	
S/S	11	2.8	

Americans (17). This allele is found almost exclusively in European or European-derived populations and its presence in non-Caucasian populations has been proposed as a marker of European admixture (17). The 347S allele, which displays a much wider distribution worldwide, was detected in about 14% of the subjects investigated (Table 1). This frequency is similar to that observed in US Hispanics (13%), and somewhat lower than that described for Afro-Americans (8). Linkage disequilibrium was observed between the two polymorphisms ($\chi^2 = 8.82$; P = 0.003; D' = 0.986, P < 0.00001). Three haplotypes were found: 360Q-347T, 360Q-347S, and 360H-347T with frequencies of 84.5, 12.7, and 2.8%, respectively.

As lipid differences between genders are well known to exist in children and adolescents (18), all association analyses were performed separately for boys and girls. Data on lipid variables according to gender and genotypes are presented in Table 2. No significant associations were observed between serum lipids and the two polymorphisms in the *APOA1* gene. The *APOC3*/-455 variant showed a significant effect on HDL cholesterol in girls (P = 0.033); the -455C carriers had higher HDL cholesterol than the TT genotype. Significantly higher plasma total (P = 0.003) and LDL cholesterol (P = 0.004) levels were observed in boys who were carriers of the G allele at the *APOC3*/3238 C>G. Girls with the *APOA4*/347S allele had significantly higher HDL cholesterol levels than girls with the other genotypes (P = 0.016). The other polymorphic sites investigated were not associated with lipid levels in this sample. All of these associations were no longer statistically significant at the haplotype level (data not shown).

Xu et al. (14) reported an association between the *APOA1* -75 polymorphism and plasma levels of total cholesterol, LDL cholesterol, apoB and apoA-I in Italian boys. Individuals with the A allele had higher mean levels of these lipid traits. Wang et al. (5) did not detect an effect for this *APOA1* polymorphism, but observed an influence of the +83 site variant on HDL cholesterol levels in Australian children of European descent. The absence of association of the two *APOA1* polymorphisms with lipid levels in Brazilian children observed in the present study is consistent with the findings of Kamboh et al. (9) in Africans. Since the estimated contribution of African genes (44%) to this population is high (10), it is possible that the genetic background of the population studied might modulate the effect of *APOA1* on lipid levels.

The effect of the *APOC3* polymorphisms on plasma lipids was modest in the children and neither polymorphism was associated with triglyceride levels, the most common finding in several studies with adults (6,7). Since no significant associations were observed at the haplotype level, the genotype results should be viewed with caution, in view of the number of tests performed and the moderate sample size. No statistically significant differences in the distribution of -455 and -482 alleles in relation to lipids were reported in Italian (19) or Hispanic children (15). However, Shoulders et al. (19) described a modest effect of the G allele at the 3238 C>G site on triglyceride levels in Italian schoolchildren. An increased frequency of the -455C allele associated with elevated triglyceride and reduced HDL cholesterol levels was also reported in a young group of native Canadians (16).

No significant influence of the 360H allele on lipid levels or BMI was observed in the present young admixed Brazilian sample. However, the number of carriers of the H allele at the Q360H locus was too small to detect small differences in lipid levels in this sample. Mean HDL cholesterol values were significantly higher in girls, who were carriers of at least one 347S allele, but this association was no longer significant at the haplotype level (data not shown).

Although we adjusted all lipid levels for

Table 2. Association of *APOA1, APOC3* and *APOA4* genotypes and lipid levels in Brazilian children.

	N	TC (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	TG+ (mg/dl)
APOA1 -75 G>A					
Boys					
G/G	156	153.31 ± 25.36	93.60 ± 21.88	43.50 ± 8.89	81.46 ± 36.69
A Carriers	65	156.26 ± 23.56	95.67 ± 20.37	44.86 ± 9.11	77.68 ± 23.80
P		0.424	0.513	0.303	0.802
Girls					
G/G	121	159.17 ± 26.46	96.35 ± 23.38	45.70 ± 10.17	85.61 ± 31.58
A Carriers	72	160.81 ± 30.40	98.78 ± 26.66	43.75 ± 8.74	91.35 ± 47.28
P		0.693	0.508	0.177	0.906
APOA1 +83 C>T					
Boys					
C/C	168	153.53 ± 24.33	93.49 ± 20.58	43.88 ± 9.09	80.55 ± 35.04
T Carriers	53	156.24 ± 26.47	96.51 ± 23.96	43.97 ± 8.60	79.72 ± 27.90
P		0.490	0.372	0.950	0.928
Girls					
C/C	161	159.86 ± 27.67	97.92 ± 24.49	44.45 ± 9.51	87.70 ± 39.53
T Carriers	32	159.40 ± 29.62	93.89 ± 25.37	47.60 ± 10.24	88.03 ± 31.07
P		0.933	0.398	0.093	0.699
APOC3 -482 C>T					
Boys					
C/C	77	152.53 ± 24.55	92.70 ± 21.42	43.76 ± 8.71	80.60 ± 33.06
T Carriers	144	155.06 ± 25.02	95.02 ± 21.45	43.98 ± 9.12	80.22 ± 33.71
P		0.470	0.444	0.862	0.954
Girls					
C/C	68	156.12 ± 26.97	95.66 ± 22.59	43.22 ± 9.35	85.07 ± 38.32
T Carriers	125	161.77 ± 28.34	98.12 ± 25.69	45.92 ± 9.76	89.21 ± 38.18
P		0.180	0.507	0.064	0.656
APOC3 -455 T>C					
Boys					
T/T	64	151.17 ± 23.06	91.40 ± 19.87	43.94 ± 8.72	80.11 ± 34.98
C Carriers	157	155.40 ± 25.48	35.36 ± 21.98	43.88 ± 9.08	80.45 ± 32.87
P		0.252	0.214	0.964	0.713
Girls					
T/T	63	155.76 ± 26.68	95.49 ± 21.69	42.84 ± 9.02	85.81 ± 39.60
C Carriers	130	161.73 ± 28.41	98.11 ± 25.95	46.00 ± 9.85	88.69 ± 37.60
P		0.164	0.957++	0.033	0.789
APOC3 3238 C>G					
Boys					
C/C	168	151.45 ± 23.39	91.77 ± 20.34	43.89 ± 8.48	78.99 ± 32.62
G Carriers	53	162.85 ± 27.38	101.95 ± 23.08	43.92 ± 10.41	84.64 ± 35.78
P		0.003	0.002	0.981	0.251
Girls					
C/C	144	159.72 ± 28.30	96.85 ± 24.45	44.98 ± 9.76	88.76 ± 38.65
G Carriers	49	160.55 ± 27.07	98.43 ± 25.32	44.94 ± 9.53	84.80 ± 37.01
P		0.824	0.699	0.981	0.672
APOA4 T347S					
Boys					
T/T	163	155.43 ± 25.93	95.20 ± 22.58	43.84 ± 8.83	82.39 ± 35.27
S Carriers	58	150.67 ± 21.25	91.43 ± 17.65	44.08 ± 9.37	74.62 ± 26.99
P		0.210	0.250	0.860	0.222
Girls					
T/T	146	158.50 ± 28.50	96.77 ± 25.02	44.02 ± 9.12	88.12 ± 39.55
S Carriers	47	163.76 ± 25.95	98.77 ± 23.50	47.91 ± 10.82	86.62 ± 33.98
P		0.263	0.628	0.016	0.762
APOA4 Q360H					
Boys					
Q/Q	209	154.51 ± 25.18	94.51 ± 21.62	43.90 ± 8.98	80.44 ± 33.67
Q/H	12	148.48 ± 17.33	88.99 ± 17.43	43.80 ± 8.86	78.75 ± 29.75
P		0.415	0.386	0.970	0.815
Girls					
Q/Q	182	159.98 ± 27.86	97.71 ± 24.87	44.91 ± 9.48	86.68 ± 36.44
Q/H	11	156.44 ± 30.26	89.71 ± 19.14	46.03 ± 13.11	105.45 ± 59.95
P		0.684	0.296	0.708	0.389++

Data are reported as means ± SD. Statistical analysis performed with ANOVA or ++Kruskal-Wallis test. +Unadjusted means, but test performed on log-transformed values.

age and BMI, we did not ascertain the children's Tanner stage (which measures pubertal status); therefore, we cannot exclude an allelic effect of interaction between sexual maturity and lipid levels. However, it has been shown (20) that BMI closely correlates with the Tanner scale classification of the subjects, minimizing any interference by puberty with lipoprotein levels. Mean BMI was 17.52 ± 3.34 and 17.76 ± 3.40 in boys and girls, respectively. No association between *APOA1/C3/A4* gene cluster polymorphisms and BMI was detected in this sample (data not shown). Therefore, if some effect of pubertal status on the associations observed was present, it should have been small.

In this highly heterogeneous population, all comparisons were performed among genotypes that are more robust for the effects of population stratification than in case-control studies. The children studied here have been exposed to much fewer environmental factors than adults, providing fewer opportunities for interactions between these polymorphisms and the environment. As pointed out by Sing et al. (21), the effects of few genes will be invariant across populations and environmental strata; most will be context-dependent, as defined by gender, age, and other measures of exposure to environmental factors both internal and external to the individual. Therefore, if the contribution of these polymorphisms to the modulation of lipid levels depends on these exposures, the overall absence of associations with lipids in children was not an unexpected finding. The observed associations could also be spurious due to multiple statistical comparisons. Although the possibility of type II error could not be excluded, this study may be considered preliminary and suggests candidate genes for future association studies in this population. Therefore, before reaching definitive conclusions, the results of this study need to be confirmed in larger samples from the study population.

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