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Apolipoprotein E Polymorphism in Vietnamese Children and Its Relationship to Plasma Lipid and Lipoprotein Levels

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We investigated the frequency of apolipoprotein E (apoE) polymorphism and the effect of apoE polymorphism on plasma lipid and lipoprotein levels under different nutritional statuses in Vietnamese children living in urban and rural areas. Three hundred and forty-eight girls (aged 7 to 9 years) were randomly selected from urban and rural areas in southern Vietnam. Their apoE genotypes were analyzed by an Invader assay, and the plasma lipid and lipoprotein levels were determined by enzymatic methods using fasting blood samples. Dietary intake and anthropometry of children were also measured. The frequency of the allele ϵ2 and ϵ4 of the Vietnamese girls was 0.09 and 0.12, respectively. The levels of low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) of the allele ϵ2 carriers were significantly lower than those of the allele ϵ3 carriers ($P < .0001$) in both the urban and rural groups. In contrast, the allele ϵ4 carriers tended to show a higher LDL-C level than the allele ϵ3 carriers, especially in subjects with a higher fat intake in urban area. The allele ϵ2 carriers had the same high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) level as the allele ϵ3 carriers, but the allele ϵ4 carriers with a higher fat intake living in urban areas had lower HDL-C and higher TG level than allele ϵ3 carriers. In conclusion, our findings showed that the LDL-C lowering effect of allele ϵ2 was independent of the nutritional status, while allele ϵ4 tended to lower HDL-C and increase the LDL-C level in a high-fat intake population. Therefore, the plasma lipid profiles of apoE ϵ4 carriers may be a risk factor of atherogenesis in Vietnamese, who tend to have a westernized eating habit.

APOLIPOPROTEIN E (apoE) is known to be an important factor for regulating the metabolism of lipoproteins. The apoE gene is polymorphic, and it has 3 common alleles ϵ2, ϵ3, and ϵ4 that code for 3 major isoforms, thus resulting in 6 common genotypes.[1-3] ApoE facilitates the binding of triglyceride (TG)-rich lipoprotein remnants to receptors that determine their clearance. The isoforms vary in their receptor-binding activity, therefore individuals with allele ϵ2 have higher TG levels, while individuals with ϵ4 tend to have higher levels of low-density lipoprotein cholesterol (LDL-C) than those with ϵ2 and ϵ3. The increase is due, in part, to a more efficient absorption of dietary cholesterol and perhaps a down-regulation of LDL receptor.[2] As a result, the apoE gene is an important candidate for cardiovascular disease.[1-5]

In general, the nutritional status of the Vietnamese is still inadequate, thus resulting in the high prevalence of malnutrition and low plasma lipids profiles.[6] However, a gap has recently developed in the dietary patterns between inhabitants in urban areas and those in rural areas. A more westernized dietary pattern has been observed in big cities, leading to a higher energy and fat intake and a higher prevalence of obesity in urban areas.[7-9] As a result, the morbidity and mortality rate of cardiovascular diseases have been increasing, especially in big cities.[10] The atherosclerosis process is considered to start early in life and has been causally linked to elevated levels of plasma lipids and lipoproteins since childhood. However, the role of genetic risk factors for atherosclerosis in the Vietnamese has not yet been investigated. The aim of this study was to identify the frequency of apoE isoforms in Vietnamese children and the effects of apoE polymorphism on the plasma lipids in individuals with different nutritional statuses.

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MATERIALS AND METHODS

Study Subjects

The study subjects included 348 schoolgirls, ranging in age from 7 to 9 years-old, who were randomly selected from 2 schools in the center of Hochiminh city (urban area) and 3 schools in the suburban areas in Hochiminh city (rural area). The characteristics of the subjects were described in details elsewhere.[9] All of them are Kinh, which is the major ethnic group in Vietnam. The research protocol was approved by the Research and Ethical Review Board of the Ho Chi Minh Child Nutrition Center. Informed consent to participate in the study was given by the parents of all subjects.

Laboratory Analyses

Clinical examinations consisted of physical examinations for the children and fasting blood samples were obtained in EDTA-coated vacutainer tubes. The samples were stored at −80°C until analysis. Total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), and TG levels were all determined by enzymatic methods (Determiner L, Kyowa Medex, Tokyo, Japan).

Anthropometric Measurement and Dietary Intake

Anthropometric measurements including weight, height, and left midarm circumference (LMAC) were examined. The body mass index (BMI) was calculated from the baseline measurements of body weight and height (kg/m^2). Body fat was measured using a bioelectric impedance method on a body fat analyzer (TBF-511; Tanita, Tokyo, Japan).

The parents were interviewed regarding the dietary intake of their children for 3 consecutive days. The nutritionists performed these interviews. The dietary intake was then calculated according to a Vietnamese food composition table.[11]

ApoE Genotyping

DNA was isolated (QIA amp Blood Kit; QIAGEN, Valencia, CA) from 2 mL whole blood of the subjects. We used the Invader assay (Third Wave Technologies, Madison, WI) to determine apoE polymorphism of the children. The Invader assay combines structure-specific cleavage enzymes and a universal fluorescent resonance energy transfer (FRET) system. The primary probes and Invader oligonucleotide for each mutation were designed using Invader Creator software to have theoretic annealing temperatures of 63°C and 77°C, respectively, using the nearest neighbor algorithm based on the final probe and target levels. FRET detection plates for Invader assay were provided by Third Wave Technologies, in which almost all the generic components of an Invader assay (Cleavase enzyme [Third Wave Technologies], FRET probes, 3-(N-morpholino)propanesulfonic acid [MOPS] buffer, and polyethylene glycol) were dried together. The biplex format of the Invader assay enables the simultaneous detection of 2 DNA sequences in a single well. In brief, the probe/Invader/$MgCl_2$ mixture was prepared by combining 3 μL of primary probe/Invader mix and 5 μL of 22.5 mmol/L $MgCl_2$ mixture per reaction. The primary probe/Invader mixture contained 3.5 μmol/L wild primary probe and 3.5 μmol/L mutant primary probe, 0.35 μmol/L Invader oligonucleotide, and a 10 mmol/L MOPS buffer. Eight microliters of primary probe/Invader /$MgCl_2$ mixture was added into a 96-well plate. Seven microliter of 5 fmol/L synthetic target oligonucleotides, 10 μg/mL yeast tRNA (no target blank), and genomic DNA samples ($\geq$15ng/μL), which were denatured by incubation at 95°C for 10 minutes, were added. After 15μL of mineral oil (Sigma, St Louis, MO) were overlaid into all reaction wells, the plate was incubated isothermally at 63°C for 4 hours in the DNA thermalcycler (PTC-200; MJ Research, Watertown, MA) and then kept at 4°C until fluorescence measurements was observed. The fluorescent intensities were measured by the fluorescence microtiter plate reader (Cytoflour 4000; Applied Biosystems, Foster City, CA) with excitation, 485 nm/20 nm (wavelength/bandwidth) and emission, 530 nm/25 nm for FAM dye; excitation, 560 nm/20 nm and emission, 620 nm/40 nm for RED dye. The genotyping was analyzed by calculating the ratios of the net counts with a wild primary probe to net counts with the mutant primary probe.[12]

Table 1. Frequencies of ApoE Genotypes in Vietnamese Children

No.	ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4
	Genotypes					
Total (348)	*3*	*46*	*10*	*219*	*62*	*8*
Rural (193)	1	28	7	111	41	5
Urban (155)	2	18	3	108	21	3
	Frequency (%)					
Total	*0.9*	*13.2*	*2.9*	*63.0*	*17.8*	*2.3*
Rural	0.5	14.4	3.6	57.7	21.1	2.6
Urban	1.3	11.6	1.9	69.7	13.5	1.9

	ε2	ε3	ε4
Total	*0.09*	*0.79*	*0.12*
Rural	0.10	0.75	0.15
Urban	0.08	0.82	0.10

NOTE. The frequency of apoE genotypes in rural and urban was not significantly different.

Statistical Analyses

Because very few subjects had ε2/ε2 (n = 3) and ε4/ε4 (n = 8), the subjects were divided into 3 groups as follows: ε2 carriers (ε2/ε2 and ε3/ε2), ε3 carriers (ε3/ε3), and ε4 carriers (ε4/ε3 and ε4/ε4) to analyze. The subjects with ε4/ε2 (n = 10) could not be assigned to any of the groups and were therefore excluded from the analyses. The apoE allele frequencies were estimated by gene counting. The Hardy-Weinberg equilibrium of the apoE polymorphism was assessed by the χ^2 test. Any significant differences in the frequencies of apoE polymorphism between the rural and urban group were also determined by the χ^2 test. The significance of the differences in the plasma lipid levels among apoE genotypes was evaluated by analysis of variance (ANOVA) and post hoc comparisons (Scheffe test). Statistical procedures were performed using the StatView statistical program 5.0 (SAS, Cary, NC). A *P* value of less than .05 was considered to be statistically significant.

RESULTS

Frequency of ApoE Genotypes in Vietnamese Children

The distribution of allele frequencies of apoE genotypes in both the rural and urban groups is shown in Table 1. The distribution of apoE polymorphism was in Hardy-Weinberg equilibrium. The majority of participants were ε3/ε3 (57.7% and 69.7%) and the frequency of allele ε3 was 0.75 and 0.82 in the rural and urban groups, respectively. The frequencies of allele ε2 in the rural and the urban groups were 0.10 and 0.08, while that of allele ε4 was 0.15 and 0.10, respectively. However, there was no significant difference in apoE distribution between the 2 groups.

Nutritional Statuses and ApoE Genotypes

The dietary intake and anthropometric parameters among apoE polymorphism are shown in Table 2. The quantity of energy intake and the percentage of energy intake from fat and protein were both significantly higher in the urban group than those in the rural group, in general. Nevertheless, the dietary

Table 2. Nutritional Status of Vietnamese Children According to ApoE Genotypes

	ε2 Carriers	ε3 Carriers	ε4 Carriers
Rural (n = 186)	*(29)*	*(111)*	*(46)*
Energy intake (kcal)	1199 ± 301†	1288 ± 313†	1285 ± 293†
Protein intake E (%)*	14.8 ± 2.2	14.4 ± 2.0†	14.0 ± 2.1†
Fat intake E (%)*	16.8 ± 5.4†	15.1 ± 5.5†	15.9 ± 5.9†
Carbohydrate intake E (%)*	68.4 ± 6.0†	70.5 ± 6.3†	70.1 ± 6.9†
BMI (kg/m^2)	13.7 ± 1.3†	14.0 ± 1.2†	14.0 ± 1.0†
Body fat (%)	10.4 ± 3.8‡	10.7 ± 2.8†	10.8 ± 2.6‡
LMAC (cm)	15.6 ± 1.5†	16.1 ± 1.3†	16.0 ± 1.2†
Urban (n = 152)	*(20)*	*(108)*	*(24)*
Energy intake (kcal)	1895 ± 368	1777 ± 365	1724 ± 257
Protein intake E (%)*	15.3 ± 2.8	15.9 ± 2.1	16.5 ± 2.3
Fat intake E (%)*	24.1 ± 4.5	22.2 ± 4.5	22.2 ± 3.3
Carbohydrate intake E (%)*	57.6 ± 6.5	59.7 ± 4.9	59.5 ± 4.9
BMI (kg/m^2)	16.2 ± 2.4	15.6 ± 2.8	16.0 ± 2.1
Body fat (%)	14.8 ± 5.1	14.8 ± 5.6	16.3 ± 7.1
LMAC (cm)	18.3 ± 2.5	17.9 ± 2.3	18.2 ± 2.0

NOTE. Values are mean ± SD. No significant difference was observed between the various genotypic groups.
*E(%) percentage of total energy intake.
†$P < .0001$ (as compared with the same genotypic subgroup in urban).
‡$P < .01$ (as compared with the same genotypic subgroup in urban).

intake was not different among genotypic subgroups in both rural and urban groups. In addition, BMI, the percentage of body fat, and LMAC were higher in the urban group than in the rural group, but no difference was observed between the various genotypic groups.

Relationship Between ApoE Genotypes and Plasma Lipid and Lipoprotein Levels

The plasma lipid and lipoprotein levels among genotypic groups from both the rural and urban areas are shown in Table 3. In comparison to ε3 carriers, ε2 carriers showed a plasma TC level, which decreased by 0.54 mmol/L (14.4%; $P < .0001$) and by 0.66 mmol/L (14.4%; $P < .0001$) and a plasma LDL-C level, which decreased by 0.53 mmol/L (24.5%; $P < .0001$) and 0.66 mmol/L (24.8%; $P < .0001$) in the rural and urban groups, respectively. Compared with ε3 carriers, no variations in the TC level in ε4 carriers were observed in either group, but the LDL-C levels of ε4 carriers had inconsiderably increased by 0.08 mmol/L (3.7%; P = not significant [NS]) and 0.18 mmol/L (6.8%; P = NS) in the rural and the urban groups, respectively. In addition, the HDL-C level of allele ε4 carriers in the urban group was significantly lower than that of the ε3 allele carriers (0.16 mmol/L or 12.4%; $P < .05$). The TG level of allele ε2 showed no difference from that of the ε3 carriers in both groups, while allele ε4 increased TG as little as 0.08 mmol/L (6.8%; P = NS) and 0.09 mmol/L (7.3%; P = NS) in the rural and urban groups, respectively. When the dietary pattern changed, the TG level of the ε2 carriers increased by 0.17mmol/L (15.6%; P = NS) (from 1.09 in rural group to 1.26 mmol/L in urban group), while the TG level of ε3 and ε4 carriers increased by only 0.04 mmol/L (3.4%; P = NS) and 0.05 mmol/L (4%; P = NS), respectively.

Table 3. Plasma Lipid and Lipoprotein Levels of Vietnamese Children According to ApoE Genotypes

Plasma Lipid and Lipoprotein	ε2 Carriers	ε3 Carriers	ε4 Carriers
Total (N = 338)	*49*	*219*	*70*
TC	3.50 ± 0.6*§	4.16 ± 0.8	4.10 ± 0.7
LDL-C	1.78 ± 0.5*§	2.41 ± 0.6	2.45 ± 0.6
HDL-C	1.13 ± 0.3§	1.14 ± 0.3	1.00† ± 0.3
TG	1.16 ± 0.5	1.19 ± 0.7	1.27 ± 0.61
Rural (n = 186)	*29*	*111*	*46*
TC	3.22 ± 0.6*§	3.76 ± 0.7	3.83 ± 0.6
LDL-C	1.63 ± 0.4*§	2.16 ± 0.5	2.24 ± 0.5
HDL-C	1.02 ± 0.3	0.99 ± 0.2	0.93 ± 0.2
TG	1.09 ± 0.5	1.18 ± 0.5	1.26 ± 0.6
Urban (n = 152)	*20*	*108*	*24*
TC	3.91 ± 0.5*§	4.57 ± 0.9	4.61 ± 0.7
LDL-C	2.00 ± 0.5*§	2.66 ± 0.6	2.84 ± 0.6
HDL-C	1.27 ± 0.4	1.29 ± 0.3	1.13‡ ± 0.2
TG	1.26 ± 0.5	1.22 ± 0.8	1.31 ± 0.6

NOTE. Values are mean ± SD.
*$P < .0001$ (as compared with ε3 carriers).
†$P < .01$ (as compared with ε3 carriers).
‡$P < .05$ (as compared with ε3 carriers).
§$P < .001$ (as compared with ε4 carriers).

DISCUSSION

The process of atherosclerosis is assumed to begin from early in life and has been linked to elevated plasma lipid and lipoprotein levels. Both nutritional and genetic factors are considered to be risk factors for atherosclerosis. In addition, the role of the apoE polymorphism as a risk factor for coronary artery disease (CAD) has been well established in the Caucasian population. Our study is the first report on the apoE allele frequencies and the effect of apoE polymorphism on plasma lipid levels in 2 groups with different nutritional profiles in the Vietnamese. Our finding was compatible to those of other studies that reported the frequency of the ε3 allele to be the highest. Compared with other Asian populations, the frequency of allele ϵ2 in the Vietnamese was nearly the same as that observed in Chinese,[13] but a little lower than that seen in Malaysians[14] and higher than those of Japanese[14] and the Koreans.[15] The allele ϵ4 frequency was also nearly the same as that seen in Malaysians,[14] but lower than that of Javanese[16] and higher than that observed in Chinese,[13] Japanese,[14] or Koreans.[15] Furthermore, the frequency of allele ϵ2 and ϵ4 was nearly equal to that in Caucasians (the frequency of allele ϵ2 and ϵ4 was 0.09 and 0.12 in the Vietnamese *v* 0.108 and 0.121 in the French,[17,18] respectively).

The present study was performed in prepubertal girls within a narrow age range (7 to 9 years) from urban and rural areas with different quantity of fat intake, which thus allowed us to investigate the effects of the apoE genotypes and nutritional factors on the plasma lipid and lipoprotein levels in children by minimizing any possible confounding factors (ie, age, gender). Up to now, the effects of apoE alleles on the serum lipids were mostly performed in populations with a normal and high-fat intake.[2-5] This study examined a role of the apoE genotypes in modulating the plasma lipid and lipoprotein levels in a representative group with a habitual diet consisting of an extremely low-fat intake. The nutritional intake was nearly the same among the apoE polymorphism groups, but allele ϵ2 carriers had significantly lower plasma TC and LDL-C levels than the allele ϵ3 and ϵ4 carriers in both urban and rural group with different quantity of dietary intake. Although the Vietnamese had an extreme low-fat intake (15% and 22% of total energy intake), the magnitude of the LDL-C lowering effect of allele ϵ2 compared with allele ϵ3 (0.53 and 0.66 mmol/L in the rural and urban groups) was similar to that in young, free-living Finnish children with 44.1% energy intake from fat,[19] while it was higher than that in Caucasian American girls (9 to 10 years) (0.25 mmol/L), and that in African American girls (9 to 10 years) (0.20 mmol/L).[20] Other studies have shown an effect of allele ϵ2 on the serum total cholesterol and LDL-C level regardless of the ethnic background on the average cholesterol level of the population.[2,21] Our results clearly proved that allele ϵ2 still affects the plasma TC and LDL-C level even in a population with a low-fat energy intake and low levels of plasma LDL-C. The ϵ2 allele is thought to have less affinity for the receptor that governs the uptake of very–low-density lipoprotein (VLDL) remnants, thus the slower hepatic clearance of dietary fat in ϵ2 allele carriers could upregulate LDL receptors, which in turn, decrease the plasma LDL-C level.[22]

Based on the current nutritional status, the LDL-C increasing effect of allele ϵ4 Vietnamese was only slight. However, it was stronger in the urban group with a higher fat intake (0.18 mmol/L) than in the rural group with a low-fat intake (0.08 mmol/L). The ϵ4 allele was thought to have a higher affinity for the receptor governing the uptake of VLDL remnants, and thereby downregulating the LDL receptors and increasing the plasma LDL-C level.[23-25] Moreover, it was shown that allele ϵ4 increases the absorption of fat intake.[23] The effect of allele ϵ4 on the cholesterol level differs significantly regarding the nutritional habit (eg, fat intake) and allele ϵ4 carriers may acquire a high cholesterol level only after a high-fat intake.[26] This phenomenon also explains the high prevalence of allele ϵ4 and the low rate of cardiovascular diseases in Africans, while the high prevalence of allele ϵ4 in the Finish population appears to cause the high rate of such diseases.[18]

No variations in the plasma HDL-C level in allele ϵ2 carriers were observed. In contrast, subjects with ϵ4 allele had significantly lower HDL-C levels (0.15mmol/L) than in the ϵ3 allele subjects in the urban group. The association of allele ϵ4 carriers with a lower HDL-C level in our findings was also compatible with other studies.[2-5]

Allele ϵ2 carriers had almost unchanged TG levels compared with those of ϵ3 in both the rural and urban groups. At present, the proposed effect of allele ϵ2 on TG metabolism may be absent in the Vietnamese whose fat intake is substantially lower than the Western counterparts. However, due to the increase in the fat and energy intake (in the urban group in comparison to the rural group), the TG level of allele ϵ2 carriers increased by 0.17 mmol/L, while that of allele ϵ3 and ϵ4 increased only by 0.04 mmol/L. Under the present nutrition status, the allele ϵ4 subjects had insignificantly higher TG levels than the ϵ3 subjects. The increase of TG level in allele ϵ4 carriers was reported to be due to a delayed catabolism relative to production in this subset.[26]

In summary, our findings demonstrated that the frequencies of the allele ϵ2 and ϵ4 in Vietnamese children were homogenous to other Asians populations. The LDL-C lowering effect of allele ϵ2 was independent of the dietary intake. A trend of higher LDL-C and lower HDL-C levels in allele ϵ4 carriers was found in children with a high-fat intake. Due to an increasingly westernized diet in Vietnam, allele ϵ4 carriers may thus show a profile of the high plasma lipids and lipoproteins, which leads to a higher risk of atherogenesis. More research on the relationship between apoE polymorphism and atherosclerosis in Vietnamese is thus called for.

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