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Relation of Apolipoprotein E Polymorphism to Serum Lipid Profiles in Obese Children

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Summary. **Obesity is a risk factor for future atherosclerosis in children. Apolipoprotein B (apoB) is the principal apolipoprotein of low-density lipoprotein, which is one of causative factors of atherosclerosis, and apolipoprotein E ε4 allele (apoE4) allele has been considered to be a risk factor for coronary artery diseases. We investigated the influence of apoE polymorphism on lipid and apolipoprotein levels in obese children. Thirty-seven Japanese obese children with a mean age of 10.1 years and 134 normal children were included in the study. Serum lipids and apolipoproteins were measured by an autoanalyzer, and apoE phenotyping was performed by isoelectric focusing of delipidated serum samples on polyacrylamide gel followed by immunoblotting. The Stat-View statistical computer package was used for data processing. Obese children with at least one apoE4 allele had significantly higher apoB levels and apoB/apoA-I ratios than E3 homozygotes ($p=0.0080$ and $p=0.0104$, respectively). Frequencies of apoE phenotypes and alleles in the obese children were not different from those in normal children. Obese children with at least one apoE4 allele had higher apoB levels and apoB/apoA-I ratios. It is suggested that obese children with at least one apoE4 allele are at a higher risk for future atherosclerosis than those without it. ApoE phenotyping may be a useful clinical test for identifying obese children who require more careful management.**

Key words—obese children, apolipoprotein E, apoE 4 allele, apolipoprotein B.

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INTRODUCTION

Obesity is a well-known risk factor for atherosclerosis or obesity-associated metabolic disorders in adults[1-5]. As obese children are at an increased risk of becoming obese adults[6], early identification of individuals at higher risk is important for the implementation of preventive measures. The major issues that confront the clinician in relation to childhood obesity are identifying the children at risk, deciding the goal and focus of therapy, and determining how to maintain weight loss[7]. In that context, it is important to assess the role of genetic and nongenetic factors in childhood obesity as well as in susceptibility to dietary variations[8].

Apolipoprotein E (apoE) is a plasma protein involved in cholesterol transport and metabolism. Three common alleles, ε 2, ε 3, and ε 4, genetically determine the six apoE phenotypes E 2/2, E 2/3, E 2/4, E 3/3, E 4/3, and E 4/4. Recent papers have demonstrated that the apoE ε 4 allele (apoE 4 allele) is associated with an increased risk for atherosclerotic vascular diseases both in adults[9-12] and in children[13].

ApoB is the principal apolipoprotein of low-density lipoprotein (LDL), which is one of causative factors of atherosclerosis. The combination of apoE 4 allele and higher serum apoB levels has been reported to further increase the risk for atherosclerosis[14]. Reiewing the literature shows that there has been only one report on the impact of apoE 4 allele on serum lipid profiles in obese children. According to this report, serum LDL cholesterol (LDLC) levels are elevated in the obese children with appoE 4 allele compared with

those without it[15]. These results seem to be very important in the management of obese children; however, no studies have confirmed these findings, and, in the study, the apoE 4 frequency was not compared with that in normal control children for a control. With this background in mind, we have undertaken the present study to investigate the influence of apoE polymorphism on lipid and apolipoprotein levels in obese children.

RESEARCH METHODS AND PROCEDURES

Subjects

Thirty-seven children (25 boys and 12 girls) under follow-up for obesity at Shonai Hospital, Tsuruoka City, Yamagata Prefecture and Niigata University Hospital, Niigata City, were included in this study. At the time of data collection, the mean (SD) age was 10.7 years (12.5 years), ranging from 4.1 to 15.3 years, and the mean body mass index (BMI) was 26.2±3.1. Informed consent was obtained from the patients' parents. To obtain apoE phenotype frequencies in normal children, 143 school children, aged from 13 to 14 years, were enrolled in this study after informed consent was obtained from their parents.

Methods

Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDLC), LDL cholesterol (LDLC) and apoA-I, apoB and apoE levels were measured on an autoanalyzer. Apolipoprotein phenotypes were determined by the isoelectric focusing of delipidated serum samples on polyacrylamide gel followed by immunoblotting using a double antibody technique. The test kits were purchased from Johoh Co. Ltd., Tokyo, Japan. Apo ε 2, ε 3, and ε 4 allele frequencies were calculated from the obtained apoE phenotypes by the gene-counting method.

Data analysis

All analyses were performed with StatView - version 4.5 - statistics computer software. Results concerning categorical variables are presented as counts and frequencies; for continuous ones (lipids and apolipoproteins), means and SD values are given. Fisher's exact test was used to compare the apoE allele frequencies in various subgroups. Analysis of variance (ANOVA) was used to compare mean values of lipids in patients with different apoE phenotypes and to compare them, in turn, in patients with at least one E 2 or E 4 isoform or E 3 homozygotes versus the means in the rest of the patients. A p value below 0.05 was considered to indicate a significant difference.

RESULTS

ApoE phenotype distribution and isoform frequencies

The apoE phenotype of the 143 normal control subjects was E 3/3 (100 subjects, percentage 70.0%); E 4/3 (29, 20.4%); E 3/2 (10, 7.0%); and E 4/2 and E 5/3 (4, 2.8%), and was in agreement with other normative data published in Japan[19]. The first three types comprised about 97% of the normal subjects. The apoE allele of the 143 control subjects was ε 3 (241, 84.3%), ε 4 (31, 10.8%), and ε 2 (12, 4.2%), ε 5 and ε 7 (2, 0.7%). In the obese children, apoE phenotype distribution and isoform frequencies were as follows: phenotype E 3/3 (27 pts, 72.9%), E 4/3 (7 pts, 18.9%), E 3/2 (2 pts, 5.4%), and E4/4 (1 pt, 2.7%); allele ε 3 (85.1%), ε 4 (12.2%), and ε 2 (2.7%). These values were not statistically different from those in normal children.

Apolipoprotein E phenotypes and serum lipids and apolipoproteins (Table 1)

Means and standard deviation of serum TC, TG, HDLC and LDLC levels in our patients are presented in Table 1. Although all values, with the exception of TG, were within normal limits, 40.5% had TC > 200 mg/dl, 41.2% had TG >150 mg/dl, and 45.5% had LDLC >120 mg/dl among the obese children. Atherogenic index (AI), calculated as the (TC-HDLC)/HDLC ratio, was >3.0 in 38.9% of the patients, with the highest mean levels recorded in children with the E 4/3 phenotype E.

Children with apo E 4/3 phenotype had higher mean TC and TG levels and AI than those in the E3/3 group, but these differences did not reach a statistical significance. Carriers of at least one E 4 allele had higher mean serum LDLC levels and similar mean HDLC levels. Odds ratio analysis revealed that children with at least one E 4 isoform had a 3.16 times higher risk of hypercholesterolemia (33.3% of the hypercholesterolemic children had at least one E 4 isoform, versus only 13.6% of the normocholesterolemic ones) and a 2.26 times higher risk of hypertriglyceridemia.

Table 1. Mean serum lipid values by apoE polymorphism

ApoE phenotypes	TC N	TC Mean (SD)	TG N	TG Mean (SD)	HDLC N	HDLC Mean (SD)	LDLC N	LDLC Mean (SD)	AI N	AI Mean (SD)
E 3/2	2	4.85 (1.15)	2	1.85 (0.11)	2	1.42 (0.48)	2	2.58 (1.68)	2	2.8 (2.1)
E 3/3	27	4.85 (0.75)	25	1.64 (0.85)	27	1.37 (0.29)	25	2.75 (0.67)	27	2.7 (0.9)
E 4/3	7	5.48 (0.91)	6	2.44 (1.21)	6	1.49 (0.48)	5	2.97 (0.80)	6	3.3 (2.4)
E 4/4	1	3.72 -	1	0.96 -	1	1.22 -	1	2.07 -	1	2.1 -
Alleles										
E 2	2	4.85 (1.15)	2	1.85 (0.11)	2	1.42 (0.48)	2	2.58 (1.68)	1	2.8 (2.1)
E 3	27	4.85 (0.75)	25	1.64 (0.85)	27	1.37 (0.29)	25	2.75 (0.67)	27	2.7 (0.9)
E 4	8	5.27 (1.04)	7	2.23 (1.24)	7	1.46 (0.45)	6	3.04 (0.85)	7	3.2 (2.2)
Total	37	4.94 (0.83)	34	1.77 (0.93)	36	1.39 (0.32)	33	2.82 (0.80)	36	2.8 (1.3)

E 2, carriers of at least one E 2 allele; E 3, children with E 3/3 phenotype; E 4, carriers of at least one E 4 allele; AI, atherogenic index. All lipids are expressed in SI units (mmol/l).

Table 2. Mean serum apolipoprotein levels by apoE polymorphism

ApoE phenotypes	ApoA-I N	ApoA-I Mean (SD)	ApoB N	ApoB Mean (SD)	ApoB/ApoA-I N	ApoB/ApoA-I Mean (SD)	ApoE N	ApoE Mean (SD)
E 3/2	1	125.0 -	1	123.0 -	1	1.0 -	1	6.9 -
E 3/3	22	130.1 (19.2)	22	92.8 (22.5)*	22	0.7 (0.2)§	22	6.1 (1.7)
E 4/3	6	131.8 (24.2)	6	124.8 (30.5)*	6	1.0 (0.5)§	6	7.3 (2.8)
E 4/4	1	109.0 -	1	85.0 -	1	0.7 -	1	3.0 -
Alleles								
E 2	1	125.0	1	123.0 -	1	1.0 -	1	6.9 -
E 3	22	130.1 (19.2)	22	92.8 (22.5)**	22	0.7 (0.2)§§	22	6.1 (1.7)
E 4	7	128.6 (23.7)	7	117.4 (34.0)**	7	1.0 (0.4)§§	7	6.7 (3.0)
Total	30	129.6 (19.6)	30	99.5 (27.1)	30	0.8 (0.3)	30	6.2 (2.0)

E 2, carriers of at least one E 2 allele; E 3, children with E 3/3 phenotype; E 4, carriers of at least one E 4 allele;*, The E 4/3 vs E 3/3 difference in apoB levels - p=0.0080;** The E 4 vs E 3 difference in apoB levels - p=0.0344;§, The E 4/3 vs E 3/3 difference in apoB/apoA-I ratio - p=0.0104;§§ The E 4 vs E 3 difference in apoB/apoA-I ratio - p=0.0198. Apolipoprotein serum levels are expressed in mg/dl.

Serum apolipoproteins by apoE polymorphism (Table 2)

Statistically significant differences were noted in apoB levels among the different phenotype groups (p=0.0306). The largest difference was observed between patients with apo E 4/3 and E 3/3 phenotypes: 124.8±30.5 vs 92.8±22.5 mg/dl (p=0.0080). Similar significant differences existed between children with at least one E 4 isoform and E 3 homozygotes and between those with at least one E 4 isoform and those lacking it (i. e. E 3/2 and E 3/3 phenotypes taken together) - p=0.0344 and p=0.0442 respectively. Differences were also noted with respect to the apoB/apoA 1 ratio: children with E 4/3 phenotype had higher values of this ratio, 1.0±0.5 vs 0.7±0.2, p=0.0104, than children with E 3/3 phenotype; carriers of at least one E 4 isoform had higher apoB/apoA-I than E 3 homozygotes (p=0.0198) and in comparison with those lacking this isoform (p=0.0250). Serum apoA-I and E levels did not seem to be influenced by apoE phenotypes.

DISCUSSION

Among diverse ethnic groups, frequency of the apoE 4 allele varies but stays within a very small range: 0.061[16], 0.070[17], 0.083 and 0.085[18], 0.108(our data), 0.110[19], or 0.114[20]. This means that only 6-11% of children in most countries, whether obese or not, have the apoE 4 allele. As is known, when comparing some obtained data with controls, the smaller the number of subjects, the harder it is for the difference to reach a statistical significance. This probably is one reason why this kind of study on apoE 4 allele has not been reported in obese children.

In our study, although the number of subjects was not so large, statistical analyses revealed that obese children with at least one apoE ε 4 allele had higher apoB levels and apoB/apolA-I ratio. ApoE phenotype distribution and isoform frequency in our obese patients did not significantly differ from that in a control group. Although this seems quite natural, there has been no previous report on this issue in obese children.

The observed elevation of apoB (a major apolipoprotein of LDL) levels in our obese children with at least one apoE 4 seems to support the results of a previous study by Parlier et al. who first disclosed that obese children with the apoE 4 allele are more likely to have a LDLC elevation than those without it[15]. With regard to non-obese children[21] and obese adults[22,23], the presence of the apoE 4 allele has been shown to be associated with higher serum TC, LDLC, and apoB levels. Taken altogether, possesing the apoE 4 allele seems to be a genetically determined risk factor for future atherosclerosis in obese children.

Various mechanisms have been proposed to explain the influence of apoE polymorphism on lipid and apolipoprotein serum levels. Persons who carry the ε 4 allele (apoE 4 positive) absorb cholesterol from the intestine more effectively than those who are apoE 4 negative[24]. ApoE further influences serum cholesterol concentrations by acting as a ligand for LDL receptors and possibly other receptors and by being involved in the conversion of intermediate-density lipoprotein[25]. ApoE 4-containing lipid particles effectively bind to LDL receptors, down-regulate their expression, and subsequently raise the plasma LDL concentration[26]. In a more recent study, Woollett et al. demonstrated that apoE-containing lipoproteins can act as potent competitive inhibitors of hepatic LDLC transport and so can significantly increase steady-state plasma LDLC levels[27]. Taking these proposed mechanisms and our results into account, we speculate that, in some obese children, well-known hypercholesterolemia might be genetically determined through the effective binding of apoE 4-containing lipid particles to LDL receptors and would expose the apoE 4 positive obese children to a higher risk for developing future atherosclerosis through the higher apoB, implying higher LDLC levels.

Viewing recent papers, the combination of apoE 4 allele and higher apoB levels has been reported to further increase the risk for atherosclerosis. According to a report by Sanghera et al.[28], in both African American and White 9-10-year-old girls, the apoE 4 allele is significantly associated with higher levels of LDLC and apoB, and the apoE 2 allele with lower mean levels of LDLC and apoB. When adult patients (survivors of stroke or a transient ischaemic attack) with at least one apoE 4 allele and one X 2 allele of apoB are combined and compared with those without either of them (E 2 E 3 or E 3 E 3 and X 1 X 1), the interaction of common apoB and apoE alleles increases the risk of atherosclerosis in cervical arteries[14]. Furthermore, the effect of diets seems to be associated with apoE polymorphism. According to a report by Dreon et al., reduction of LDLC in a low-fat diet is greater for apoE 4/3, 4/4 than apoE 3/3, and reduced dietary fat lowers levels of large, buoyant LDL particles by an apoE-dependent mechanism[29]. Since obesity among older children is an increasingly important predictor of adult obesity[6], it is important to identify those obese children who are at a higher risk for future atherosclerosis.

In summary, obese children with at least one apoE ε 4 allele had higher apoB levels and apoB/apoA-I ratio. Based on our results, it is suggested that obese children with at least one apoE ε 4 allele are at a higher risk for future atherosclerosis than those without it. ApoE phenotyping may be one useful clinical test for identifying obese children who require more careful management. Since our study remains preliminary, further studies from other institutions are needed to confirm our results.

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