No Association found between the Ala54Thr Polymorphism of FABP2 Gene and Obesity and Obesity with Dyslipidemia in Japanese Schoolchildren

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To investigate whether the Ala54Thr polymorphism of the fatty acid binding protein 2 gene is associated with obesity and obesity with dyslipidemia in Japanese schoolchildren, we analyzed 370 children with morbid obesity and 463 control children of normal weight. The allele frequencies did not differ significantly between the control group and the morbidly obese group. The odds ratio (95% confidence interval CI) in obesity of the The54 allele was 1.0 (0.9-1.3). There were no significant differences in obesity index and metabolic characteristics between the two groups. The odds ratio (95% CI) in dyslipidemia of the Thr54 allele was 1.1 (0.8-1.4) in the morbidly obese group. Our data suggested that Ala54Thr polymorphism of the FABP2 gene is not a major contributing factor for obesity and obesity with dyslipidemia in Japanese children. J Atheroscler Thromb, 2001; 8: 80-83.

Key words: Fatty acid binding protein 2 gene, Obesity, Dyslipidemia, Schoolchildren

Introduction

Obesity is a well-known risk factor for dyslipidemia, type 2 diabetes mellitus (Type 2 DM), cardiovascular disease, and hypertension (1). Yamamoto et al. noted that about 50% of hypertriglyceridemic children are obese (2). Childhood obesity increases the risk of obesity in adulthood (3). Therefore, it is important to identify the causes of obesity and dyslipidemia in childhood.

The intestinal fatty acid binding protein 2 (FABP2) is expressed only in the columnar absorptive epithelial cells of the small intestine villus, and plays a role in the absorption and intracellular transport of dietary long-chain fatty acids (4, 5). Recently, a substitution of alanine (Ala) by threonine (Thr) at position 54 of the FABP2 gene was reported to be associated with insulin resistance and increased fat oxidation in vivo in the Pima Indians (6). In aboriginal Canadians, the presence of the threonine allele was shown to be associated with variations in body mass and triglyceride (TG) (7, 8). In Japanese, subjects homozygous for the threonine allele showed a greater accumulation of intra-abdominal fat (9). In this study, we investigated the relationship between the FABP2 gene polymorphism and obesity or obesity with dyslipidemia in Japanese schoolchildren.

Subjects

This study is based on school medical examinations in a rural town located ~50 km northeast of Tokyo. Three hundred and seventy Japanese schoolchildren (220 boys and 150 girls), aged 9 to 13 years, with a morbid obesity index level (≥ +50%) were selected as the morbid obesity group and 463 Japanese schoolchildren (246 boys and 217 girls), aged 9 to 15 years, with a normal obesity index level (≥ -20% and < +20%) were used as controls.

The obesity index [(real weight-standard weight)/standard weight × 100] was calculated using the standard
weight scale for Japanese children, which was proposed by the Ministry of Education, Culture, Sports, Science and Technology in Japan and determined according to age, gender, and stature (10).

Informed consent was obtained from the students, their parents, and the school principals prior to participation in this study.

**Methods**

Blood samples were obtained in the morning after an overnight fast. Total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-C), and alanine aminotransferase (ALT) were measured using an autoanalyzer (HITACHI7350). Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald’s formula (11). The atherosclerosis index (AI) was calculated according to the following formula: \(\frac{(TC - HDL-C)}{HDL-C}\). Dyslipidemia was defined as TC ≥ 5.17 mmol/L or TG ≥ 1.47 mmol/L or HDL-C ≤ 0.93 mmol/L or AI ≥ 3.0, which values were proposed by the Ministry of Health, Labor and Welfare in Japan (12).

The FABP2 genotype was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis as described by Baier et al. (6). The primers were as follows: upstream 5’-ACAGGT-GTTAATAGTGAAAAG-3’ and downstream 5’-TACC-CTGAGTTCAGTTCCGTC-3’. The PCRs were started with denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 52°C for 1 minute and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes. The amplified PCR products were digested with Hha I at 37°C overnight and were separated by electrophoresis through 4% agarose gel containing 2% agarose S and 2% nusieve GTG agarose. DNA was visualized by staining with ethidium bromide. Digestion of PCR products yielded fragments of the following size: 99 and 81 base pairs (bp) in Ala/Ala; 180, 99 and 81 bp in Ala/Thr; and 180 bp in Thr/Thr.

All data are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA), unpaired t-test, and chi-squared test were used to estimate the effects of each genotype on quantitative variables and qualitative variables. TG was logarithmically transformed before the analysis. Statistical analyses were performed using the StatView 5.0 software. Statistical significance was established at the p < 0.05 level.

**Results**

The allelic frequencies of Ala54 and Thr54 were 0.639 and 0.361, respectively, in control subjects. In morbidly obese subjects, the allelic frequencies of Ala54 and Thr54 were 0.626 and 0.374, respectively. The frequency did not differ significantly between the two groups (\(\chi^2 = 0.33\), \(p = 0.57\)). The odds ratio (95% CI) in obesity of the Thr54 allele was 1.0 (0.9-1.3).

Clinical and metabolic characteristics of the subjects according to the Ala54Thr genotype of the FABP2 gene are shown in Table 1. There were no significant differences in obesity index, TC, TG, HDL-C, LDL-C, AI, or ALT between the two groups. The frequency of dyslipidemia and the odds ratio for dyslipidemia according to the Ala54Thr genotype of the FABP2 gene are shown in Table 2. The frequencies of dyslipidemia of the control and morbidly obese groups were 106 (22.9%) and 186 (50.3%), respectively. The odds ratio (95% CI) in dyslipidemia of the Thr54 allele was 1.1 (0.8-1.4) in the morbidly obese group.

**Discussion**

Associations of the Ala54Thr polymorphism of the FABP2 gene with obesity or variation in serum lipid levels have already been investigated in previous studies, several of which found a significant association between the Ala54Thr polymorphism of the FABP2 gene and variation in body mass or TG (7-9, 13, 14). On the other hand, the

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**Table 1.** Clinical and metabolic characteristics according to Ala54Thr genotype of the FABP2 gene in control and morbidly obese groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n = 463)</th>
<th>Morbidly obese (n = 370)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ala/Ala (n = 196)</td>
<td>Ala/Thr (n = 200)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>11.9 ± 1.8</td>
<td>12.0 ± 1.8</td>
</tr>
<tr>
<td>Obesity index (%)</td>
<td>-0.3 ± 9.5</td>
<td>0.4 ± 9.3</td>
</tr>
<tr>
<td>Serum lipids (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>4.37 ± 0.61</td>
<td>4.38 ± 0.60</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.83 ± 0.39</td>
<td>0.88 ± 0.52</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.41 ± 0.29</td>
<td>1.44 ± 0.28</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>2.58 ± 0.53</td>
<td>2.54 ± 0.53</td>
</tr>
<tr>
<td>Atherosclerosis index</td>
<td>2.2 ± 0.7</td>
<td>2.1 ± 0.6</td>
</tr>
<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>12.0 ± 4.6</td>
<td>11.7 ± 4.1</td>
</tr>
</tbody>
</table>

Data are mean ± SD. *: ANOVA was used for comparisons among the genotypic groups.
polymorphism of the FABP2 gene was not associated with the insulin sensitivity, the risk of type 2 diabetes, severe obesity, and hyperinsulinemia (15-22). Thus, association of this polymorphism with obesity and dyslipidemia remains controversial. Few studies have been conducted on the effects of this polymorphism on obesity or dyslipidemia in children. Obesity and dyslipidemia are influenced by both environmental and genetic factors. Few Japanese schoolchildren have a habit of drinking and smoking at this age. They usually take exercise regularly at school. Thus, in children, the effects of environmental factors such as drinking, smoking, and exercise are probably much lower than in adults. This study was conducted to ascertain whether the Ala54Thr polymorphism of the FABP2 gene influences obesity and obesity with dyslipidemia in children.

Baier et al. showed that the threonine-containing protein of FABP2 had a two-fold greater affinity for long-chain fatty acids than that of the alanine-containing protein (6). Actually, Agren et al. reported that the threonine-encoding allele of the FABP2 gene was associated with increased postprandial lipemia, with an increased postprandial response of 14-18-carbon fatty acids (18, 23). These findings suggest that this polymorphism may play an important role in obesity and variation of serum lipid levels. In Japanese adults, other studies reported that the FABP2 genotype was not associated with obesity and type 2 DM (9, 21, 22, 24, 25). In the present study, to investigate the association between this polymorphism and serum lipid levels in morbidly obese children, serum lipid levels were evaluated in control and morbidly obese children, respectively. We found no association between this polymorphism and serum lipid levels in the control or in morbidly obese children. In aboriginal Canadians, Hegele et al. reported that the Ala54Thr polymorphism of the FABP2 gene was associated with variation in TG in young subjects (8). The difference between the previous results and our data may be due to ethnicity or to the small scale of the study population in aboriginal Canadians. In the present study, it was impossible to reveal the association between this polymorphism and postprandial lipemia or insulin resistance, because we could not measure postprandial TG, insulin levels, and glucose levels. Insulin resistance is a major risk factor for type 2 DM. Since other studies reported that genetic variation in the FABP2 gene influenced insulin resistance (9, 26), further studies are required to clarify the association between this polymorphism and insulin resistance.

In conclusion, our data suggested that the Ala54Thr polymorphism of the FABP2 gene is not a major contributing factor for obesity and obesity with dyslipidemia in Japanese children.

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References

Table 2. Frequencies and odds ratio for dyslipidemia according to Ala54Thr genotype of the FABP2 gene

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequencies</th>
<th>Odds ratio (95% CI)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Ala/Ala</td>
<td>Ala/Thr</td>
</tr>
<tr>
<td>Normal lipid level (n = 357)</td>
<td>147 (41.2%)</td>
<td>156 (43.7%)</td>
</tr>
<tr>
<td>Dyslipidemia (n = 106)</td>
<td>49 (46.2%)</td>
<td>44 (41.5%)</td>
</tr>
<tr>
<td>Normal lipid level (n = 184)</td>
<td>76 (41.3%)</td>
<td>81 (44.0%)</td>
</tr>
<tr>
<td>Dyslipidemia (n = 186)</td>
<td>73 (39.2%)</td>
<td>84 (45.2%)</td>
</tr>
</tbody>
</table>

Dyslipidemia was defined as TC ≥ 5.17 mmol/L or TG ≥ 1.47 mmol/L or HDL-C ≤ 1.03 mmol/L or AI ≥ 3.0.


