Variation of the Fatty Acid Binding Protein 2 Gene Is Not Associated With Obesity and Insulin Resistance in Japanese Subjects

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An alanine to threonine substitution at codon 54 of the fatty acid binding protein 2 (FABP2) gene has been associated with insulin resistance in Pima Indians and with obesity in aboriginal Canadians. We investigated whether this polymorphism contributes to obesity and insulin resistance in 258 Japanese subjects. Thirty-six subjects (13.9%) were homozygous for the Thr54 allele, 106 (41.1%) were heterozygous for the Ala54/Thr54 allele, and 116 (45.0%) were homozygous for the Ala54 allele. The frequency of the Thr54 allele was 0.34 and did not differ significantly between men and women. The incidence of non–insulin-dependent diabetes mellitus (NIDDM) was not different among the three genotypes. The variation at codon 54 of the FABP2 gene was not associated with obesity, hypertension, dyslipidemia, hyperuricemia, or hyperinsulinemia. These results suggest that the polymorphism at codon 54 of the FABP2 gene is not a major contributing factor to obesity and insulin resistance in Japanese subjects.

SINCE RANDLE et al[1] proposed the glucose–fatty acid cycle, free fatty acids (FFAs) have been thought to play an important role in the development of insulin resistance in obesity and non–insulin-dependent diabetes mellitus (NIDDM). Intestinal fatty acid binding protein (FABP), the product of the FABPi gene, is expressed only in the columnar absorptive epithelial cells of the small intestine villus[2] and plays a role in the absorption and intracellular transport of dietary long-chain fatty acids.[3] In Pima Indians, an alanine to threonine substitution at codon 54 (Ala54Thr) of the FABP2 gene was shown to be associated with increased fatty acid binding, increased fat oxidation, and insulin resistance.[4] In aboriginal Canadians, the presence of this variation was shown to be associated with obesity and an increase of the fasting plasma triglyceride concentration.[5] On the other hand, in Finnish nondiabetic and NIDDM subjects, the polymorphism of the FABP2 gene did not influence insulin sensitivity.[6]

The polymorphism of the FABP2 gene appears to effect obesity and insulin resistance differently among races, and in the Japanese, the effect of insulin resistance is controversial.[7,8] Thus, we investigated the association of genetic variation in the FABP2 gene with obesity, dyslipidemia, hypertension, insulin resistance, and NIDDM in Japanese subjects.

SUBJECTS AND METHODS

Three hundred eleven Japanese subjects aged 30 to 71 years (mean ± SD, 52.2 ± 7.1) were recruited from individuals who underwent general health examinations at Kanazawa Municipal Hospital from December 1995 to April 1997. Since 53 subjects used prescribed medicine for diabetes mellitus (DM), hyperlipidemia, hypertension, or hyperuricemia, we excluded them, and the remaining 258 subjects aged 30 to 68 years (51.5 ± 7.1) were investigated. There were a total of 214 men and 44 women. All subjects underwent a 75-g oral glucose tolerance test after an overnight fast, and 205 were classified as having normal glucose tolerance (NGT), 38 impaired glucose tolerance (IGT), and 15 NIDDM by World Health Organization criteria. Informed consent was obtained from all subjects.

Blood samples were taken in the morning after an overnight fast. The body mass index (BMI), waist to hip ratio, systolic and diastolic blood pressure, serum total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, uric acid, FFA, fasting plasma glucose, and fasting serum insulin were investigated in all subjects. The waist to hip ratio was determined as the ratio of the waist circumference at the umbilical level to the maximum hip circumference. Serum total cholesterol and triglyceride levels were determined by enzymatic methods, and the HDL cholesterol level was measured by the heparin-manganese method. The serum uric acid level was measured by the uricase-peroxidase method. Serum FFAs were assayed by an enzymatic method based on the activity of acyl-coenzyme A synthetase with a NEFA-HR kit (Wako Pure Chemical, Osaka, Japan). Plasma glucose was determined by the glucose oxidase method. The serum insulin level was measured by radioimmunoassay with a Phadeseph Insulin RIA kit (Pharmacia Diagnostics, Uppsala, Sweden). Insulin resistance was assessed by homeostasis model assessment (HOMA).[9]

Genomic DNA was extracted from peripheral blood leukocytes. To detect Ala54Thr polymorphism, we performed a polymerase chain reaction (PCR) with primers previously described.[4] The Ala for Thr substitution at codon 54 was verified by digestion of the PCR product of 180 base pairs (bp) using the restriction enzyme *Hha*I and by electrophoresis on a 3% agarose gel. PCR products containing an intact *Hha*I site are cleaved into 99- and 81-bp fragments, and Ala54Thr substitution abolished the restriction site.

Statistical Analysis

All data are expressed as the mean ± SD. Statistical analysis was performed using the StatView II statistical package (Abacus Concepts, Berkeley, CA). Differences between group means were tested by Bonferroni *t* test after justification by one-way ANOVA. The χ^2 test was used to compare frequencies. A P level $< .05$ indicated statistical significance.

RESULTS

Among 258 subjects, 36 (13.9%) were homozygous for the Thr54 allele, 106 (41.1%) were heterozygous for the Ala54/Thr54 allele, and 116 (45.0%) were homozygous for the Ala54 allele. The frequency of the Thr54 allele was 0.34 in all subjects, 0.35 in men, and 0.33 in women. The frequency did not differ significantly between men and women. Thr54 allele frequency in NIDDM, IGT, and NGT was 0.43, 0.30, and 0.35,

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respectively. Although the frequency of the Thr54 allele was higher in NIDDM versus IGT or NGT, the differences were not significant ($P = .19$ for NIDDM *v* IGT and $P = .34$ for NIDDM*v* NGT) (Table 1).

The gender distribution, age, BMI, blood pressure, serum lipids, uric acid, FFA, fasting plasma glucose, fasting serum insulin, and insulin resistance as measured by HOMA were not different between subjects carrying the Thr/Thr or Ala/Thr genotype and those carrying the Ala/Ala genotype (Table 2). Similarly, gender differences were not observed for clinical and metabolic characteristics between the three genotypes (data not shown).

DISCUSSION

In this study of Japanese subjects, the variation at codon 54 of the FABP2 gene was not associated with obesity, hypertension, dyslipidemia, hyperuricemia, insulin resistance, or NIDDM. The frequency of the Thr54 allele in the Japanese was 0.34, which is the same as the previously reported rate[7,8] and slightly higher than the frequency reported in Pima Indians (0.29)[4] and aboriginal Canadians (0.14).[5] This finding suggests racial differences in the frequency of the Thr54 FABP2 allele.

Using linkage analyses, the FABP2 gene was shown to be linked with insulin resistance in Pima Indians[10] and Mexican-Americans,[11] but not in Finnish and UK Caucasians.[12] On the other hand, no linkage was found between the FABP2 gene and NIDDM in Caucasians,[13] Mexican-Americans,[14] French,[15] and Japanese.[16]

In Caco-2 cells, the human colonic carcinoma cell line, cells expressing Thr54 intestinal FABP were reported to transport long-chain fatty acids and secrete triglycerides to a greater degree than cells expressing Ala54 intestinal FABP,[17] and in Pima Indians, Thr-coding protein was reported to increase fatty acid binding. Thus, in Pima Indians, the Thr54 FABP2 genotype was associated with fasting lipid oxidation rates and insulin resistance but not with body weight, percent body fat, and DM.[4] In aboriginal Canadians, the presence of this variation was associated with significant increases in the BMI, percent body fat, and fasting plasma triglyceride concentration, but it was not associated with the presence of NIDDM and insulin resistance.[5] In Finns, this polymorphism did not influence the severity of obesity, insulin sensitivity, fasting lipid oxidation rates, fatty acid composition of serum lipids, and NIDDM.[6,18,19] Although these studies suggest that the FABP2 gene is not a major contributor to the pathogenesis of NIDDM, the results are controversial with regard to obesity and insulin resistance, and racial differences may exist.

Table 1. Sex and Glucose Tolerance Pattern According to Codon 54 Polymorphism of the FABP2 Gene

Parameter	Thr/Thr No.	Thr/Thr %	Ala/Thr No.	Ala/Thr %	Ala/Ala No.	Ala/Ala %	Allele Frequency of Thr54
Sex							
Men	30	14.0	89	41.6	95	44.4	0.35
Women	6	13.6	17	38.7	21	47.7	0.33
Glucose tolerance pattern							
NIDDM	4	26.7	5	33.3	6	40.0	0.43
IGT	4	10.5	15	39.5	19	50.0	0.30
NGT	28	13.7	86	41.9	91	44.4	0.35
Total	36	13.9	106	41.1	116	45.0	0.34

Table 2. Clinical and Metabolic Characteristics According to Polymorphism of the FABP2 Gene

Characteristic	Thr/Thr	Ala/Thr	Ala/Ala
No. of subjects	36	106	116
Sex (men/women)	30/6	89/17	95/21
Age (yr)	50.9 ± 8.1	51.6 ± 6.8	51.6 ± 7.2
BMI (kg/m^2)	22.9 ± 3.1	23.8 ± 2.9	23.4 ± 2.4
Wait to hip ratio	0.87 ± 0.07	0.89 ± 0.06	0.88 ± 0.06
Blood pressure (mm Hg)			
Systolic	114 ± 14	116 ± 16	114 ± 13
Diastolic	71 ± 10	73 ± 11	73 ± 10
Serum lipids (mmol/L)			
Total cholesterol	5.58 ± 0.86	5.31 ± 0.87	5.49 ± 1.05
Triglyceride	1.53 ± 0.61	1.54 ± 0.62	1.65 ± 0.66
HDL cholesterol	1.41 ± 0.29	1.38 ± 0.35	1.42 ± 0.42
Serum uric acid (μmol/L)	353.5 ± 72.9	343.0 ± 81.3	349.7 ± 69.9
Serum FFA (mEq/L)	0.50 ± 0.20	0.47 ± 0.20	0.47 ± 0.17
Fasting plasma glucose (mmol/L)	5.50 ± 1.04	5.46 ± 0.72	5.49 ± 0.89
Fasting serum insulin (pmol/L)	36.0 ± 17.0	39.7 ± 17.4	34.9 ± 13.7
HOMA insulin resistance	1.47 ± 0.70	1.50 ± 0.69	1.42 ± 0.58

NOTE. Data are the mean ± SD. All differences between subjects carrying the Thr/Thr or Ala/Thr genotype and those carrying the Ala/Ala genotype were not significant.

In 395 Japanese men, Yamada et al[7] reported that the Thr54 allele was associated with insulin resistance and intraabdominal fat thickness but not with NIDDM, BMI, blood pressure, or serum lipid levels. In 118 Japanese men and 15 women, Mochizuki et al[8] reported that FABP2 polymorphism did not show any association with the BMI, percent body fat, fasting and 2-hour serum insulin, serum lipid levels, or glucose tolerance patterns. Our results are consistent with the results for all parameters from Mochizuki et al and with the results from Yamada et al except insulin resistance. While Yamada et al[7] reported that subjects who were homozygous for the Thr54 allele showed higher fasting serum insulin and homozygous and heterozygous subjects showed greater insulin resistance as measured by HOMA, we found no differences in fasting serum insulin and insulin resistance between genotypes in our study. This discrepancy is unclear, but it may be partly attributable to differences in the study populations, as their subjects were selected at random from people attending a health screening, whereas we excluded subjects who were prescribed medicine for DM, hyperlipidemia, hypertension, or hyperuricemia. Another reason is that since the BMI of our subjects and subjects reported by Mochizuki et al is lower than the BMI of subjects reported by Yamada et al, the difference in the degree of obesity might produce different results. We cannot entirely rule out the possibility of a relationship between the Thr54 FABP2 genotype and insulin resistance in moderately fat Japanese, but FABP2 polymorphism is not associated with insulin resistance in Japanese subjects of normal body weight.

In conclusion, a variation at codon 54 of the FABP2 gene is not a major contributing factor to obesity, dyslipidemia, hypertension, insulin resistance, or NIDDM in the Japanese.

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