Effects of Intestinal Fatty Acid-Binding Protein Gene Ala54Thr Polymorphism and β_3-Adrenergic Receptor Gene Trp64Arg Polymorphism on Insulin Resistance and Fasting Plasma Glucose in Young to Older Japanese Men

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The present study was performed to investigate the effects of the intestinal fatty acid-binding protein (*FABP2*) gene Ala54Thr polymorphism and the β_3-adrenergic receptor (*β3AR*) gene Trp64Arg polymorphism on body mass index (BMI), blood pressure, heart rate, glucose and lipid profiles, and serum leptin level in 196 young men aged 21 to 39 years, 186 older normoglycemic men (fasting plasma glucose [FPG] < 110 mg/dL) aged 40 to 65 years, and 122 older hyperglycemic men, including 77 type 2 diabetic patients. Genomic DNA was extracted from the peripheral blood, and these polymorphisms were assessed by the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method. In the older groups, the *β3AR* Arg64-allele frequency tended to be lower and the *FABP2* Thr/Thr54 genotype frequency tended to be higher in hyperglycemic patients, although these differences did not reach statistical significance. Also, there were no significant differences in the genotype or allele frequency of either variant between the 27 hyperlipidemic and 204 normolipidemic subjects. In the younger group, there were no significant differences in any of the parameters measured between the genotypes of *β3AR* or *FABP2*. In the older normoglycemic subjects, heart rate was significantly lower ($P = .037$) in *β3AR* Arg64-positive subjects, and FPG was significantly higher in subjects with the *FABP2* Thr/Thr genotype than the other genotypes (99.8 ± 5.6 *v* 96.5 ± 5.6 mg/dL, $P = .010$). In the older hyperglycemic group, the *β3AR* Arg64-positive group had significantly lower high-density lipoprotein (HDL) cholesterol and free fatty acid (FFA) levels ($P = .024$ and $P = .043$, respectively). There were no synergistic effects of these 2 variants on any measured parameter, but only the *FABP2* Thr/Thr genotype was related to a higher FPG in the older normoglycemic men. In conclusion, no major difference was associated with the *β3AR* Trp64Arg or *FABP2* Ala54Thr polymorphism in terms of type 2 diabetes or hyperlipidemia in young to older Japanese men. However, a slight but significant increase in FPG was observed in older Japanese men with the *FABP2* Thr/Thr genotype.

OBESITY, hypertension, diabetes mellitus, and dyslipidemia are important predisposing factors for the development of atherosclerotic macrovascular disease on the basis of insulin resistance.[1-3] In addition, an increased intake of dietary fat can worsen insulin resistance. Since the average dietary fat intake in Japanese is increasing, the incidence of type 2 diabetes mellitus has also increased. Furthermore, insulin sensitivity has been shown to decrease in experimental animals fed a high-fat diet.[4-8] These findings suggest that a high-fat diet and hyperlipidemia, including high serum free fatty acid (FFA) level, could be associated with the pathogenesis of type 2 diabetes.[9,10]

The intestinal fatty acid-binding protein (FABP2) plays an important role in the absorption of FFA from the intestine.[11] Prochazka et al[12] have shown linkage of chromosomal markers on 4q near *FABP2* and annexin V genes determining maximal insulin action in Pima Indians. Furthermore, Baier et al[13] identified the Ala54Thr polymorphism on the *FABP2* gene in Pima Indians and suggested that it was strongly associated with insulin resistance (Thr54 allele frequency was 0.29). They showed that homozygotes (Thr/Thr) had higher fasting plasma insulin and FFA levels and lower glucose uptake, as measured by the glucose clamp method. The Ala54Thr mutation in *FABP2* has been reported to enhance affinity for FFA,[13] suggesting a possible increase in fat absorption from the intestine. Yamada et al[14] have shown that the frequency of the Thr54 allele is relatively high in Japanese (0.34), and that this mutation was associated with insulin resistance and accumulation of visceral fat, but that the allele frequency was not different between type 2 diabetic and normal subjects. Yagi et al[15] also investigated the link between the locus of the *FABP2* gene and diabetes mellitus using polymorphic markers but did not find any significant association.

In the regulation of adipose tissue, the sympathetic nervous system plays an important role. β-adrenergic receptors (β3AR) mediate the action of epinephrine and norepinephrine on lipolysis. The β3AR are specifically expressed in adipose tissue and play a role in lipolysis in white adipose tissue, together with β_1- and β_2-receptors.[16] On the other hand, energy utilization (heat production) is increased via β3AR in brown adipose tissue.[17] Because the β3AR system forms a negative feedback loop that can inhibit obesity, impairment in any portion of this feedback loop could result in obesity. In 1995, the Trp64Arg missense mutation in the 64th codon of the *β3AR* gene was identified in Pima Indians,[18] who have a high prevalence of obesity and type 2 diabetes. The frequency of the Arg allele was high (31%) in this population, and the mutation was associated with a higher body mass index (BMI), 5-year earlier onset of type 2 diabetes,

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and hypertension. Widén et al[19] reported that the frequency of the Arg64 allele in the Finnish population was 12%, and the Arg allele was associated with upper body obesity, hypertension, and hyperinsulinemia. This mutation in the *β3AR* gene has also been associated with gain in body weight after the age of 20 years in severely obese French Caucasian patients.[20]

The purpose of the present study was to investigate the effects of both the *FABP2* and *β3AR* polymorphisms, and possible synergistic effects, on features of the insulin resistance syndrome[3] (BMI, blood pressure, glucose and lipid profiles) and serum leptin level in younger to older Japanese men.

SUBJECTS AND METHODS

This study included 196 young men aged 21 to 39 years, 186 men aged 40 to 65 years with normal fasting plasma glucose (FPG, <110 mg/dL), and 45 men with hyperglycemia (FPG ≥ 110 mg/dL). All of the men had received an annual health check-up. Seventy-seven male type 2 diabetic patients, who were diagnosed and followed up since the age less than 65 years at Keio University Hospital were also included as the hyperglycemic group. Subjects with endocrine disease, or significant renal or hepatic disease and those receiving systemic corticosteroids were excluded. Furthermore, we divided the older 231 men into the following subgroups. The hyperlipidemic group consisted of subjects with low-density lipoprotein (LDL) cholesterol level ≥160 mg/dL and those receiving antihyperlipidemic medication such as hepatic hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor (n = 18). The normolipidemic group consisted of male subjects with an LDL cholesterol level less than 160 mg/dL without medication. Informed consent was obtained from each subject before the study, and the protocol was approved by the ethical review committee of the Health Center and the Department of Internal Medicine, School of Medicine, Keio University, Tokyo, Japan.

Measurements

Height, body weight, blood pressure (systolic [SBP] and diastolic [DBP]), and heart rate were measured. Plasma glucose, serum lipids (total cholesterol, triglycerides (TG), high–density lipoprotein [HDL] cholesterol, and LDL cholesterol), and uric acid levels were measured using peripheral blood samples obtained after an overnight fast. Serum FFA level was measured only in the young male subjects aged 21 to 29 years and in the type 2 diabetic patients. Serum insulin and leptin concentrations were measured by radioimmunoassay, as described previously.[24,25] The insulin resistance index was assessed by the homeostasis model assessment (HOMA-IR).[26,27]

Sample Collection and Polymorphism Analyses

A 5-mL sample of venous blood was collected in a vacutainer containing EDTA. Genomic DNA was extracted from the peripheral white blood cell fraction by the standard method. Approximately 500 ng of extracted DNA was used as a template, as we have described previously.[28]

A transition polymorphism of Trp to Arg at codon 64 of the *β3AR* gene was detected as follows. The primers were 5'-CGC CCA ATA CCG CCA ACA C-3' and 5'-CCA CCA GGA GTC CCA TCA CC-3'. The reaction was performed in a final volume of 25 μL containing 2 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 5 pmol of each primer, and 0.5 U Taq DNA polymerase (Takara, Tokyo, Japan). DNA was denatured for 5 minutes at 94°C, and amplified for 35 cycles (30 seconds each) at 94°C, 61°C, and 72°C. After the cycles, there was 10-minute extension at 72°C.

A transition polymorphism of alanine to threonine at codon 54 of the *FABP2* gene was detected as follows. The primers were 5'-ACA GGT GTT AAT ATA GTG AAA AG-3' and 5'-TAC CCT GAG TTC AGT TCC GTC-3', as described by Baier et al.[13] The reaction was performed in a final volume of 20 μL containing 2 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 5 pmol of each primer and 0.5 U Taq DNA polymerase. DNA was denatured for 2 minutes at 94°C, and amplified for 35 cycles of 45 seconds at 94°C, 1 minute of annealing at 55°C, and 45 seconds of extension at 72°C. After the cycles, there was 10-minute extension at 72°C.

Polymerase chain reaction (PCR) products were digested with a 10-fold excess of restriction enzymes, either *Mva*I (Takara) for *β3AR* or *Hha*I (Takara) for *FABP2* at 37°C for 3 hours. They were then electrophoresed on 3% agarose gel with 0.5X Tris-borate-EDTA buffer and stained with ethidium bromide for visualization. The PCR products for *β3AR* with an intact *Mva*I site were cleaved into 99- and 62-bp fragments, but the Trp64Arg substitution abolished the restriction site. The PCR products for *FABP2* with an intact *Hha*I site were cleaved into 99-bp and 81-bp fragments, while the Ala54Thr substitution abolished the restriction site.

Statistical Analysis

Data are expressed as the mean ± SD. The Mann-Whitney *U* test was used to compare quantitative variables in 2 groups, Kruskal-Wallis test in 3 groups, and chi-square test to compare proportions. Furthermore, 2-way analysis of variance (ANOVA) was used to test for synergistic effects of the *β3AR* and *FABP2* variants. Because serum insulin, leptin and TG levels, and HOMA-IR were normally distributed after log-transformation, the logarithms of these parameters were used for the analyses. A *P* value less than .05 was considered statistically significant.

RESULTS

Association Study of β3AR and FABP2 Polymorphisms With Type 2 Diabetes and Hyperlipidemia

As shown in Table 1, we compared the genotype and allele frequencies of the *β3AR* and *FABP2* polymorphisms between the older groups of hyperglycemic and normoglycemic men. Although the *β3AR* Arg64 allele frequency tended to be lower, and the *FABP2* Thr/Thr54 genotype frequency tended to be

Table 1. Prevalence of Genotype and Allele Frequencies of *β3AR* and *FABP2* Genes in 122 Hyperglycemic Male Subjects, Including Type 2 Diabetic Patients (n = 77), and 186 Normoglycemic Japanese Men Aged 40 to 65 Years

	Subjects		
	Hyperglycemic	Normoglycemic	
β3AR genotype			
Trp/Trp	89 (73.0%)	125 (67.2%)	$\chi^2 = 1.15$
Trp/Arg + Arg/Arg	33 (27.0%)	61 (32.8%)	$P = .284$
Total (n)	122	186	
β3AR allele			
64Trp	210 (86.1%)	304 (81.7%)	$\chi^2 = 2.01$
64Arg	34 (13.9%)	68 (18.3%)	$P = .156$
FABP2 genotype			
Ala/Ala	50 (41.0%)	66 (33.5%)	
Ala/Thr	53 (43.4%)	106 (53.8%)	$\chi^2 = 3.24$
Thr/Thr	19 (15.6%)	25 (12.7%)	$P = .198$
Total (n)	122	197	
FABP2 allele			
54Ala	153 (62.7%)	238 (60.4%)	$\chi^2 = 0.34$
54Thr	91 (37.3%)	156 (39.6%)	$P = .562$

Table 2. Prevalence of Genotype and Allele Frequencies of *β3AR* and *FABP2* Genes in 27 Hyperlipidemic and 204 Normolipidemic Japanese Men Aged 40 to 65 Years

	Subjects		
	Hyperlipidemic	Normolipidemic	
β3AR genotype			
Trp/Trp	20 (74.1%)	139 (68.1%)	$\chi^2 = 0.39$
Trp/Arg + Arg/Arg	7 (25.9%)	65 (31.9%)	$P = .531$
Total (n)	27	204	
β3AR allele			
Trp	45 (83.3%)	337 (82.6%)	$\chi^2 = 0.18$
Arg	9 (16.7%)	71 (17.4%)	$P = .893$
FABP2 genotype			
Ala/Ala	11 (40.7%)	60 (29.4%)	
Ala/Thr	13 (48.1%)	112 (54.9%)	$\chi^2 = 1.53$
Thr/Thr	3 (11.1%)	32 (15.7%)	$P = .466$
Total (n)	27	204	
FABP2 allele			
Ala	35 (64.8%)	232 (56.9%)	$\chi^2 = 1.24$
Thr	19 (35.2%)	176 (43.1%)	$P = .266$

NOTE. Values are n (%).

higher in the hyperglycemic group, these differences did not reach statistical significance.

We also compared the genotype and allele frequencies of these 2 variants between the hyperlipidemic and normolipidemic groups (Table 2). However, there were no significant differences in the genotype or allele frequency of either variant between the hyperlipidemic and normolipidemic subjects.

Next, we compared clinical and metabolic parameters among these genotypes in both the younger men and the older normoglycemic male subjects.

β3AR Gene Trp64Arg Polymorphism

In the younger group, as shown in Table 3, 137 subjects were normozygotes, 55 were heterozygotes (28.1%), and 4 were homozygotes (2.0%) for the Trp64Arg polymorphism in the *β3AR* gene. The genotype frequencies were governed by Hardy-Weinberg law in all the subject groups in this study. In this younger group (Table 3), serum TG and leptin levels tended to be higher in the Trp/Arg heterozygotes than the normozygotes, and both insulin and HOMA-IR tended to be higher in Arg/Arg homozygotes, but these differences did not reach statistical significance. There were no significant differences in any of the parameters measured between the genotypes.

In the older normoglycemic subjects aged 40 to 65 years (Table 4), 125 were normozygotes, 54 were heterozygotes (29.0%), and 7 were homozygotes (3.8%). In this group, the Arg64-positive subjects (Trp/Arg or Arg/Arg) had a significantly lower heart rate ($P = .037$), and tended to have a lower HDL-cholesterol level, although this was not significant ($P = .217$).

In the older hyperglycemic group (Table 5), the Arg64-positive group had significantly lower HDL cholesterol (43.5 ± 8.5 *v* 49.7 ± 14.1 mg/dL in Trp/Trp genotype, $P = .024$) and lower FFA ($P = .043$). There were no significant differences in any other parameter measured among the genotypes (Table 5).

FABP2 Gene Ala54Thr Polymorphism

The genotype frequencies were governed by Hardy-Weinberg law in all the subject groups in this study.

In the younger group, as shown in Table 6, subjects with a Thr/Thr genotype tended to have higher TG than in the other genotypes, although the difference did not reach statistical significance ($P = .106$).

In the older normoglycemic men (Table 7), FPG was significantly higher in the subjects with Thr/Thr genotype than in those with Ala/Ala or Ala/Thr genotype (99.8 ± 5.6 *v* 96.5 ± 5.6 mg/dL, $P = .010$).

In the older hyperglycemic group, there were no significant differences in any of the parameters measured among the genotypes (data not shown).

Table 3. Relationship Between *β3AR* Trp64Arg Genotype and Subject Profile and Metabolic Variables in 196 Men Aged 21 to 39 Years

Parameter	*β3AR* Genotype			*P* for 3 Groups	*P* for Arg-Positive
	Trp/Trp	Trp/Arg	Arg/Arg		
No. of subjects	137 (69.9%)	55 (28.1%)	4 (2.0%)		
Age (yr)	25.9 ± 5.0	25.2 ± 4.9	27.0 ± 6.2	NS	NS
BMI (kg/m^2)	22.0 ± 3.0	21.9 ± 3.3	21.3 ± 2.5	NS	NS
SBP (mm Hg)	123 ± 17	123 ± 12	112 ± 11	NS	NS
DBP (mm Hg)	72 ± 10	72 ± 9	65 ± 10	NS	NS
Heart rate (/min)	75 ± 12	79 ± 15	69 ± 10	NS	NS
Glucose (mg/dL)	89.2 ± 8.0	89.2 ± 8.2	91.5 ± 6.6	NS	NS
Insulin (μU/mL)	5.6 ± 3.5	5.7 ± 4.1	7.7 ± 7.3	NS	NS
HOMA-IR	1.2 ± 0.8	1.3 ± 0.9	1.8 ± 1.7	NS	NS
Leptin (ng/mL)	2.5 ± 2.0	3.1 ± 3.0	2.3 ± 1.5	NS	NS
Total cholesterol (mg/dL)	172 ± 31	175 ± 30	157 ± 9	NS	NS
TG (mg/dL)	85 ± 62	91 ± 38	75 ± 36	.147	.067
HDL cholesterol (mg/dL)	53 ± 10	54 ± 13	51 ± 14	NS	NS
LDL cholesterol (mg/dL)	106 ± 25	105 ± 27	91 ± 14	NS	NS
FFA (mEq/L)	0.36 ± 0.15	0.35 ± 0.17	0.32 ± 0.13	NS	NS
Uric acid (mg/dL)	5.6 ± 1.2	5.7 ± 1.0	6.0 ± 1.6	NS	NS

NOTE. Values are mean ± SD; n = 85 for heart rate and n = 159 for LDL cholesterol and FFA.
Abbreviation: NS, not significant ($P > .15$).

Table 4. Relationship Between *β3AR* Trp64Arg Genotype and Subject Profile and Metabolic Variables in 186 Normoglycemic Men Aged 40 to 65 Years

Parameter	*β3AR* Genotype			*P* for 3 Groups	*P* for Arg-Positive
	Trp/Trp	Trp/Arg	Arg/Arg		
No. of subjects	125 (67.2%)	54 (29.0%)	7 (3.8%)		
Age (yr)	51.9 ± 7.4	52.1 ± 7.4	50.4 ± 5.9	NS	NS
BMI (kg/m^2)	23.1 ± 2.7	23.5 ± 2.8	23.1 ± 2.4	NS	NS
SBP (mm Hg)	119 ± 16	121 ± 21	121 ± 9	NS	NS
DBP (mm Hg)	75 ± 11	78 ± 14	76 ± 3	NS	NS
Heart rate (/min)	75 ± 12	72 ± 12	68 ± 17	.111	.037
Glucose (mg/dL)	96.6 ± 5.8	97.8 ± 6.5	95.0 ± 4.8	NS	NS
Insulin (μU/mL)	7.6 ± 4.4	8.2 ± 3.8	5.7 ± 1.6	NS	NS
HOMA-IR	1.8 ± 1.1	2.0 ± 0.9	1.3 ± 0.4	NS	NS
Leptin (ng/mL)	3.7 ± 1.7	3.7 ± 1.7	4.6 ± 3.7	NS	NS
Total cholesterol (mg/dL)	206 ± 31	199 ± 29	210 ± 27	NS	NS
TG (mg/dL)	134 ± 92	149 ± 151	156 ± 102	NS	NS
HDL cholesterol (mg/dL)	55 ± 16	52 ± 13	53 ± 20	NS	NS
LDL cholesterol (mg/dL)	124 ± 29	118 ± 30	126 ± 25	NS	NS
Uric acid (mg/dL)	6.3 ± 1.3	6.3 ± 1.3	6.7 ± 0.9	NS	NS

Values are mean ± SD; n = 108 for insulin, HOMA-IR, and leptin.

Furthermore, we examined possible synergistic effects of these 2 variants, but the results were negative, and only the *FABP2* Thr/Thr genotype was related to a higher FPG in the older normoglycemic men.

DISCUSSION

Trp64Arg Polymorphism in β3AR Gene

As for the *β3AR* Trp64Arg polymorphism, some reports have suggested an association with obesity and insulin resistance,[21] while others did not find the associations to be significant.[22] In the Japanese population, Kadowaki et al[23] examined a group of 159 diabetic and 191 nondiabetic subjects and found no difference in allele frequency between the groups (21% *v* 20%). Yoshida et al[29] suggested that obese Japanese women with this mutation have a lower basal metabolic rate and that the effects of diet or exercise therapy are weakened, even in the heterozygotes. Some reports have suggested an association between this mutation and type 2 diabetes,[18,30] but many other reports have failed to show a significant association.[22,23] Kawamura et al[31] reported that this mutation was associated with serum insulin level and HOMA-IR in Japanese-American subjects with impaired glucose tolerance.

In the present study, serum TG and leptin levels tended to be higher (but not significantly) only in the younger male subjects with the *β3AR* Trp64/Arg64 heterozygous mutation. In the older normoglycemic men, on the other hand, *β3AR* Arg64-positive subjects had a significantly lower heart rate. Although the mechanisms by which this mutation may cause insulin

Table 5. Relationship Between *β3AR* Trp64Arg Genotype and Subject Profile and Metabolic Variables in 122 Hyperglycemic Men Aged 40 to 78 Years

Parameter	*β3AR* Genotype		*P* for Arg-Positive
	Trp/Trp	Trp/Arg + Arg/Arg	
No. of subjects	89 (73.0%)	32 ± 1 (27.0%)	
Age (yr)	59.9 ± 8.2	58.3 ± 7.6	NS
Duration of diabetes (yr)	7.4 ± 6.9	7.3 ± 5.7	NS
BMI (kg/m^2)	23.7 ± 2.9	23.9 ± 2.5	NS
SBP (mm Hg)	132 ± 19	133 ± 24	NS
DBP (mm Hg)	78 ± 11	76 ± 13	NS
Heart rate (/min)	82 ± 14	79 ± 20	.129
Glucose (mg/dL)	144 ± 40	136 ± 34	NS
HbA_{1c} (%)	7.4 ± 1.6	7.3 ± 1.4	NS
Insulin (μU/mL)	9.8 ± 6.8	10.5 ± 6.7	NS
Leptin (ng/mL)	3.7 ± 1.4	4.5 ± 1.8	NS
Total cholesterol (mg/dL)	198 ± 28	201 ± 33	NS
TG (mg/dL)	160 ± 96	151 ± 70	NS
HDL cholesterol (mg/dL)	50 ± 14	43 ± 8	.024
LDL cholesterol (mg/dL)	123 ± 25	134 ± 29	.115
FFA (mEq/L)	0.53 ± 0.26	0.40 ± 0.19	.043
Uric acid (mg/dL)	5.7 ± 1.4	6.1 ± 1.7	NS

NOTE. Values are mean ± SD; n = 43 for insulin and leptin, and n = 69 for duration of diabetes, HbA_{1c}, and FFA.

Table 6. Relationship Between *FABP2* Ala54Thr Genotype and Subject Profile and Metabolic Variables in 196 Men Aged 21 to 39 Years

Parameter	*FABP2* Genotype			*P* for 3 Groups	*P* for Thr/Thr
	Ala/Ala	Ala/Thr	Thr/Thr		
No. of subjects	83 (42.3%)	78 (39.8%)	35 (17.9%)		
Age (yr)	25.6 ± 5.2	25.9 ± 5.0	25.6 ± 4.6	NS	NS
BMI (kg/m^2)	22.1 ± 2.5	22.1 ± 3.7	21.3 ± 2.7	NS	NS
SBP (mm Hg)	123 ± 13	122 ± 18	123 ± 13	NS	NS
DBP (mm Hg)	71 ± 10	72 ± 10	71 ± 8	NS	NS
Heart rate (/min)	74 ± 13	79 ± 17	76 ± 9	NS	NS
Glucose (mg/dL)	89.6 ± 7.6	89.5 ± 8.2	87.8 ± 8.7	NS	NS
Insulin (μU/mL)	5.8 ± 4.2	5.5 ± 3.3	5.7 ± 3.8	NS	NS
HOMA-IR	1.3 ± 0.9	1.2 ± 0.8	1.2 ± 0.7	NS	NS
Leptin (ng/mL)	2.4 ± 1.7	3.0 ± 2.9	2.5 ± 2.2	NS	NS
Total cholesterol (mg/dL)	173 ± 31	172 ± 29	173 ± 33	NS	NS
TG (mg/dL)	90 ± 43	76 ± 36	102 ± 99	NS	NS
HDL cholesterol (mg/dL)	54 ± 13	53 ± 9	54 ± 10	NS	NS
LDL cholesterol (mg/dL)	105 ± 26	106 ± 25	105 ± 26	NS	NS
FFA (mEq/L)	0.36 ± 0.15	0.36 ± 0.14	0.35 ± 0.20	NS	NS
Uric acid (mg/dL)	5.5 ± 1.1	5.8 ± 1.2	5.7 ± 1.0	NS	NS

NOTE. Values are mean ± SD; n = 85 for heart rate, and n = 159 for LDL cholesterol and FFA.
Abbreviation: NS, not significant ($P > .10$).

resistance are unclear, this mutation is reported to reduce intracellular cyclic adenosine monophospate level.[32] We also speculate that this reduced function may lead to a decrease in energy production and in lipolysis, causing accumulation of visceral fat and worsening of insulin resistance.[23,33]

Contrary to other reports[23,29,30] and a meta-analysis,[34] however, our results did not demonstrate a significant difference in BMI. Mitchell et al[35] reported that a quantitative trait locus influencing BMI in 470 Mexican-Americans is mapped to the region of *β3AR*. It is also possible that the Arg64 variant is in linkage disequilibrium with functional mutation(s) elsewhere in the gene.[35,36]

In the Japanese population, the frequency of this allele (0.17 to 0.21) is relatively high, and the mutation may be associated with obesity, insulin resistance, and the accumulation of visceral fat.[23,33] Although our results were negative and there were no synergistic effects with *FABP2* variants, there is a possibility that this *β3AR* Trp64Arg mutation might contribute to the development of obesity through a multifactorial inheritance mechanism, together with other genetic and/or environmental factors.

Ala54Thr Polymorphism in FABP2 Gene

In this study, the *FABP2* Thr/Thr genotype was related to a significantly higher FPG level in older normoglycemic men aged 40 to 65 years. Because Yamada et al(14) have shown that this *FABP2* gene Ala54Thr polymorphism could lead to accumulation of body fat, we hypothesized that this variant may result in increased insulin resistance and a consequent elevation of FPG level. In this study, however, there were no differences

Table 7. Relationship Between *FABP2* Ala54Thr Genotype and Subject Profile and Metabolic Variables in 186 Normoglycemic Men Aged 40 to 65 Years

Parameter	*FABP2* Genotype			*P* for 3 Groups	*P* for Thr/Thr
	Ala/Ala	Ala/Thr	Thr/Thr		
No. of subjects	60 (32.3%)	101 (54.3%)	25 (13.4%)		
Age (yr)	51.7 ± 7.8	51.7 ± 6.9	52.9 ± 7.8	NS	NS
BMI (kg/m^2)	23.1 ± 2.4	23.4 ± 2.6	23.0 ± 3.7	NS	NS
SBP (mm Hg)	118 ± 17	120 ± 17	121 ± 20	NS	NS
DBP (mm Hg)	75 ± 11	76 ± 12	78 ± 12	NS	NS
Heart rate (/min)	74 ± 11	73 ± 13	77 ± 16	NS	NS
Glucose (mg/dL)	96.5 ± 6.1	96.5 ± 5.9	99.8 ± 5.6	.037	.010
Insulin (μU/mL)	7.4 ± 3.8	8.1 ± 4.5	6.3 ± 2.5	NS	NS
HOMA-IR	1.8 ± 0.9	1.9 ± 1.1	1.5 ± 0.6	NS	NS
Leptin (ng/mL)	3.5 ± 1.5	3.8 ± 2.0	4.0 ± 1.9	NS	NS
Total cholesterol (mg/dL)	205 ± 33	203 ± 29	207 ± 27	NS	NS
TG (mg/dL)	147 ± 154	130 ± 73	159 ± 126	NS	NS
HDL cholesterol (mg/dL)	54 ± 16	53 ± 14	58 ± 15	NS	NS
LDL cholesterol (mg/dL)	122 ± 32	124 ± 28	117 ± 26	NS	NS
Uric acid (mg/dL)	6.5 ± 1.2	6.4 ± 1.3	5.9 ± 1.6	NS	NS

NOTE. Values are mean ± SD; n = 108 for insulin, HOMA-IR, and leptin.

in serum insulin level or HOMA-IR among the genotypes, which differs from the findings of Baier's report in Pima Indians.[13] It is unfortunate that we were not able to measure any parameter such as abdominal fat mass or waist to hip ratio in this study. Serum leptin level tended to be higher in the older male subjects with the *FABP2* Thr54 variant, but the difference was not statistically significant.

FABP2 Thr54 allele frequency is relatively high in the Japanese population compared with Pima Indians (0.29); Thr54 frequency was 0.34 in Yamada's report[13] and 0.37 to 0.40 in the 3 groups in this study. The difference between our and Yamada's results is not so large, but the reason for the difference is not clear.

In the older hyperglycemic men, however, no significant differences among the genotypes were found for any measured parameter. We speculate that this discrepancy occurred because the *FABP2* mutation may gradually cause insulin resistance, but insulin resistance was already established in hyperglycemic subjects. Further studies will be needed to clarify the significance of these variants using different age, sex, and ethnic groups in combination with other variant alleles.

In conclusion, no major difference was associated with the *β3AR* Trp64Arg or *FABP2* Ala54Thr polymorphism in terms of type 2 diabetes or hyperlipidemia in young to older Japanese men. However, a slight but significant increase in FPG was observed in older Japanese men with the *FABP2* Thr/Thr genotype.

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