

Genotype Arg/Arg, but not Trp/Arg, of the Trp64Arg Polymorphism of the β_3 -Adrenergic Receptor Is Associated With Type 2 Diabetes and Obesity in a Large Japanese Sample

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OBJECTIVE — Despite a large number of studies, no association of the Trp64Arg polymorphism of the β_3 -adrenergic receptor gene with obesity and type 2 diabetes has yet to be clearly elucidated. We examined the associations in a large population-based sample.

RESEARCH DESIGN AND METHODS — A total of 1,685 subjects (935 women and 750 men, aged 58.7 ± 12.4 years) from a cohort population ($n = 3,706$) of the Funagata Diabetes Study were divided into three groups according to genotypes: Trp/Trp ($n = 1,155$), Trp/Arg ($n = 486$), and Arg/Arg ($n = 44$). Glucose tolerance was diagnosed according to the 1985 World Health Organization criteria. Subjects who had a BMI ≥ 30 kg/m² were considered obese. Associations with the traits related to obesity, diabetes, hypertension, and dyslipidemia were also examined. The χ^2 test and analysis of variance were used for the association studies and to assess the differences in the traits' values, respectively.

RESULTS — More subjects with genotype Arg/Arg were obese and had diabetes (13.6% for each) than those with genotype Trp/Trp (3.29%, $P < 0.001$; and 4.16%, $P = 0.007$, respectively) or genotype Trp/Arg (2.06%, $P < 0.001$; and 5.97%, $P = 0.051$, respectively). No significant differences in the frequencies of occurrence of these conditions were observed between genotypes Trp/Arg and Trp/Trp. Traits related to obesity, such as percent body fat (28.82 ± 7.95 vs. 25.93 ± 7.21 , $P = 0.038$) and BMI (25.07 ± 3.84 vs. 23.63 ± 3.18 , $P = 0.018$), were higher in the genotype Arg/Arg than in the genotype Trp/Trp groups.

CONCLUSIONS — Genotype Arg/Arg, but not Trp/Arg, of the β_3 -adrenergic receptor was associated with both obesity and type 2 diabetes in a large Japanese sample.

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Obesity causes insulin resistance and is a major risk factor for type 2 diabetes. Obesity has been reported to be determined by the interplay of both environmental and genetic factors (1). However, attempts to identify the genes causing obesity in humans using the candidate gene approach or linkage studies in

obese families have been rather unsuccessful (2). The β_3 -adrenergic receptor gene has been postulated as a factor. It is predominantly expressed in adipose tissue and regulates lipid metabolism and thermogenesis, so its impairment may lead to obesity through its effect on the energy expenditure of fat tissue (3). Indeed, the gene polymorphism Trp64Arg of the β_3 -adrenergic receptor was reported to be associated with a tendency toward weight gain, insulin resistance, and the earlier onset of type 2 diabetes (4–6). Since then, a large number of studies have shown either an association between obesity and the gene polymorphism (4,7–16) or a lack thereof (5,6,17–25). The reason for this discrepancy is not clear. The small number of subjects examined rather than differences in ethnic background or sex, may be the cause (26). Few studies included >600 subjects, and the numbers of subjects with genotype Arg/Arg were not significant (9,17,18,24). The Arg allele might have a weak influence on the pathogenesis of obesity and type 2 diabetes; as a result, it would only be possible to determine any association in a study with a large sample. Therefore, a study with a substantial number of subjects with genotype Arg/Arg was needed. We examined the association of the genotype with obesity and type 2 diabetes in a large population-based Japanese sample. This study was expected to provide an even larger number of subjects with genotype Arg/Arg, since the frequency of the Arg allele seemed to be common in Japan (7,8,12,14). The gene polymorphism might affect only a fraction of subjects; thus, the differences in the proportion of the fraction among the studies might have led to the different results (27). Therefore, we also examined the differences in the distributions for the traits related to obesity and diabetes among the genotypes. Subsequently, we were able to make a con-

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Abbreviations: FPG, fasting plasma glucose.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

clusion regarding whether the gene polymorphism is a risk factor for obesity and type 2 diabetes.

RESEARCH DESIGN AND METHODS

The Funagata Diabetes Study was a population-based study that was undertaken to clarify the risk factors, related conditions, and consequences for type 2 diabetes from an epidemiological point of view (28). In Funagata, an agricultural area located about 400 km north of Tokyo, there were 4,183 people aged >35 years in 1995. Subjects ($n = 377$) with cerebrovascular diseases or other disabilities who were unable to attend the study were excluded. One hundred residents who had been identified as having diabetes by public health nurses and through contacts with outpatient clinics were also excluded. Therefore, the number of residents registered for the study was 3,706. From 1995 to 1997, 2,013 residents attended the study. Among them, 1,685 were enrolled for a genetic analysis for the Trp64Arg variation of the β_3 -adrenergic receptor; the participation rate for this genetic analysis was 45.5%.

This study was approved by the Ethical Committee of Yamagata University School of Medicine. Written consent was obtained from the participants. Along with the genetic analysis, the following traits were analyzed: height, body weight, 75-g oral glucose tolerance test, HbA_{1c}, waist circumference, hip circumference, waist-to-hip ratio, BMI, percent body fat, systolic blood pressure, diastolic blood pressure, and total serum cholesterol, triglyceride, and HDL cholesterol. Percent body fat was assessed based on the principles of bioelectrical impedance (29).

The mean age (\pm SD) and the sex ratio (female/male) of the study group were 58.7 ± 12.4 and 935/750, respectively. Glucose tolerance was diagnosed according to 1985 World Health Organization criteria (30). The numbers of subjects with normal glucose tolerance, impaired glucose tolerance, and type 2 diabetes were 1,377, 225, and 83, respectively. Subjects who had a BMI ≥ 30 kg/m² were considered obese. The number of obese and nonobese subjects was 64 and 1,621, respectively.

Genetic analysis

Genomic DNA was isolated from peripheral blood leukocytes by proteinase K and the phenol/chloroform extraction proce-

dures. The genotyping for the Trp64Arg variation of the β_3 -adrenergic receptor was performed by polymerase chain reaction–restriction fragment-length polymorphism analysis as previously described (6). The study population was divided into three groups according to genotypes Trp/Trp ($n = 1,155$), Trp/Arg ($n = 486$), and Arg/Arg ($n = 44$). The mean age and the sex ratio of the groups (Trp/Trp, Trp/Arg, and Arg/Arg) were 58.8 ± 12.7 and 616/539, 58.5 ± 11.8 and 293/193, and 57.7 ± 11.3 and 26/18, respectively. No statistical differences in age or sex ratio were observed among the groups.

Statistic analysis

χ^2 Tests were performed for the association studies. Data are given as the means \pm SD. The statistical significances of the differences of the trait values between two groups (Trp/Trp versus Trp/Arg or Trp/Trp versus Arg/Arg) were assessed by analysis of variance. The Scheffe's *F* test was used for post hoc analysis. Stepwise linear regression analysis with BMI as a covariant was performed to examine the relation between fasting plasma glucose (FPG) and BMI. $P < 0.05$ was accepted as statistically significant.

RESULTS

Distribution of genotypes

The distribution of the genotypes defined by the Trp64Arg polymorphism of the β_3 -adrenergic receptor was examined. The frequencies of the Trp and Arg alleles were 83.0 and 17.0%, respectively. The frequencies of genotypes Trp/Trp, Trp/Arg, and Arg/Arg were 68.5, 28.8, and 2.6%, respectively. The genotypes were found to be in Hardy-Weinberg equilibrium. The frequencies of alleles and genotypes were in concordance with other observations in Japan (7,8,12,14); therefore, our study population seemed to be genetically similar to others reported in Japan but different in regard to sample size.

Association of genotype Arg/Arg, but not Trp/Arg, with obesity and type 2 diabetes

The associations of genotype with obesity and type 2 diabetes are shown in Tables 1 and 2, respectively. More subjects with genotype Arg/Arg were obese (13.6%) than those with genotypes Trp/Trp

Table 1—Frequency of obesity and Trp64Arg mutation of β_3 -adrenergic receptor gene

	Genotypes		
	Arg/Arg	Trp/Arg	Trp/Trp
Obese	6 (13.6)	10 (2.1)	38 (3.3)
Nonobese	38 (86.4)	466 (95.9)	1,117 (96.7)

Data are n (%). Frequency of obesity in Arg/Arg subgroup was significantly different from those in Trp/Trp or Trp/Arg subgroup ($P < 0.001$).

(3.29%, $P < 0.001$) or Trp/Arg (2.06%, $P < 0.001$) (Table 1). In regard to the frequency of obese subjects, there were no differences between genotypes Trp/Arg and Trp/Trp ($P = 0.258$). More subjects with genotype Arg/Arg were more likely to have diabetes (13.6%) than those with genotype Trp/Trp (4.16%, $P = 0.007$) and those with genotype Trp/Arg (5.97%, $P = 0.051$) (Table 2). No difference was observed in terms of the frequency of diabetic subjects between genotypes Trp/Arg and Trp/Trp ($P = 0.168$). Genotype Arg/Arg was associated with obesity and type 2 diabetes, whereas Trp/Arg was not. We also examined whether genotype Arg/Arg was associated with an early mortality by comparing the frequencies of Arg/Arg between subjects aged <45 and ≥ 45 years, <50 and ≥ 50 years, <55 and ≥ 55 years, <60 and ≥ 60 years, and <65 and ≥ 65 years. The frequency of genotype Arg/Arg should be lower in the elder group if it is associated with an early mortality. However, no statistically significant differences were observed between any groups in terms of the frequency of the genotype (2.32 vs. 2.67%, $P = 0.799$; 2.47 vs. 2.63%, $P = 0.410$; 2.78 vs. 2.51, $P = 0.949$; 2.77 vs. 2.47, $P = 0.814$; and 2.67 vs. 2.52, $P = 0.748$ for subjects aged <45 and ≥ 45 years, <50 and ≥ 50 years, <55 and ≥ 55 years, <60 and ≥ 60 years, and <65 and ≥ 65 years, respectively).

Relation of genotype Arg/Arg, but not Trp/Arg, to traits related to obesity

As described in RESEARCH DESIGN AND METHODS, the traits related to obesity, diabetes, hypertension, and dyslipidemia were measured. Subjects with genotype Arg/Arg had higher fasting serum insulin levels (5.84 ± 3.76 vs. 4.50 ± 3.30 , $P = 0.036$), higher BMI (25.07 ± 3.84 vs. 23.63 ± 3.18 , $P = 0.018$), and higher

Table 2—Frequency of type 2 diabetes and the Trp64Arg mutation of β_3 -adrenergic receptor gene

	Genotype		
	Arg/Arg*	Trp/Arg	Trp/Trp
Diabetes	6 (13.6)	29 (6.0)	48 (4.2)
Impaired glucose tolerance	8 (18.2)	57 (11.7)	160 (13.9)
Normal glucose tolerance	30 (68.2)	400 (82.3)	947 (82.0)

Data are means \pm SD or n (%). Frequency of diabetes in the Arg/Arg subgroup was significantly different from that in the Trp/Trp subgroup ($P = 0.0067$).

percent body fat (28.82 ± 7.95 vs. 25.96 ± 7.21 , $P = 0.038$) than those with genotype Trp/Trp (Table 3). The two groups showed no significant differences in the other traits examined (Table 3). The traits that differed between the two groups were those related to obesity or insulin resistance but not to diabetes, hypertension, or dyslipidemia. Subjects with genotype Trp/Arg did not show any significant differences from those with genotype Trp/Trp for the traits examined (Table 3).

Possible contribution of genotype Arg/Arg to pathogenesis of obesity and type 2 diabetes in only a fraction of the population

The distributions of subjects with each genotype for the traits related to obesity and diabetes were examined. Distribu-

tions for BMI and FPG levels are shown in Fig. 1. These distributions were not normal and seemed to be divided into two fractions (major and minor fractions). The nonobese subjects seemed to compose the major fraction (drawn as a curve in the figure), and the distributions were normal in all genotypes and were not significantly different among genotypes ($P = 0.394$). The means \pm SD of the BMI of the nonobese subjects were 23.4 ± 2.8 , 23.3 ± 2.9 , and 23.9 ± 2.5 for genotypes Trp/Trp, Trp/Arg, and Arg/Arg, respectively. The major fractions for BMI did not shift among genotypes, although the proportion of obese subjects (the minor fraction) was significantly higher in genotype Arg/Arg than in genotype Trp/Trp. This indicated that genotype Arg/Arg contributed to the pathogenesis of obesity in only a fraction of subjects. The proportion of

the minor fraction for genotype Trp/Arg was not different from that in genotype Trp/Trp. The distributions of FPG levels were similar to those for BMI. Subjects with FPG levels <110 mg/dl seemed to compose a major fraction of the population. The distributions were normal in all genotypes and not significantly different among genotypes ($P = 0.347$). The means \pm SD of the FPG levels in subjects with FPG levels <110 mg/dl were 92.3 ± 7.9 , 91.7 ± 8.5 , and 91.6 ± 8.2 for genotypes Trp/Trp, Trp/Arg, and Arg/Arg, respectively. As with BMI, genotype Arg/Arg did not seem to contribute to the pathogenesis of increased FPG levels in most cases. Only a minor fraction of the cases seemed to be affected by genotype. The proportions of the minor fraction did not differ between genotypes Trp/Arg and Trp/Trp.

Although the significant association of genotype Arg/Arg with obesity and type 2 diabetes seemed to be driven by the minor fractions, the proportions of the minor fraction seemed to not be large enough to influence the traits of the whole study population. Namely, stepwise linear regression analysis did not show any significant relation between FPG and BMI in subjects with genotype Arg/Arg ($P = 0.680$).

Table 3—Effect of the Trp64Arg mutation of β_3 -adrenergic receptor gene

Parameter	Genotype					
	Arg/Arg		Trp/Arg		Trp/Trp	
	Value	<i>P</i>	Value	<i>P</i>	Value	
Sex (F/M)	26/18		293/193		616/539	
Age (years)	57.7 ± 11.3	0.846	58.5 ± 11.8	0.911	58.8 ± 12.7	
Height (cm)	154.7 ± 8.7	0.876	154.4 ± 8.6	0.121	155.4 ± 9.0	
Body weight (kg)	60.1 ± 11.0	0.172	56.5 ± 9.9	0.430	57.2 ± 10.0	
Fasting plasma glucose (mmol/l)	97.6 ± 18.0	0.435	94.9 ± 15.0	0.999	94.9 ± 13.1	
2-h plasma glucose (mmol/l)	122.0 ± 52.4	0.522	114.2 ± 47.9	0.997	114.0 ± 44.4	
Fasting serum insulin (μ U/ml)	5.84 ± 3.76	0.036*	4.48 ± 3.49	0.990	4.50 ± 3.30	
HbA _{1c} (%)	5.57 ± 0.72	0.371	5.45 ± 0.53	0.999	5.45 ± 0.53	
Waist circumference (cm)	82.4 ± 8.82	0.058	78.5 ± 8.94	0.464	79.1 ± 9.03	
Hip circumference (cm)	94.3 ± 6.40	0.072	91.9 ± 6.20	0.645	92.2 ± 5.92	
Waist-to-hip ratio	0.873 ± 0.067	0.334	0.853 ± 0.069	0.656	0.857 ± 0.070	
BMI (kg/m^2)	25.07 ± 3.84	0.018*	23.66 ± 3.44	0.988	23.63 ± 3.18	
Percent body fat	28.82 ± 7.95	0.038*	26.46 ± 7.32	0.454	25.96 ± 7.21	
Systolic blood pressure (mmHg)	128.5 ± 17.9	0.848	125.4 ± 17.2	0.228	127.0 ± 17.3	
Diastolic blood pressure (mmHg)	74.0 ± 10.6	0.904	73.8 ± 9.44	0.275	74.6 ± 9.84	
Total cholesterol (mg/dl)	207.3 ± 35.5	0.916	204.5 ± 35.9	0.951	205.1 ± 35.0	
Triglycerides (mg/dl)	149.0 ± 104.0	0.114	111.6 ± 78.0	0.441	118.3 ± 102.1	
HDL cholesterol (mg/dl)	53.1 ± 16.0	0.136	58.3 ± 15.3	0.650	57.6 ± 14.3	

Data are *n* or means \pm SD. *P* values compared subjects carrying Arg/Arg or Trp/Arg genotype with subjects carrying Trp/Trp genotype. * $P < 0.05$.

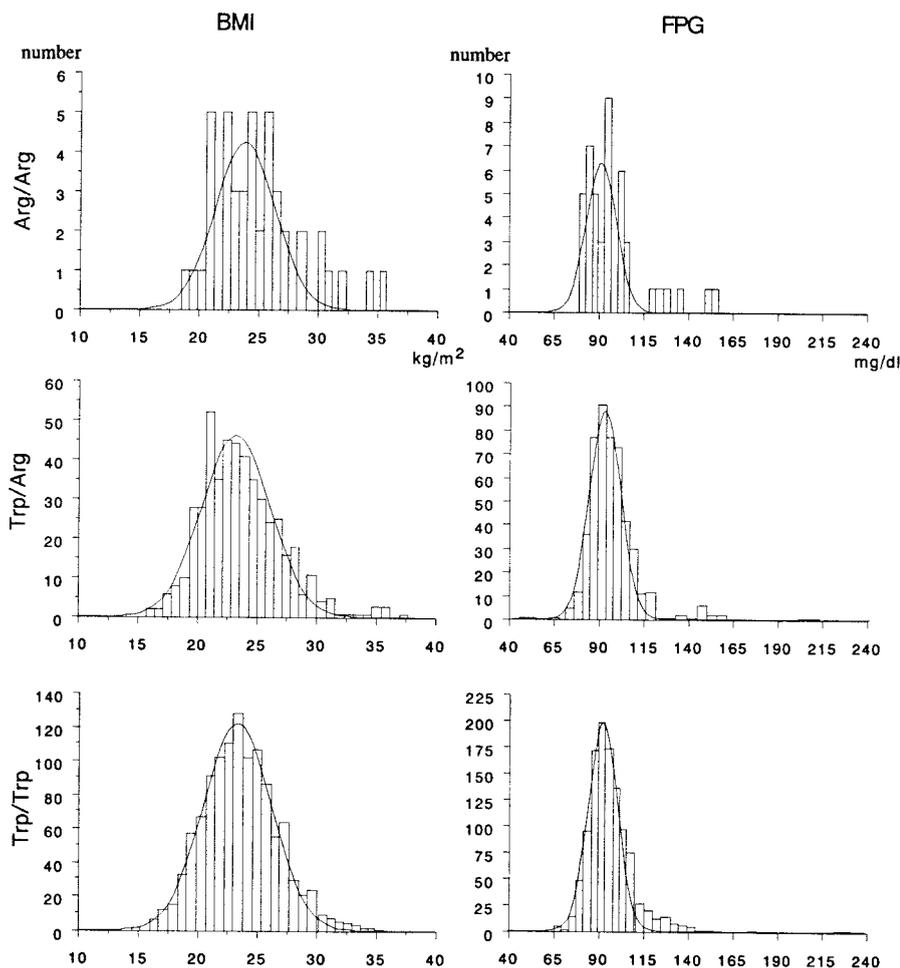


Figure 1—The distributions of subjects with each genotype for BMI and FPG levels are shown. These distributions seemed to be divided into two fractions (major and minor fractions). The distributions of the major fractions were normal and are shown as a curve.

CONCLUSIONS— Genotype Arg/Arg, but not Trp/Arg, of the Trp64Arg polymorphism of the β_3 -adrenergic receptor was associated with obesity and type 2 diabetes. No single study has ever shown an association of genotype Arg/Arg with type 2 diabetes. This finding indicates that genotype Arg/Arg is a risk factor for obesity and type 2 diabetes and that—if genotype Trp/Arg contributes at all to the pathogenesis of these conditions—the contribution was not detected in our large study sample. To further confirm these findings, we examined the relations of the genotypes to the traits related to obesity and type 2 diabetes. Traits related to insulin resistance, such as hypertension and dyslipidemia, were also examined. Subjects with genotype Arg/Arg, but not Trp/Arg, had higher BMIs and percent body fat than those with genotype Trp/Trp. The traits that differed

between genotypes were those related to obesity but not to diabetes, hypertension, or dyslipidemia. These results indicate that genotype Arg/Arg is a risk factor for obesity but not for diabetes. Genotype Trp/Arg did not, however, seem to be a risk factor for the conditions examined, or at least it did not seem to be a large enough factor to be significant. These results were somewhat confusing. Genotype Arg/Arg was associated with type 2 diabetes but not related to the traits associated with type 2 diabetes. This discrepancy might be due to the small number of subjects who had increased plasma glucose levels and to the low severity of their diabetes. The number of subjects with type 2 diabetes in the genotype Arg/Arg group was high enough to indicate an association but not high enough to increase the mean values of the plasma glucose levels in the study group. As shown in Fig. 1,

most subjects (the major fraction) with genotype Arg/Arg had FPG levels similar to those subjects with other genotypes, and only a small fraction of them (the minor fraction) had higher FPG levels. These facts seemed to affect our results. Altogether, our results indicate that genotype Arg/Arg, but not Trp/Arg, is a risk factor for obesity and type 2 diabetes.

In most studies, the frequencies of genotypes have been compared between a normal (control) group and a study group with conditions such as obesity or type 2 diabetes in order to examine the association of the genotypes with those conditions. However, we chose another method in this study. When the association of some genotype with some condition is examined, the difference in the frequency of occurrence of the condition among genotypes should be examined. However, in most studies on the β_3 -adrenergic receptor gene polymorphism, it is the difference between the genotypes of subjects with the condition under investigation and those in the control group that has been examined. We compared the frequencies of the conditions among genotypes and found that the frequencies of obesity and type 2 diabetes were significantly higher in subjects with genotype Arg/Arg than in those with the other genotypes.

The frequency of genotype Arg/Arg is generally low. Therefore, the number of subjects with this genotype was small in previous studies, and the results reported seemed to be less reliable. In some studies (17,18,21,23), subjects with the genotype were grouped with those with genotype Trp/Arg to make the results more reliable. However, in those cases, the results obtained seemed to be a mixture of the influences of the two genotypes. In this study, we were able to examine the differences between genotypes Arg/Arg and Trp/Arg with confidence because the sample was much larger.

The distributions of BMI and FPG levels in subjects with each genotype were not normal and seemed to fall into two fractions. Nonobese subjects or subjects with FPG levels <110 mg/dl seemed to compose the major fraction. The values in these fractions were not significantly different among genotypes. Subjects who showed higher values for BMI or FPG levels composed the minor fraction. The proportion of the minor fraction was higher for genotype Arg/Arg than for the other genotypes and did not differ be-

tween genotypes Trp/Arg and Trp/Trp. The distribution of subjects with genotype Arg/Arg did not simply shift to the higher range. The proportion of the fraction of subjects who had increased BMIs or higher FPG levels increased. Therefore, genotype Arg/Arg seemed to contribute to the pathogenesis of obesity and type 2 diabetes in only a fraction of the population. This indicated that there may have been some other factors that made these subjects sensitive to the genotype. Genotype Arg/Arg may only affect those subjects who have such factors. This study revealed that genotype Arg/Arg, but not Trp/Arg, was a risk factor for obesity and type 2 diabetes in only certain subjects. Therefore, differences in the proportion of these subjects among the study populations might have led to different results in previous reports.

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