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Association of Trp64Arg mutation of the β3-adrenergic-receptor with NIDDM and body weight gain

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Summary A possible pathogenic mutation in the β3-adrenergic-receptor gene (Trp64Arg) has been reported to be associated with an earlier age of onset of non-insulin-dependent diabetes mellitus (NIDDM) and clinical features of the insulin resistance syndrome in Pima Indian, Finnish and French subjects. Since marked heterogeneity has been reported in the association of mutations of candidate genes with NIDDM between Japanese and other ethnic groups, we investigated the association of Trp64Arg with NIDDM in Japanese subjects. The allele frequency of the mutation (Arg) was slightly, but not significantly, higher in NIDDM than in control subjects (70 out of 342 alleles [20.5 %] vs 40 out of 248 [16.1 %], respectively, $p > 0.2$). When our data were combined with those of Pima Indian and Finnish subjects, however, the Arg/Arg genotype was significantly associated with NIDDM as compared with the other two genotypes ($p < 0.005$, relative risk [RR] 2.13, 95 % confidence interval [CI] 1.28–3.55). The Arg allele was also associated with NIDDM ($p < 0.05$, RR 1.27, 95 % CI 1.06–1.52). Japanese subjects homozygous for the mutation had a significantly higher body mass index (mean ± SD: 25.5 ± 3.9 kg/m^2) than heterozygotes (22.6 ± 4.1, $p < 0.05$) and normal homozygotes (22.8 ± 3.8, $p < 0.05$). NIDDM patients homozygous for the mutation tended to have an earlier age of onset of NIDDM than those with other genotypes. These data suggest that the Trp64Arg mutation not only contributes to weight gain and age-at-onset of NIDDM but is also associated with susceptibility to NIDDM. [Diabetologia (1996) 39: 349–352]

Keywords β3-adrenergic-receptor gene, susceptibility, missense mutation, non-insulin-dependent diabetes mellitus, insulin resistance syndrome.

Genetic predisposition is important in the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM) and obesity, but the genes responsible for the common forms of obesity, insulin resistance and NIDDM are largely unknown. The β-3-adrenergic-receptor (β3-AR) is predominantly expressed in adipose tissue and plays an important role in lipid metabolism and metabolic rate by mediating lipolysis and thermogenesis, and thus, molecular abnormalities of β3-AR may lead to obesity and insulin resistance [1]. Recently, three groups [2–4] reported the association of a mutation in the β3-adrenergic-receptor gene causing a Trp to Arg change at codon 64 (Trp64Arg) with an earlier age of onset of NIDDM [2, 3], clinical features of the insulin resistance syndrome [3] and an increased tendency to gain weight [4].

The marked genetic heterogeneity in the association of NIDDM with mutations in the glycogen syn-

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Abbreviations: NIDDM, Non-insulin-dependent diabetes mellitus; β3-AR, β3-adrenergic-receptor; Trp64Arg, a mutation in the β3-adrenergic-receptor gene causing a Trp to Arg change at codon 64; BMI, body mass index; ADRB3, β3-adrenergic receptor gene; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RR, relative risk; CI, confidence interval.

Table 1. Genotype frequency of the β3-adrenergic receptor in NIDDM patients and control subjects

Genotype	Japanese		Pima Indian[a]		Finnish[b]		Total	
	NIDDM	Control	NIDDM	Control	NIDDM	Control	NIDDM	Control
Arg/Arg	7 (4.1)	3 (2.4)	41 (10.5)	16 (6.3)	2 (1.0)	2 (0.7)	50 (6.5)[d, e]	21 (3.2)
Trp/Arg	56 (32.8)	34 (27.4)	173 (44.4)	117 (46.4)	36 (17.6)	68 (23.8)	265 (34.6)	219 (33.1)
Trp/Trp	108 (63.1)	87 (70.2)	176 (45.1)	119 (47.3)	167 (81.4)	216 (75.5)	451 (58.9)	422 (63.7)
Relative Risk[c]		1.72		1.73		1.40		2.13
95 % CI		0.44–6.71		0.96–3.14		0.2–9.9		1.28–3.55

Data are N (%).
[a, b] Data from references [2] and [3], respectively
[c] Relative risks of NIDDM were calculated between subjects with the Arg/Arg genotype and subjects with the Trp/Arg or Trp/Trp genotype.
[d] $p < 0.05$ vs Trp/Arg genotype (RR 1.97, 95 % CI 1.15–3.35);
[e] $p < 0.005$ vs Trp/Trp genotype (RR 2.22, 95 % CI 1.33–3.73)

Table 2. Body mass index and age-at-onset of NIDDM in β3-adrenergic receptor genotypes

Genotype	Arg/Arg	Trp/Arg	Trp/Trp
Body mass index, (kg/m²)			
Japanese	25.5 ± 3.9	22.6 ± 4.1[d]	22.8 ± 3.8[d]
Pima[a]	35.2 ± 8.0	34.1 ± 7.9	33.9 ± 7.5
Finn, NIDDM[b]	–	29.1 ± 4.6	27.7 ± 4.0
French, Obese[c]	–	51 ± 9	47 ± 7
Age-at-onset of NIDDM (years)			
Japanese	40.4 ± 19.4	43.3 ± 14.7	43.4 ± 13.3
Pima[a]	36 ± 10	40 ± 10	41 ± 11
Finn[b]	–	56 ± 11	61 ± 11

Data are mean ± SD.
[a] Data from reference [2];
[b] Data of western Finland, reference [3];
[c] Data from reference [4];
[d] $p < 0.05$ vs Arg/Arg genotype

thase gene [5] and the glucagon receptor gene [6] between Japanese and other ethnic groups prompted us to investigate the contribution of this mutation with NIDDM and weight gain in a large number of Japanese subjects.

Subjects and methods

Two hundred and ninety-five unrelated Japanese subjects (NIDDM patients: $n = 171$, non-diabetic subjects: $n = 124$) were investigated. NIDDM was diagnosed on the basis of the World Health Organization criteria [7]. Informed consent was obtained from the subjects. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Age-at-onset of diabetes was estimated from the time of the first symptoms attributable to the disease and/or the time of first detection of glycosuria.

Genomic DNA was extracted from peripheral blood leukocytes. The Bst NI polymorphism of β3-adrenergic receptor gene (ADRB3), which detects the Trp64Arg mutation, was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis according to the method of Widén et al. [3].

Statistical analysis

Results are given as mean ± SD. Differences between group means were tested by the Student's t-test. The chi-square test was used to compare frequencies. Relative risk (RR) and 95 % confidence interval (CI) were also calculated.

Results

A Trp64Arg mutation of the β3-AR was observed in 33.9 % of the subjects, including 10 homozygotes (3.4 %) for the mutation. Overall frequency of the mutated allele in Japanese subjects (18.6 %) was much higher than in Finnish subjects (11.4 %, $p < 1 \times 10^{-4}$) [3], French subjects (4.1 %, $p < 1 \times 10^{-7}$) [4] and Caucasians in the USA (9.1 %, $p < 0.02$) [2], but not Pima Indians (31.5 %, $p < 1 \times 10^{-7}$) [2].

The frequencies of mutated (Arg) allele and Arg/Arg genotype of ADRB3 were slightly, but not significantly, higher in NIDDM than in control subjects (20.5 vs 16.1 %, $p > 0.2$, 4.1 vs 2.4 %, $p > 0.4$, respectively) (Table 1). Since a similar tendency was observed in the Pima Indian and Finnish populations [2, 3] in which the frequencies of the mutation in both NIDDM and control subjects are known, we combined the data of the three population groups (Japanese 295, Pima Indian 642 and Finnish 491). The Arg/Arg genotype was significantly associated with NIDDM as compared with the other two genotypes ($p < 0.005$), with an RR of 2.13 (95 % CI 1.28–3.55). The Arg allele was also significantly associated with NIDDM ($p < 0.05$, RR 1.27, 95 % CI 1.06–1.52) (Table 1).

The BMI (mean ± SD) of the subjects was 22.8 ± 3.9 kg/m², with a range of 14.4–38.3. Japanese subjects with the Arg/Arg genotype had a significantly higher BMI than those with the Trp/Arg genotype ($p < 0.05$) or the Trp/Trp genotype ($p < 0.05$), as was also observed in Pima Indians [2] and in morbidly obese patients in France [4] (Table 2). Similarly, Japanese NIDDM patients homozygous for the Trp64Arg mutation tended to have an earlier age of onset of NIDDM (Table 2), as was also observed in Pima Indians [2] and Finns [3] (Table 2).

Discussion

Although there were similar tendencies towards a higher frequency of the Arg/Arg genotype of ADRB3 among NIDDM patients in the Japanese, Pima Indian and Finnish populations, this mutated genotype was not significantly associated with NIDDM (Table 1). Analysis of the combined data, however, suggests that the Arg/Arg genotype and the Arg allele were positively associated with NIDDM. The weak association observed within each population may be due to the small numbers of subjects homozygous for the mutation.

With respect to age-at-onset of NIDDM, Japanese patients homozygous for the Trp64Arg mutation tended to have an earlier onset of NIDDM, which was similar to those reported in Pima Indians [2] and Finns [3] (Table 2). Thus, this mutation appears to accelerate the course of NIDDM as well as contribute to its development, and this effect seems to be universal.

The frequency of the mutated allele in the Japanese was higher than those reported in other populations [2–4] except for Pima Indians [2]. The high frequency of the mutated allele in the Japanese whose BMI (22.8 ± 3.9 kg/m^2) was lower than those of several other ethnic groups suggests that the mutation itself appears not to be a major determinant of obesity in explaining the racial difference.

Although the Trp64Arg mutation of ADRB3 may not be a major determinant of obesity, it was associated with BMI in Japanese as well as in Pima Indians [2] and in morbidly obese patients in France [4] (Table 2), indicating that it may contribute to weight gain within each population. Taken together, these data suggest that the association of this mutation with weight gain is universal, but the effect is not particularly strong. Given that this mutation predisposes individuals to obesity early in life, it would in turn result in an early onset of NIDDM, but prospective studies in younger subjects are needed to further understand the influence of this mutation on the development of obesity and NIDDM.

The genetic heterogeneity in NIDDM, as suggested by previous studies, emphasizes the importance of genetic studies in NIDDM and other complex traits in different ethnic groups. In contrast to an almost complete lack of the Gly40Ser mutation of the glucagon receptor in Japanese [6] as compared with a 5–8% frequency in French and Sardinians with NIDDM [8], the Trp64Arg mutation of ADRB3 appears to be universally associated with susceptibility to and age-at-onset of NIDDM as well as BMI. Not all the subjects homozygous for Arg/Arg, however, were diabetic, indicating that the mutation itself is not sufficient for the development of NIDDM. These observations suggest that the influence of the Trp64Arg mutation on the development of NIDDM is not so strong compared with those of other candidate genes, such as the 3243 mutation of mitochondrial DNA in Japanese [9] and Gly40Ser mutation of glucagon receptor gene in French and Sardinians [8]. Thus, the Trp64Arg mutation of ADRB3 appears not to determine, but rather to modify the susceptibility to NIDDM, probably through its effect on metabolic rate and fat accumulation. The calculated relative risk of 2 in Trp64Arg homozygotes for NIDDM seems rather impressive in the light of the complex traits of polygenic disorders such as NIDDM.

Recently, Kadowaki et al. [10] reported similar results; i.e. this mutation is associated with higher BMI in non-diabetic Japanese subjects. In addition, they showed that the mutation was also associated with insulin levels, both fasting and 2-h after glucose load. A lack of detailed information on genotype frequencies in NIDDM patients in their study, however, prevented us from comparing their results with those of our association study with NIDDM.

In conclusion, the high frequency of the Trp64Arg mutation in non-obese Japanese subjects suggests that the mutation itself is not a major determinant of obesity. Within the Japanese population, however, this mutation was associated with higher BMI and earlier age-at-onset of NIDDM, as in other ethnic groups, suggesting that the contribution of this mutation to weight gain and age-at-onset of NIDDM appears to be universal. In addition, combined data analysis suggested that the Trp64Arg mutation is also significantly associated with the development of NIDDM.

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