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Association of the Trp64Arg Mutation of the β_3-Adrenergic Receptor with Diabetes Mellitus, Impaired Glucose Tolerance and Lifestyle in Japanese Workers

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In order to investigate whether the Trp64Arg (a missense mutation of tryptophan for arginine at position 64 codon) polymorphism of the β_3-adrenergic receptor (β_3-AR) gene is related to the incidence of non-insulin-dependent diabetes mellitus (NIDDM) and impaired glucose tolerance (IGT), a retrospective cohort study among Japanese workers was conducted. The subjects were Japanese workers at an occupational site in Shimane Prefecture. Informed consent was obtained from 492 workers. The baseline data were obtained at the regular health examination in 1992 and a retrospective cohort study was performed for analyzing the incidence of NIDDM and IGT in 1998. The Trp64Arg polymorphism β_3-AR gene for each worker was detected by the single strand conformation polymerase analysis. Relative risks were calculated by the logistic regression analysis. The rates of Trp64Trp (TT), Trp64Arg (TA) and Arg64Arg (AA) genotypes were 66.3%, 31.1% and 2.6%, respectively. The relative risk of (TA + AA) against TT for the incidence of NIDDM and IGT by univariate analysis was 1.37 (95% confidence interval, 0.69–2.72). The relative risk of (TA + AA) against TT for the incidence of NIDDM and IGT adjusted for confounders in a multiple logistic regression model including age, gender, family history, body mass index, alcohol consumption, eating habits and exercise was 1.31 (95% confidence interval, 0.65–2.67). The present findings suggested that a weak association between Trp64Arg polymorphism of the β_3-AR gene and the incidence of NIDDM and IGT.

Key words: β_3-Adrenergic receptor; impaired glucose tolerance; multiple risk factors; non-insulin-dependent diabetes mellitus; Trp64Arg polymorphism

Several case-control studies revealed that the polymorphism of the β_3-adrenergic receptor (β_3-AR) gene (Trp64Arg) is associated with the development of non-insulin-dependent diabetes mellitus (NIDDM), insulin resistance, obesity and syndrome X (Reaven, 1988). It was also reported that the Trp64Arg of the β_3-AR gene is significantly associated with the development of NIDDM and obesity in Pima Indians (Wallston et al., 1995), Finns (Widen et al., 1995) and French Caucasians (Clement et al., 1995). Moreover, the locus of Trp64Arg mutation was reported to be associated with the development of obesity and hyperinsulinemia in Japanese (Yoshida et al., 1995; Kadowaki et al., 1995; Fujisawa et al., 1996). However, other studies suggested that the polymorphism of the β_3-AR gene (Trp64Arg mutation) is not associated with the development of NIDDM and obesity (Li et al., 1996; Oksanen et al., 1996; Urhammer et al., 1996; Nagase et al., 1997; Ueda et al., 1997; Azuma et al., 1998). Although many

Abbreviations: β_3-AR, β_3-aderenergic receptor; BMI, body mass index; CI, confidence interval; IGT, impaired glucose tolerance; NIDDM, non-insulin-dependent diabetes mellitus; PCR, polymerase chain reaction; RR, relative risk; Trp64Arg, a missense mutation of arginine for tryptophan at position 64 codon

case-control studies regarding Trp64Arg have been conducted, there have been few cohort studies. Moreover, various lifestyle-related habits such as eating and drinking habits are associated with the development of NIDDM, in addition to a hereditary predisposition. Therefore, we conducted a cohort study concerning possible lifestyle habits relating NIDDM in Japanese workers with similar occupations to clarify the genetic risk factors for NIDDM.

Subjects and Methods

The ethics committee of the Tottori University Faculty of Medicine approved this research. The subjects were 492 staff members at an occupational site in Shimane Prefecture above 40 years of age (301 males and 191 females) who gave informed consent for gene examinations during a regular health examination in 1998. Baseline data were obtained at consultation in 1992, and a retrospective cohort study was conducted to investigate the incidence of NIDDM and impaired glucose tolerance (IGT) over a 7-year observation period. Those who had NIDDM or IGT in 1992 were excluded from the study.

As possible risk factors for NIDDM and IGT, drinking habits, frequency of exercise, eating habits between meals, food intake, midnight meals and preference of seasoning of meals were evaluated. As described below, hereditary predispositions for NIDDM were evaluated by identifying the polymorphism of the β_3-AR gene (Trp64Arg) using the genomic DNA. According to the diagnostic criteria proposed by the American Diabetes Association, the development of NIDDM and IGT was defined as the exhibition of fasting blood glucose levels above 126 mg/dL at least once during the 7-year observation period. The body mass index (BMI) was calculated by dividing body weight (kg) by a square of height (m).

Blood samples were collected in a tube containing EDTA-Na as an anticoagulant, and the genomic DNA was extracted from 100 μL of whole blood using MagExtractor, a full automatic DNA extractor (MFX-2000, Toyobo, Osaka, Japan). Subsequently, the β_3-AR gene was amplified by the standard polymerase chain reaction (PCR) method. PCR was performed in total of 10 μL, containing 0.04 μg of DNA, batch of 1 μmol/L of sense and antisense primers (sense: 5'-TTC CGT GGG AGG CGG CCC TAG-3', antisense: 5'-CAC CAC CAG GAG TCC CAT CAC C-3'), AmpliTaq Gold 0.1 U (Applied Biosystems Japan, Inc., Tokyo, Japan), dNTPs 200 μmol/L (Applied Biosystems Japan), and 10 × buffer 1 μL recommended by manufacture's company. After initial heat denaturation at 95˚C for 10 min, 35 cycles of 3 steps each, consisting of heat denaturation at 95˚C for 30 s, annealing at 65˚C for 30 s and extension at 72˚C for 30 s, were carried out, followed by extension at 72˚C for 5 min. Allelic genotypes were determined by single strand conformational polymorphism analysis (Fig. 1). One microgram each of the PCR products was mixed with denaturing solution, and denatured at 95˚C for 5 min. Subsequently, the PCR products were rapidly cooled on ice, and then electrophoresed by using mini gels containing 12% of acrylamide and 5% of glycerol, at 24˚C for 6 h. After electrophoresis, bands were visualized by standard silver staining. Initially, by sequencing after cloning into a TA vector, each band pattern was confirmed as to whether it had a wild type allele or polymorphism. After that, diagnosis was made by comparison of the band pattern with initial data.

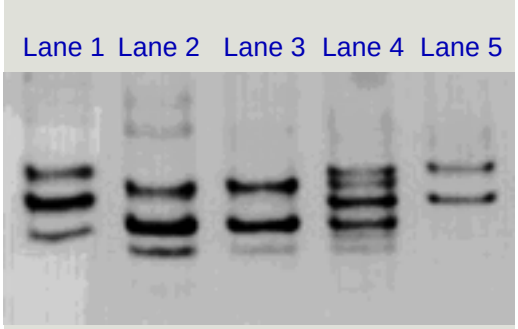

Fig. 1. Genotype determination of the β_3-adrenergic receptor with the polymerase chain reaction-single strand conformation polymorphism analysis. Lane 1 is the plasmid of a wild type and lane 2 is the polymorphism type. Three allelic genotypes were determined as to whether they had a wild type allele or a polymorphism. Lane 3 is Arg64Arg, lane 4 is Trp64Arg and lane 5 is Trp64Trp.

Table 1. Properties of cohort and baseline lifestyle statuses

Items		Trp64Trp	Trp64Arg	Arg64Arg
Sample size		326 (66.3)	153 (31.1)	13 (2.6)
Age	(year)	42.33 ± 5.33	42.52 ± 5.12	43.92 ± 6.18
BMI	(kg/m^2)	22.57 ± 2.57	22.94 ± 2.80	22.85 ± 2.87
Presence of family history		26 (8.0)	10 (6.5)	0 (0.0)
Presence of drinking habits		202 (62.0)	108 (70.6)	6 (46.2)
Absence of exercise		298 (91.4)	137 (89.5)	13 (100.0)
Skipping meals		267 (81.9)	135 (88.2)	11 (84.6)
Eating between meals		219 (67.2)	99 (64.7)	8 (61.5)
Neglect the balance of meals		284 (87.1)	132 (86.3)	12 (92.3)

( ), the percentage of indivisual item.
BMI, body mass index.

Statistical analysis was performed using StatView-J 5.0 for windows (Abacus Concepts, Inc., Berkeley, CA). Each value for age and BMI was expressed as mean ± SD. Relative risks (RRs) of Trp64Arg and lifestyles for NIDDM and IGT were calculated using the multiple logistic regression analysis.

Results

The frequency of Trp64Arg mutation was investigated in 492 Japanese workers using genomic DNAs. As a result, the allelic frequency of Trp64Arg was 20.8%. The proportions of genotype for TT, TA and AA were 66.3%, 31.1% and 2.6%, respectively.

As shown in Table 1, the total mean age was 42.43 ± 5.30 years old in 1992, and there were no significant differences in age among subjects with the respective allelic genotypes. In addition, the total mean BMI was 22.69 ± 2.65 in 1992, and there were no significant differences in BMI by allelic genotypes. Moreover, there were no significant differences in various lifestyle-related items among subjects with the respective allelic genotypes.

As possible risk factors for NIDDM, the presence or absence of Trp64Arg, BMI, age, drinking habits, frequency of exercise, eating habits between meals, foods intake, midnight meals and preference of seasoning of meals were analyzed by both univariate and multivariate logistic analyses (Table 2). Concerning Trp64Arg, univariate analysis showed an RR of 1.37 (95% CI: 0.69–2.72), whereas multivariate analysis showed an RR of 1.31 (95% CI: 0.65–2.67). Therefore, it was considered that

Table 2. Relative risks of possible risk factors for NIDDM and IGT

Items		Univariate analysis		Multivariate analysis	
		RR	95% CI	RR	95% CI
Trp64Arg	Hetero+homo/wild	1.37	0.69–2.72	1.31	0.65–2.67
Family history	Present/absent	1.60	0.53–4.81	1.53	0.49–4.75
Age		1.06	1.00–1.12	1.06	1.00–1.12
Gender	Female/male	0.17	0.06–0.50	0.27	0.07–0.99
BMI		1.00	0.88–1.14	0.98	0.86–1.11
Presence of drinking habits	Yes/no	3.85	1.47–10.08	1.54	0.47–5.00
Meals	Eat 3 times a day/skip	1.49	0.67–3.40	1.33	0.56–3.16
Balance of meals	Neglect /consider	1.76	0.52–5.89	1.36	0.40–4.86
Eating between meals	Yes /no	0.45	0.23–0.89	0.75	0.36–1.55
Taking exercise	Yes /no	1.12	0.33–3.82	1.44	0.41–5.06

BMI, body mass index; CI, confidence interval; IGT, impaired glucose tolerance; NIDDM, non-insulin-dependent diabetes mellitus; RR, relative risk.

the Trp64Arg mutation of the β_3-AR gene slightly increases the risk of NIDDM and IGT, although the RR was not significant. Moreover, gender (female/male) was the greatest risk for NIDDM and IGT; i.e., univariate analysis showed an RR of 0.17 (95% CI: 0.06–0.50), while multivariate analysis showed an RR of 0.27 (95% CI: 0.07–0.99), demonstrating significantly higher risks for NIDDM and IGT in males than in females. Concerning drinking habits, univariate analysis alone showed a significant RR of 3.85 (95% CI: 1.47–10.08). However, no other lifestyle-related habits showed a significantly higher RR for NIDDM.

Discussion

There were no significant differences in mean age and BMI among subjects with the respective allelic genotypes in 1992. The rates of TT, TA and AA genotypes were 66.3%, 31.1% and 2.6%, respectively. These findings were compatible with Hardy-Weinberg's law. In addition, allelic frequencies in this study did not markedly differ from those reported by previous studies in Japanese.

In this cohort study, univariate analysis showed an RR of 1.37, and multivariate analysis showed an RR of 1.31, suggesting a possible relationship between the Trp64Arg gene and the development of NIDDM and IGT, although the RR was not significant. Among various lifestyle-related habits, none showed any significant association with NIDDM and IGT. This was probably because the relationship between NIDDM and IGT and various lifestyle-related habits were evaluated together with gender; i.e., gender is closely associated with lifestyle relating NIDDM and IGT. Therefore, we reanalyzed these habits excluding gender. As a result, drinking habits showed an RR of 3.11 (95% CI: 1.13–8.58), suggesting that drinking habits may be a significant risk factor for NIDDM and IGT. However, Trp64Arg showed an RR of 1.29 (95% CI: 0.64–2.62), and this level of RR was similar to the results of analysis including gender. Therefore, the results of this cohort study suggest that the Trp64Arg mutation may slightly increase the risk of NIDDM and IGT, because the propriety of this analytical model was confirmed.

However, this cohort study has some controversial issues because the amount of food ingestion including salt and sugar, and the amount of alcohol intake and exercise in individual subjects depended on subjective data based on self-assessment, and were not numerically evaluated during a questionnaire survey.

In the present study, none of these items including the Trp64Arg and lifestyle-related items such as age, family history and BMI showed any significant association with NIDDM and IGT, except for gender. In addition, the results of other case-control studies on allelic frequencies in Japanese were similar to ours (Awata and Katayama, 1996; Nagase et al., 1997; Ueda et al., 1997; Azuma et al., 1998). Fujisawa et al. (1996) also reported results similar to those of previous case-control studies, except for a slightly higher odds ratio of 1.72. Therefore, it is considered that this cohort study using a superior methodology reconfirmed the results of previous case-control studies.

Currently, we have investigated a total of 492 subjects. However, further accumulation of cases and long-term follow-up may reveal significant associations of certain lifestyle-related items with NIDDM and IGT.

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