Contribution of a Missense Mutation (Trp64Arg) in β3-Adrenergic Receptor Gene to Multiple Risk Factors in Japanese Men with Hyperuricemia


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Abstract. Epidemiological data reveal that hyperuricemia is a risk factor of atherosclerosis. The risk is possibly caused by a link between hyperuricemia and insulin resistance-related metabolic syndrome. Recently it has been proposed that a missense mutation (Trp64Arg) in the β3-adrenergic receptor (β3-AR) gene may contribute to the accumulation of multiple risk factors related to insulin resistance. The present study was undertaken to further clarify an association between the Trp64Arg mutation and the metabolic syndrome in 47 Japanese men with hyperuricemia, who are substantially at high risk of atherosclerosis. One patient (2%) had the homozygous mutation, 12 (26%) were heterozygous for the mutation, and 31 (72%) had no mutation found by the PCR-RFLP analysis. The Trp64Arg mutation was not related to past maximal body mass index (BMI), BMI and waist/hip ratio. The subjects with the heterozygous mutation showed a slightly higher incidence of impaired glucose tolerance and diabetes mellitus in the 75 g oral glucose challenge (67%), as compared with those without the mutation (39%). Serum insulin response at 60 min and the sum of serum insulin in the glucose challenge were greater in the former subjects than those in the latter subjects (P=0.041 and 0.076, respectively). An increase in serum lipoprotein(a) was also observed in the subjects with the heterozygous mutation, but the Trp64Arg mutation was not associated with other dyslipidemia, blood pressure or ischemic changes on the electrocardiogram. These results indicate that the heterozygous mutation of Trp64Arg in the β3-AR gene partly contributes to the accumulation of multiple risk factors in male subjects with hyperuricemia. A larger prospective study is necessary to elucidate a possible role of the Trp64Arg mutation in atherosclerotic diseases in future.

Key words: Hyperuricemia, Multiple risk factors, Trp64Arg mutation, β3-Adrenergic receptor, Hyperinsulinemia

β3-ADRENERGIC receptor (β3-AR) is expressed in brown and white adipose tissues in rodents and in visceral fat in humans [1–3], and participates in the regulation of lipolysis and thermogenesis [4, 5]. A role of a missense mutation in the β3-AR gene, that results in the replacement of tryptophan by arginine (Trp64Arg) in the first intracellular loop of the receptor, has been investigated in several ethnics, including Pima Indians [6], Finns [7, 8], French Caucasians [9], Australians [10] and Japanese [11–13]. Decreased resting metabolic rate, weight gain and obesity are found in the subjects with the Trp64Arg mutation in a homozygous form.
The mutation is also associated with hyperinsulinemia, insulin resistance, hypertension and earlier onset of non-insulin-dependent diabetes mellitus (NIDDM) [6, 7, 11, 13]. Therefore, the genotype of β3-AR may be one of the genetic determinants of body weight gain, clustering of multiple risk factors and development of NIDDM.

Hyperuricemia is recognized as a risk factor in atherosclerotic diseases, especially coronary heart disease [14], although there is little evidence that uric acid accumulation can directly promote atherosclerosis. Since hyperuricemia is closely associated with insulin resistance [15, 16] and clustering of multiple risk factors such as visceral fat accumulation, hypertension and dyslipidemia [17], the metabolic syndrome may alternatively contribute to atherosclerotic sequelae. The present study was undertaken to further clarify a link between the Trp64Arg missense mutation and the metabolic syndrome in Japanese male subjects with hyperuricemia, a population at high risk of atherosclerosis.

Subjects and Methods

A total of 47 Japanese subjects with hyperuricemia were studied in the special outpatient clinic for gout in Tokyo Metropolitan Komagome Hospital between January and December, 1996; 39 subjects had been regularly treated, and the remaining 8 were newly referred to the clinic during the period of the study. They were all male, aged 23–82 years old (55 ± 12 years old, mean ± SD). A few male subjects with hyperuricemia due to diuretics and female subjects were all excluded. Six subjects were asymptomatic but having a serum uric acid level higher than 8 mg/dl before treatment, and the onset of gout in the other 41 patients varied in age from 22 to 60. Six patients had a history of urinary stones. Four patients were treated with probenecid, 7 with benz bromarone and 31 with allopurinol, when they were examined in the present study. Clearance of uric acid was 5.8 ± 1.8 ml/min before the administration of the drugs, and serum levels of uric acid were 6.2 ± 1.2 mg/dl at the time of the study. All the subjects gave their informed consent to join the present study.

We determined the past maximal body mass index (BMI), BMI, waist/hip ratio (W/H ratio) and blood pressure. Fasting blood samples were subjected to measurements of total cholesterol, HDL cholesterol, triglyceride, lipoprotein(a) [Lp(a)] and leptin. Serum uric acid was measured by the uricase method, total cholesterol and triglyceride by the enzymatic methods, and HDL cholesterol by the precipitation method, respectively, with an automatic clinical analyzer (Model 736, Hitachi Co., Katsuta, Japan). Serum Lp(a) was assayed with an immunoturbidimetric assay kit (Daichi Pure Chemicals, Co. Ltd., Tokyo, Japan). Serum leptin level was measured with a commercial radioimmunoassay kit (Linco Research Inc., St. Charles, USA) [18]. The reference ranges of male control subjects in Tokyo Metropolitan Komagome Hospital were as follows: serum uric acid, 3.5 to 8.2 mg/dl, total cholesterol, 125 to 255 mg/dl, HDL cholesterol 34 to 48 mg/dl, triglyceride, 30 to 150 mg/dl, and Lp(a), less than 20 mg/dl, respectively. Glucose tolerance was assessed by a 75 g oral glucose challenge after an overnight fast. Blood collections were made before and 30, 60, 90 and 120 min after the load. Diabetes mellitus (DM) and impaired glucose tolerance (IGT) were diagnosed by the criteria of the World Health Organization. We also evaluated urinary excretion of albumin, the cardiothoracic ratio on chest x-ray film and ischemic changes on the electrocardiogram (Minnesota code I,1-3; IV, 1-4; V, 1-3).

The Trp64Arg mutation was detected by a PCR-restriction fragment length polymorphism analysis. Briefly, the PCR was carried out with 100 ng genomic DNA from peripheral leukocytes with the primers as described previously [7]. The PCR products were digested at 37 °C for 2 h with a restriction enzyme Mva I (Takara Biomedicals, Tokyo, Japan), and the digested samples were separated by electrophoresis on 4% agarose gel and stained with ethidium bromide. The band of a 161 bp fragment instead of 99 bp and 62 bp fragments indicates the presence of the Trp64Arg mutation in the β3-AR gene.

All data are shown as means ± SD. One-way factorial ANOVA accompanied with Fisher's multiple comparison test, χ²-test and unpaired t-test were used to compare the difference. A P value of less than 5% was considered significant.
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Results

In the 47 patients with hyperuricemia, one patient (2%) had the Trp64Arg mutation in a homozygous form, 12 patients (26%) were heterozygous for the mutation, and the remaining 34 patients (72%) had no mutation in the β3-AR gene (Trp64Trp homozygote, wild type). The frequency of the mutant allele was 15% (14/94) in the present study.

Among the three genotypes there were no differences in age, age of the onset of gout, family history of NIDDM and hypertension, smoking and drinking habit, clearance of uric acid or serum uric acid levels (Table 1). No difference was observed in the past maximal BMI, BMI or W/H ratio. As only a single subject had the homozygous Trp64Arg mutation, that subject was excluded from the following analysis.

Table 2 shows the results of the 75 g glucose tolerance test. IGT and DM were found to a greater extent, but not significantly (P=0.1182), in the Trp64Arg heterozygotes (67%), as compared with the subjects without the mutation (39%). Figure 1 shows the responses of plasma glucose and serum insulin to the 75 g oral glucose challenge, excluding the subjects with DM. There was no difference between the Trp64Arg heterozygotes and the subjects without the mutation in plasma glucose levels. Serum insulin levels at 60 min were greater in the patients with the heterozygous mutation (95 ± 52 μU/ml) than those without the mutation (58 ± 23 μU/ml) (P=0.041). The sum of serum insulin at each point was also slightly higher in the former subjects (298 ± 154 μU/ml) than that in the latter subjects (225 ± 91 μU/ml) (P=0.076). Greater insulin response to the oral glucose challenge in the heterozygotes was more evident in the subjects with IGT (data not shown).

The relationships between the Trp64Arg mutation and lipid metabolism, serum leptin levels and cardiovascular and renal disorders are shown in Table 3. Serum Lp(a) levels were significantly higher in the Trp64Arg heterozygotes than those without the mutation. There was no difference in the other items listed in Table 3.

Discussion

It has been reported that the Trp64Arg mutation in the β3-AR gene is associated with obesity and earlier onset of NIDDM in Japanese [11, 13]. Conversely, other reports showed that the frequency of the Trp64Arg mutation does not differ among the patients with obesity and/or NIDDM
and the normal control subjects [12, 19]. In the present study, the frequency of the Trp64Arg mutation was similar to, or a little lower than, that in the normal Japanese subjects in the literature (17–20%) [13, 20], and the mutation was independent of the onset-age and patho-physiological features of gout.

In the present study, the Trp64Arg mutation was not related to the past maximal and present BMI and W/H ratio. The W/H ratio was great in the subjects as a whole, suggesting the presence of visceral fat accumulation. In female subjects, the mutation was closely linked to the increased BMI and W/H ratio in Finns [8], and to the lower basal metabolic rate in Australians [10] and Japanese [12]. It seems possible that the effect of the mutation is weaker in males. In Japanese men aged 50–59 years old, the mean BMI has increased from 21.3 in 1950, to 22.2 in 1975 and to 23.4 in 1994 [21], suggesting that excessive energy intake and/or decreased...
energy expenditure does occur extensively in those subjects. Such environmental factors may weaken the effect of the mutation in the male Japanese subjects with hyperuricemia.

The prevalence of IGT and DM tended to be high in the subjects with the Trp64Arg mutation in a heterozygous form. Insulin secretion in response to the 75 g oral glucose challenge was higher in those subjects than that in the subjects without the mutation, despite the similar BMI and W/H ratio in these two groups (there was also no difference in the analysis excluding diabetic subjects, data not shown). Similar results were demonstrated in non-diabetic Finnish subjects [7]. Another study revealed that the Trp64Arg mutation is associated with higher BMI and fasting serum insulin, and enhanced response of insulin to the oral glucose challenge in obese Japanese subjects [11]. Thus, the hyperinsulinemia may be associated with higher BMI in that study. The present results suggest that the Trp64Arg mutation could be directly related to hyperinsulinemia and possibly insulin resistance. The mechanism whereby the mutation is implicated in insulin resistance remains undetermined, but this mutation may affect production of free fatty acid in adipose tissues and result in insulin resistance and hyperinsulinemia [22]. Since insulin resistance plays a key role in pathogenesis of NIDDM, the Trp64Arg mutation may accelerate the onset of IGT and DM in patients with hyperuricemia.

The alterations in serum cholesterol, HDL cholesterol and triglyceride varied in previous studies [7, 19, 20]. We did not find any relation between the Trp64Arg mutation and those lipid parameters. Serum Lp(a) concentrations were high in the patients with the heterozygous Trp64Arg mutation in the present study. As serum Lp(a) levels are generally increased in diabetic patients [23, 24], the greater frequency of IGT and DM in the patients with the mutation may raise serum Lp(a) concentrations. As higher insulin levels were found in those subjects, insulin is also one of the factors that may increase Lp(a). However, there was little evidence linking hyperinsulinemia to an increase in serum Lp(a) [25]. Further study is necessary to elucidate the exact mechanism increasing Lp(a) levels in subjects with the Trp64Arg mutation.

It is recognized that patients with gout are in general at high risk for atherosclerotic diseases including hypertension, ischemic heart disease and cerebrovascular disorders [14–17]. The subjects with the Trp64Arg mutation in a heterozygous form had either higher systolic blood pressure in obese Japanese subjects [11, 20] or higher diastolic pressure in elderly female Australians [10] and in non-diabetic Finns [7]. In those studies the patients had high levels of serum insulin, which might be closely linked to hypertension [7, 11, 20]. In the present study, the Trp64Arg mutation was not associated with current hypertension or cardiovascular disorders in the patients with hyperuricemia. Since the patients with the mutation had several lines of risk factors as mentioned above, we should carefully follow the occurrence of cardio- and cerebro-vascular events in those subjects.

In conclusion, we evaluated a role of the Trp64Arg mutation of the β3-AR gene in obesity, glucose tolerance, lipid metabolism and cardiovascular disorders in the 47 Japanese male patients with hyperuricemia who are substantially at high risk of atherosclerosis. The heterozygous Trp64Arg mutation was linked to a slightly higher incidence of IGT and DM with high insulin secretion in the 75 g oral glucose tolerance test, and to increased serum Lp(a) levels, but the mutation was not related to obesity, other lipid metabolism or current cardiovascular disorders. The present results indicate that the heterozygous Trp64Arg mutation partly contributes to the accumulation of multiple risk factors in the Japanese male subjects with hyperuricemia. Larger crosssectional and prospective studies are necessary to elucidate a possible role of the Trp64Arg mutation in the accumulation of multiple risk factors and the incidence of atherosclerotic events in future.

References

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