Original Article

Association of a Sequence Variation in the Gene Encoding Adiponectin Receptor 1 (*ADIPOR1*) with Body Mass Index in the Japanese Population

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Abstract

Background: Adiponectin is a circulating peptide present in adipose tissue. It mediates its insulin-sensitizing and anti-atherogenic effects on target tissues via 2 receptors—adiponectin receptors 1 and 2 (*ADIPOR1* and *ADIPOR2*). Recent studies have reported that *ADIPOR1* deficiency in mice results in increased body weight and obesity. In the present study, we examined the association between a single nucleotide polymorphism (SNP) in the 5' flanking region of the *ADIPOR1* gene and body mass index (BMI) in the Japanese population.

Methods: Association of an SNP (rs4989513) with the BMI was examined in 774 postmenopausal Japanese women.

Results: Rs4989513 SNP was significantly associated with the BMI ($P = 0.0412$).

Conclusion: Genetic variation at the *ADIPOR1* gene locus is associated with BMI, suggesting that the *ADIPOR1* gene is involved in the development of obesity.

KEY WORDS: single nucleotide polymorphism (SNP), body mass index (BMI), obesity, adiponectin, adiponectin receptor 1 (*ADIPOR1*)

Introduction

Obesity is a risk factor for diabetes, hypertension, dyslipidemia, and cardio-vascular disease and is associated with physical function or health perception ^{1,2)}. The data of the National Health and Nutrition Examination Survey (NHANES) showed that 66% of adults in the United States were overweight (body mass index (BMI) > 25 kg/m²) and 32% were obese (BMI > 30 kg/m²) in 2003–2004, while 56% were overweight and 20% were obese in 1993–1994 ^{3,4)}. In 2004, the Ministry of Health and Welfare of Japan reported that 27% of males and 21% of females over 14 years old were obese according to the Japanese criteria for determining obesity (BMI > 25 kg/m²) ⁵⁾. With more than 1 billion overweight or obese individuals, the World Health Organization has declared obesity as a global epidemic ^{6,7)}. These data suggest that obesity is a serious health problem in industrialized as well as developing countries, and its prevention is an important issue.

Adipose tissue is a major reservoir for energy storage and secretes multiple factors that regulate energy homeostasis in the body ⁸⁾. The importance of adipose tissue as an endocrine organ was highlighted by the discovery of the leptin hormone ⁹⁾. Another hormone that is exclusively secreted by the adipose tissue is adiponectin (Acrop30, AdipoQ, apM1, or GBP28) ^{10–13)}. Low

adiponectin levels are found in obesity, and reduced adiponectin levels have been correlated with an increased risk of developing obesity and related diseases ^{11,14,15)}. Treatment with adiponectin has both anti-atherosclerotic and anti-diabetic effects in animals ^{16–20)}.

Two adiponectin receptors—*ADIPOR1* and *ADIPOR2*—have been identified ²¹⁾. These receptors mediate fatty acid oxidation and glucose uptake by adiponectin, resulting in increased AMP kinase and peroxisome proliferator-activated receptor α ligand activities ^{14,15)}. *ADIPOR1* is highly expressed in human adipose tissue. *ADIPOR1* expression in the adipose tissue is reduced in obese subjects and increases after weight loss ²²⁾. A recent study has shown that *ADIPOR1* knockout in mice resulted in increased body weight and obesity ²³⁾. These data suggest that the expression level of *ADIPOR1* may affect the development of overweight and obesity. The present study aims to determine the putative contribution of a genetic variation of the *ADIPOR1* gene to BMI in the Japanese population.

Materials and Methods

Subjects

We analyzed the genotypes of DNA samples obtained from 774 healthy postmenopausal Japanese women (mean age (SD), 65.7 (7.2)) living in central Japan. The exclusion criteria included the presence of endocrine and metabolic disorders such as hyperthyroidism, diabetes mellitus, liver disease, and renal disease; use of medications known to affect body size (e.g. corticosteroids); or unusual gynecological history. Their mean weight was 51.2 ± 7.3 kg; mean height, 150.8 ± 5.7 cm; and mean BMI, 22.5 ± 3.1 kg/m². All were non-related volunteers and provided informed consent before the study was initiated. Ethical approval for the study was obtained from the ethics committees of the University of Tokyo and the Research Institute and Practice for Involuntal Diseases.

Determination of a single nucleotide polymorphism in the 5' flanking region of the human *ADIPOR1* gene

We extracted a polymorphic variation at the 5' flanking region of the *ADIPOR1* gene (rs4989513, -9388) from the Assays-on-Demand SNP Genotyping Products database (Applied Biosystems, Foster City, CA). We determined the rs4989513 polymorphism using the TaqMan (Applied Biosystems) polymerase chain reaction (PCR) method²⁴. To determine the rs4989513 single nucleotide polymorphism (SNP), we used Assays-on-Demand SNP Genotyping Products C_454718_10 (Applied BioSystems), which contains sequence-specific forward and reverse primers and 2 TaqMan MGB probes for detecting alleles. During the PCR cycle, 2 TaqMan probes competitively hybridize to a specific sequence of the target DNA, and the reporter dye is separated from the quencher dye, resulting in increased fluorescence of the reporter dye. The fluorescence levels of the PCR products were measured using the ABI 7500 Fast Real-Time PCR System (Applied Biosystems), using which 3 genotypes of the SNP were clearly identified.

Measurement of BMI and biochemical markers and statistical analysis

In addition to body measurements, we measured the serum concentrations of total cholesterol (TC) and triglyceride (TG). The subjects were classified into groups on the basis of the *ADIPOR1*

SNP genotypes; age, height, body weight, BMI, TC, and TG in these groups were compared using the Kruskal-Wallis test and Mann-Whitney U test. *p* values less than 0.05 were considered significant. Analysis was performed using the StatView-J 4.5 software (SAS Institute Inc., Cary, NC).

Results

We analyzed the genotypes for the SNP in the 5' flanking region of the *ADIPOR1* gene (rs4989513) in 774 subjects by using the TaqMan method. Among these postmenopausal Japanese women, 241 were GG homozygotes (31.1%), 338 were GA heterozygotes (43.7%), and 195 were AA homozygotes (25.2%) (Table 1). The allele frequencies of this SNP in the present study were in the Hardy-Weinberg equilibrium. The data of the allele frequencies were consistent with those reported in the HapMap project for the Japanese (www.hapmap.org, accessed 8th January 2009). According to the HapMap project data, in the Japanese population, 35.6% individuals are GG homozygotes; 44.4%, GA heterozygotes; and 20.0%, AA homozygotes.

The baseline characteristics of the subjects on the basis of the SNP rs4989513 are shown in Table 1. A significant difference was observed in the BMI of subjects with different genotypes of rs4989513 ($p = 0.0412$ by the Kruskal-Wallis test among the 3 genotype groups and $p = 0.0105$ by the Mann-Whitney U test between the GG and AA genotype groups; Table 1 and Figure 1A). These data suggest that the A allele may be the risk allele for the development of overweight or obesity. We also compared the BMI between the subjects bearing at least one risk allele (AA + GA) and those without any risk allele (GG). The BMI in the AA + GA group was significantly higher than that in the GG group ($22.7 (3.1)$ vs. $22.1 (2.9)$; $p = 0.0270$; Fig 1B). Furthermore, we compared age, height, and body weight among the 3 genotypes. We found that subjects with the GG genotype were taller than those with the GA genotype ($p = 0.0207$ by the Mann-Whitney U test). However, age, body weight, TC, and TG were not statistically different among the 3 genotypes (Table 1).

Table 1 Distribution of characteristics in the present study divided according to the genotypes of rs4989513 at the 5' flanking region of *ADIPOR1* gene

Items	Genotype (mean $\pm$ SD)			<i>p</i> value (Kruskal-Wallis)	<i>p</i> value (Mann-Whitney U test)
	GG	GA	AA		
Number of subjects	241	338	195		
Age (years)	65.6 $\pm$ 7.1	65.9 $\pm$ 7.1	65.3 $\pm$ 7.4	NS	NS
Height (cm)	151.4 $\pm$ 5.6	150.3 $\pm$ 5.7	150.7 $\pm$ 5.8	NS	0.0207 (GG vs GA)
Body weight (kg)	50.7 $\pm$ 7.4	51.0 $\pm$ 8.1	51.9 $\pm$ 7.5	NS	NS
BMI (kg/m ²)	22.1 $\pm$ 2.9	22.6 $\pm$ 3.3	22.8 $\pm$ 2.8	0.0412	0.0141 (GG vs AA)
TC (mg/dl)	197.2 $\pm$ 35.1	197.5 $\pm$ 35.7	198.6 $\pm$ 35.9	NS	NS
TG (mg/dl)	138.0 $\pm$ 66.2	145.3 $\pm$ 81.6	136.2 $\pm$ 78.4	NS	NS

Statistical analysis was performed according to the method described in the text.
BMI; body mass index, TC; total cholesterol, TG; triglyceride, NS; not significant

Fig. 1 Association between the single nucleotide polymorphism (SNP) genotypes (GG, GA, and AA genotypes) in the 5' flanking region of the *ADIPOR1* gene and the body mass index (BMI).

- (A) BMI values for the GG, GA, and AA genotypes are shown. The BMI is expressed as mean (SE). The number of subjects is shown in parentheses. The association of this SNP with the BMI was determined by performing the Kruskal-Wallis test among the 3 genotype groups or the Mann-Whitney U test between the GG and GA genotype groups.
- (B) The association of this SNP with the BMI was also determined by performing the Mann-Whitney U test between the GG and AA + GA genotype groups.

Discussion

Adiponectin is a circulating peptide belonging to the complement-1q family, and it is mostly expressed in and secreted by adipose tissue^{14,15}. Many studies have suggested that adiponectin acts as an anti-atherogenic and anti-diabetic adipokine. Plasma levels of adiponectin are decreased in individuals with obesity and with diseases related to obesity^{14,15}. In contrast, weight loss increases the plasma adiponectin levels in obese humans¹¹. Furthermore, the role of adiponectin in health and disease is supported by the fact that the adiponectin gene, *ADIPOQ*, is located on chromosome 3q27, where genomewide scans have mapped a susceptibility locus for type 2 diabetes and the metabolic syndrome²⁵. Since *ADIPOR1* and *ADIPOR2* mediate the effects of adiponectin on target tissues, they are considered as potential genetic markers for obesity, type 2 diabetes, and the metabolic syndrome. However, previous studies have found inconsistent associations between the SNPs in *ADIPOR1* and type 2 diabetes²⁶. In contrast, recent studies have reported that SNPs in the *ADIPOR1* gene are associated with BMI in Finnish and Mexican Americans^{27,28}. The rs6666089 SNP at 5' flanking region of the *ADIPOR1* gene was significantly associated with BMI in Finnish population²⁷. Minor allele frequency (MAF) of the SNP in Caucasian is 0.325 in HapMap SNP database and MAF in Japanese is only 0.056. Thus, we searched for another common SNP in the HapMap SNP database at the 5' flanking region of the *ADIPOR1* gene in the HapMap SNP database. We found the rs4989513 SNP which located 883 base upstream from the rs6666089. The MAF of the SNP is 0.422 in the HapMap Japanese database. Therefore, we selected a common rs4989513 SNP of which the MAF is 0.470 in the present analysis. In the present study, we found a significant association of an *ADIPOR1* variation with BMI in postmenopausal Japanese women. To the

best of our knowledge, the present study is the first to investigate the influence of a polymorphism of the *ADIPOR1* gene on the BMI in the Japanese population.

ADIPOR1 deficiency in mice results in increased body weight and obesity; this finding is consistent with the negative correlation of the *ADIPOR1* mRNA levels in human adipose tissue with BMI^{22,23}. The obese phenotype of *ADIPOR1* knockout mice was associated with decreased whole-body energy expenditure²³. These data suggest that the expression levels of *ADIPOR1* may affect the BMI and development of obesity. We found that postmenopausal Japanese women with a G-A transition in the 5' flanking region of the *ADIPOR1* gene showed significantly higher BMI. Higher BMI in postmenopausal women may be associated with decreased whole-body energy expenditure. The SNP analyzed in the present study may be a genetic marker for higher BMI and susceptibility to obesity. Although functional studies are required to investigate the biological function of this polymorphism, the presence of the rs4989513 SNP in the 5' flanking region of the *ADIPOR1* gene may modulate the BMI by influencing transcription and/or the expression levels of *ADIPOR1*. To search for a role of the *ADIPOR1* gene in the homeostasis of adipose tissues, especially in the case of obese individuals, our study has some limitations. In the present study, we analyzed the healthy postmenopausal women. Therefore, we could not assess the association between the SNP and the risk of obesity or type 2 diabetes. In this regard, case-control study will be required. Moreover, we only measured BMI, total cholesterol and triglyceride. To uncover the effects of the SNP on central adiposity and insulin resistance, we need to assess the association between the SNP and waist-to-hip ratio, % of body fat mass, serum insulin, plasma glucose or HbA1c in the future.

In conclusion, our findings suggest that the *ADIPOR1* gene may be a genetic determinant of BMI in postmenopausal Japanese

women. It could be applicable as a genetic marker for predicting future obesity or metabolic diseases in younger age. Further studies on variations of the *ADIPOR1* gene may enable us to elucidate one of the mechanisms underlying obesity.

Acknowledgments

We thank Ms. M. Iwashita for expert technical assistances. This work was partly supported by a grant from the Japanese Osteoporosis Foundation. This work was partly supported by grants from the Japanese Ministry of Health, Labor, Welfare, Japan Society for the Promotion of Science and a grant of the Genome Network Project from the Ministry of Education, Culture, Sports, Science and Technology of Japan. We appreciate all the volunteers for precious clinical data and samples.

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