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Angiotensinogen gene polymorphism (Met235Thr) influences visceral obesity and insulin resistance in obese Japanese women

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Abstract

To investigate the relationship between angiotensinogen (AGT) Met235Thr polymorphism (M235T) and human obesity, because AGT is regarded as one of the cytokines produced from adipocytes and serum AGT concentrations are reported to be positively correlated with body mass index. One hundred and twenty obese Japanese women (age, 58.8 ± 9.4 years; body mass index, 32.2 ± 4.9 kg/m^2) were enrolled. Angiotensinogen genotypes were determined with a fluorescent allele-specific DNA primer assay system. Subjects were divided into M/M, M/T, and T/T groups. Control subjects comprised 146 healthy age-matched women. Clinical characteristics and the effects of diet and exercise therapy for 6 months were compared among the 3 genotypes. The genotype frequencies of AGT M235T polymorphism were in accordance with the Hardy-Weinberg equation (obese: M/M, 6.7%; M/T, 27.5%; T/T, 65.8%; control: M/M, 6.8%; M/T, 21.2%; T/T, 71.9%). The frequency of the T allele did not differ between obese and control subjects (0.80 vs 0.83). As the number of obese women with M/M genotype was only 8, comparisons of the characteristics and outcomes of weight reduction therapy were performed only between subjects with M/T genotype and T/T genotype. In the T/T group, % body fat and waist circumference at baseline were significantly greater than in the M/T group (36.3% ± 4.8% vs 33.8% ± 4.7%, $P = .0105$; 107.9 ± 10.9 vs 102.6 ± 7.9 cm, $P = .0428$, respectively). Before the weight reduction therapy, significantly higher insulin and higher homeostasis model assessment (HOMA-R) were demonstrated in the T/T group than in the M/T group (9.1 ± 5.5 μU/mL vs 5.9 ± 4.4 μU/mL, $P = .0056$; 2.3 ± 1.4 vs 1.6 ± 1.3, $P = .0252$, respectively). Both systolic and diastolic blood pressure at baseline in the T/T group tended to be higher than those in the M/T group, but the differences were not significant. No genotype-dependent difference in energy expenditure or outcome of weight reduction therapy was observed with respect to AGT M235T polymorphism. After the diet and exercise therapy, the blood pressure in the T/T group tended to be higher than that in the M/T group, but the difference was not significant. We demonstrated that the T/T genotype of the AGT M235T gene polymorphism was positively related to visceral obesity and hyperinsulinemia in obese Japanese women. Blood pressure did not show genotype-specific differences before or after the treatment. Further studies of the association between obesity and this gene polymorphism should contribute to understanding and treating obesity-related diseases.

1. Introduction

The renin-angiotensinogen (AGT) system is recognized as an important regulator of systemic blood pressure and renal electrolyte homeostasis. In humans, the association between obesity and hypertension has been confirmed [1], and plasma renin activity, plasma AGT levels, and blood pressure level are positively related to body mass index (BMI) [2]. It has been reported that the AGT gene is expressed in adipose tissue [3-5] and the AGT mRNA level is significantly higher in visceral adipose tissue than in subcutaneous adipose tissue [5]. In addition, AGT plays a role in regulating the growth and differentiation of adipose

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tissue [6], and AGT synthesis in the liver is regulated by insulin, so it is likely that AGT may affect insulin resistance [7,8]. These facts suggest that the renin-AGT system contributes to obesity-related diseases, and some variations of the ATG gene may influence the development of such diseases. The substitution polymorphisms Met235Thr and Thr174Met located in AGT were identified by Jeunemaitre et al [9], and significant differences in the frequencies of the corresponding alleles between hypertensive subjects and normal subjects have been demonstrated. Many researchers have tried to elucidate how these substitutions affect the incidence of hypertension, but the results are controversial. Some reports showed a positive association between the Thr235 allele and hypertension [9-13], whereas others did not [14-19]. The heterogeneity of study design factors such as age, sex, race, and obesity may explain the different results. In particular, obesity is presumed to be one of the most important factors affecting hypertension, as greater drops in blood pressure with weight reduction therapy [20,21] and low-fat diet [22] were demonstrated in individuals with the homozygous A/A genotype of the A(−6)G polymorphism of the AGT gene, which is in very tight linkage with the AGT Met235Thr substitution. Therefore, we investigated obese Japanese women to determine how the Met235Thr substitution of the AGT gene influences obesity, obesity-related clinical characteristics, and weight reduction induced by diet and exercise therapy.

2. Research design and methods

2.1. Study population

A total of 120 obese women who visited the Outpatient Obesity Clinic of Kyoto Prefectural University of Medicine and who had BMI higher than 25 kg/m^2 were screened for the variants of the AGT gene. Control subjects comprised 146 healthy age-matched women with BMI lower than 25 kg/m^2 who visited the Sakazaki Clinic to have a medical examination. The study was approved by the Ethics Committee of Kyoto Prefectural University of Medicine. Informed written consent was obtained from all participants. Most of the patients who visited the Outpatient Obesity Clinic of Kyoto Prefectural University of Medicine were female. Because of the gap in sex between the obese subjects and control subjects, it was difficult to adjust for difference in sex. Therefore, we limited the subjects to females to exclude bias regarding sex from the statistical analysis.

2.2. Measurement of metabolic rate

The resting metabolic rate (RMR) was measured using a closed-circuit indirect calorimeter (Sanborn wedge-type spirometer: metabograph, Model SS-80; Fukada Medical Laboratory, Tokyo, Japan) with a mouthpiece, in a temperature-controlled room (22°C-24°C) after an overnight fast [23]. The subjects wore light indoor clothing. They were supine and were asked to remain motionless and awake during the test. The RMR was measured as the mean of a 30-minute measurement. The expected RMR was calculated as measured RMR times the standard coefficient of RMR which was reported by the Japan National Nutritional Research report in 1979 and depends on sex, age, and body surface area [24]. Then, the expected RMR of each subject was subtracted from the measured value to obtain the "ΔRMR" [25,26].

2.3. Measurement of blood pressure

Blood pressure was measured from the right arm using a mercury sphygmomanometer in a sitting position after 5 minutes of rest. Systolic blood pressure and diastolic blood pressure were measured according to Korotkoff phase I and phase V, respectively.

2.4. Measurement of visceral and subcutaneous white adipose tissue

Visceral white adipose tissue (VWAT) was measured by computed tomography according to a previous report [27]. The subjects were examined in the supine position with both arms stretched above their head. The position of the scan was established at the slice level of the umbilicus. The VWAT area was quantified by delineating the intra-abdominal cavity at the internal-most aspect of the abdominal and oblique muscle walls surrounding the cavity and the posterior aspect of the vertebral body. The subcutaneous white adipose tissue area was quantified between the skin and the external-most aspect of the abdominal muscle wall.

2.5. Diet and exercise therapy

All the subjects underwent diet therapy by limiting their energy intake to 5021 kJ/d (about 60% carbohydrate, about 20% fat) for 6 months. The subjects were educated to reduce sugar and fat intake as much as possible and to eat a large amount of raw vegetables before each meal. They were also asked to take more than 30 minutes for each meal and allowed to eat only raw vegetables after 8:00 PM. According to the report of Nakagawa et al [28] and Tanizaki et al [29], the average daily intake of NaCl of a Japanese is about 12 g. As we wanted to investigate the effect of weight reduction therapy on blood pressure independent from the limitation of sodium intake, we decided to limit the daily NaCl intake to less than 10 g, which is close to the ordinary sodium intake of the Japanese. The energy intake of each subject was calculated from her daily food record for the first 7 days and last 7 days of the observation period. In the food record, subjects wrote the weight of each type of food eaten or the energy of each menu item on a list provided beforehand when eating out. The education of the subjects and the calculation of energy intake were managed by 4 trained physicians. Subjects were also instructed to walk 10 000 steps per day, and their steps were checked by a pedometer. They were asked to measure their weight and record portion sizes every week. Daily counts of steps measured by the

Table 1
Clinical characteristics of control subjects and obese subjects in this study

	Control subjects	Obese subjects
n	146	120
Age (y)	58.2 ± 10.2	58.8 ± 9.4
Body weight (kg)	58.7 ± 8.7	77.6 ± 11.6*
Height (cm)	156.9 ± 6.7	155.4 ± 5.4
BMI (kg/m^2)	21.7 ± 2.3	32.2 ± 4.9*
Systolic blood pressure (mm Hg)	119 ± 15	152 ± 20*
Diastolic blood pressure (mm Hg)	71 ± 10	89 ± 13*

Data are mean ± SD.
* $P < .05$.

pedometer were also recorded for the first 7 days and last 7 days of the observation period.

2.6. Gene analysis

Genomic DNA was extracted from peripheral blood leukocytes. Angiotensinogen genotypes were determined with a fluorescent allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan) [30]. The polymorphic region of the gene was amplified by polymerase chain reaction with A allele–specific sense primers labeled at the 5′ end with fluorescein isothiocyanate (5′ -GTC-CAC-ACT-GGC-TCC-xGT-3′) or G allele–specific sense primers labeled at the 5′ end with Texas red (5′ -CTG-TCC-ACA-CTG-GCT-CCx-AT-3′) and an antisense primer labeled at the 5′ end with biotin (5′ -GGC-TGT-GAC-AGG-ATG-GAA-GAC-T-3′). The amplification protocol and method of measurement were the same as previously reported [31].

2.7. Statistical analysis

Values are given as mean ± SD. Genotype and allele frequencies between different groups were assessed by the χ^2 test. Comparisons of clinical characteristics between 2 genotypes were analyzed by an unpaired *t* test, but parameters that did not have a regular distribution were analyzed by the Mann-Whitney *U* test. Parameters whose logarithm is known to fit a regular distribution were tested after log transformation. A *P* value less than .05 was considered statistically significant. The StatView 5.0 program package (SAS Institute, Cary, NC) was used for statistical analysis.

Table 2
Differences in clinical characteristics before the diet and exercise therapy between M/T and T/T genotypes of AGT Met235Thr polymorphism in obese Japanese women

	M/T	T/T	*P*
n	33	79	
Age	57.9 ± 8.3	59.3 ± 10.2	.5628
Body weight (kg)	74.4 ± 7.2	78.9 ± 12.8	.2790
BMI (kg/m^2)	30.5 ± 3.1	32.9 ± 5.3	.1350
Body fat (%)	33.8 ± 4.7	36.3 ± 4.8	.0105*
Waist circumference (cm)	102.6 ± 7.9	107.9 ± 10.9	.0428*
W/H ratio	1.0 ± 0.1	1.0 ± 0.1	.5184
VWAT (cm^2)	140.6 ± 75.0	139.2 ± 53.3	.5932
V/S ratio	0.56 ± 0.5	0.43 ± 0.2	.3432

Data are mean ± SD. W/H ratio indicates waist-to-hip ratio; V/S ratio, ratio of visceral white adipose tissue to subcutaneous white adipose tissue.
* $P < .05$.

Table 3
Comparison of blood pressure, glucose tolerance, and lipid profiles in obese Japanese women between M/T and T/T genotypes of AGT Met235Thr polymorphism before and after the diet and exercise therapy

	M/T	T/T	*P*
Before the diet and exercise therapy			
SBP (mm Hg)	148.6 ± 15.1	154.2 ± 22.3	.1130
DBP (mm Hg)	86.3 ± 9.5	90.2 ± 14.1	.0690
FPG (mg/dL)	108.8 ± 11.0	101.2 ± 9.7	.0009*
HbA_{1c} (%)	5.5 ± 0.5	5.4 ± 0.7	.2777
Fasting insulin (μU/mL)	5.9 ± 4.4	9.1± 5.5	.0056*
HOMA-R	1.6 ± 1.3	2.3 ± 1.4	.0252*
T-Chol (mg/dL)	223.1 ± 29.6	228.7 ± 37.5	.5111
TG (mg/dL)	178.8 ± 96.4	166.3 ± 93.6	.4095
After the diet and exercise therapy			
SBP (mm Hg)	127.3 ± 15.3	133.0 ± 15.1	.0900
DBP (mm Hg)	76.8 ± 9.8	78.0 ± 8.7	.5553
FPG (mg/dL)	105.9 ± 15.2	99.6 ± 10.0	.1129
HbA_{1c} (%)	5.5 ± 0.4	5.4 ± 0.6	.7163
T-Chol (mg/dL)	198.0 ± 18.9	201.6 ± 24.9	.4973
TG (mg/dL)	105.0 ± 47.0	107.6 ± 46.5	.7772

Data are mean ± SD. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HOMA-R, homeostasis model assessment; T-Chol, total cholesterol; TG, triglyceride.
* $P < .05$.

3. Results

The characteristics of the subjects are shown in Table 1.

To describe the results more simply, we use the abbreviations M and T instead of Met and Thr, respectively. The allele and genotype frequencies of the AGT M235T polymorphism did not differ between Japanese obese women (M/M, 6.7%; M/T, 27.5%; T/T, 65.8%; T-allele frequency, 0.80) and nonobese controls (M/M, 6.9%; M/T, 21.2%; T/T, 71.9%; T-allele frequency, 0.83). The genotype frequencies of these samples were in accordance with Hardy-Weinberg equilibrium and similar to the frequencies previously reported in Japanese subjects [12,13].

According to the food record of each subject, the average daily energy intake during the first 7 days (M/M group 5230 ± 418 kcal, M/T group 5042 ± 218 kcal, T/T group 5134 ± 653 kcal) and last 7 days (M/M group 5230 ± 502 kcal, M/T group 5121 ± 628 kcal, T/T group 5059 ± 561 kcal) did not show a significant difference among the 3 groups. Counts of the pedometer during the first 7 days (M/M group, 6250 ± 2872; M/T group, 6947 ± 3689; T/T group, 6317 ± 3926) and last 7 days (M/M group, 6015 ± 3525; M/T group, 7241 ± 4248; T/T group, 5928 ± 3316) showed no significant differences either among the 3 groups. These data are expressed as mean ± SD.

As the number of the subjects with M/M genotype was only 8, further comparisons were performed only between subjects with M/T genotype and T/T genotype. Before the diet and exercise therapy, % body fat, and waist circumference were

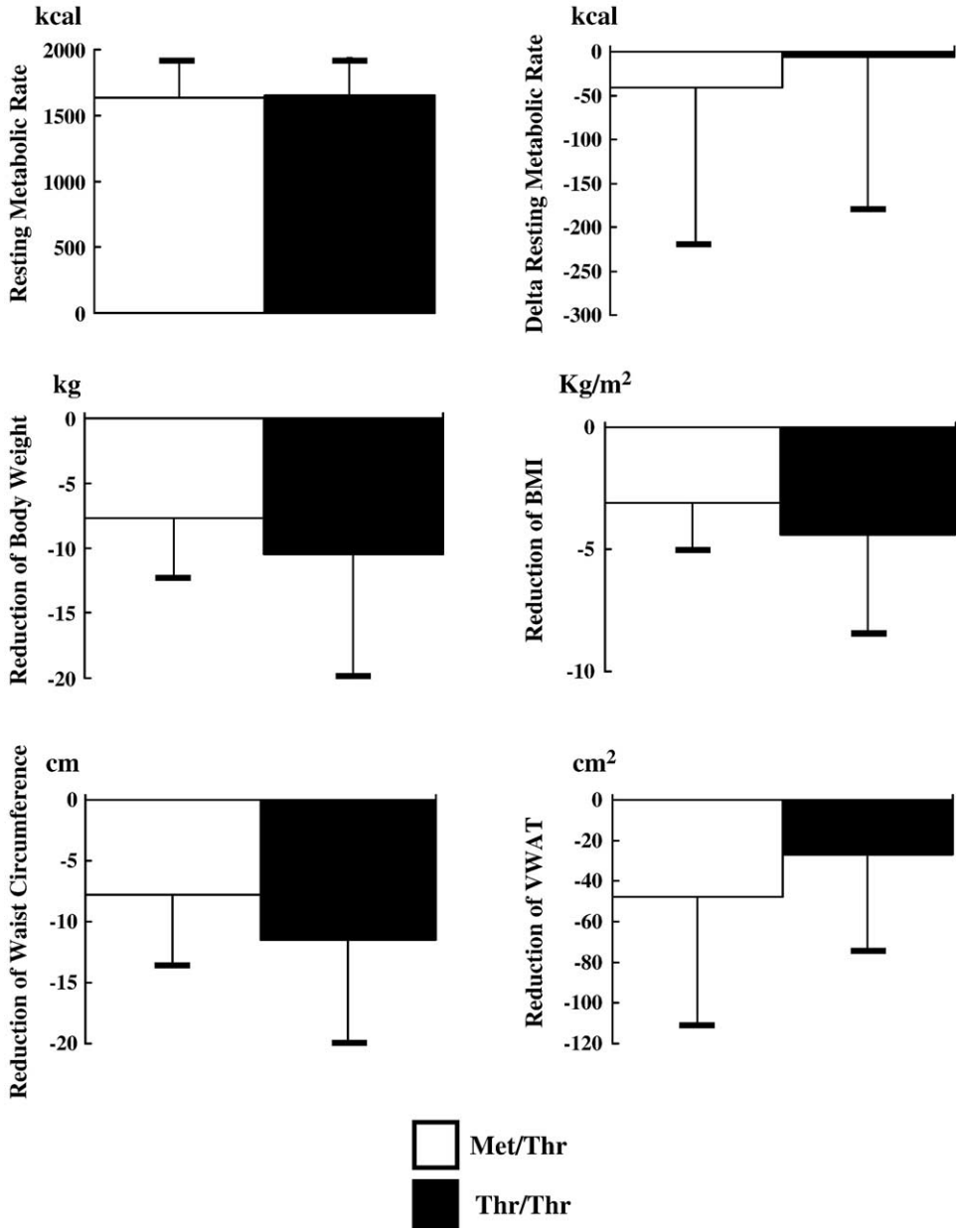

Fig. 1. Comparison of the energy expenditure and reduction of the weight, BMI, waist circumference, and VWAT after diet and exercise therapy between the M/T and T/T genotypes of the AGT Met235Thr polymorphism in obese Japanese women. No significant difference in RMR or ΔRMR was observed between the 2 genotypes. We could not demonstrate any significant differences in the reduction of body weight, BMI, waist circumference, or VWAT between the M/T and T/T groups.

significantly greater in the T/T genotype group than in the M/T genotype group despite the lack of a significant difference in body weight, BMI, and VWAT between the 2 groups (Table 2). In the T/T group, fasting blood glucose was significantly lower and insulin and homeostasis model assessment (HOMA-R) were significantly higher than in the M/T group at baseline. No significant differences were observed in blood pressure or lipid profiles before the weight reduction therapy (Table 3). The values of RMR and delta RMR demonstrated that there was no significant difference in energy expenditure between the 2 groups. We did not find any significant differences between the M/T and T/T groups in the reduction of body weight, waist circumference, or VWAT after the diet and exercise therapy (Fig. 1). After the weight reduction therapy, there were no differences in blood pressure, glucose tolerance, or lipid profiles between the subjects with M/T genotype and T/T genotype (Table 3).

4. Discussion

Obesity is known to be associated with hypertension, diabetes, and hyperlipidemia. Angiotensinogen, an important regulator of blood pressure, is synthesized not only in the liver but also in adipose tissue [3-5] and AGT itself is

known to regulate the growth of adipose tissue [6]. Several variants of the AGT gene have been investigated with respect to their association with hypertension. To clarify the mechanism of this relationship, we examined how the AGT Met235Thr substitution is related to blood pressure, glucose tolerance, and lipid profiles in obese Japanese women. The greater initial % body fat and waist circumference in obese Japanese women with T/T genotype demonstrated by our results suggest that there is a positive relationship between the T/T genotype of AGT Met235Thr polymorphism and visceral obesity. The Met235Thr polymorphism has been reported to be in tight linkage disequilibrium with a G-to-A substitution located at position −6 upstream of the initial transcription site generally described as A(−6)G [32-34]. Met235Thr polymorphism was reported to have a positive relationship with plasma AGT concentration, and it was thought likely that the A(−6)G variation influenced the basal transcription rate of the AGT gene [32]. Subjects with the A allele of the A(−6)G substitution were prone to gain greater body weight than homozygous G/G subjects during 3 years without strong intervention of a low-energy diet [35], supporting our findings that subjects with the T/T genotype of the Met235Thr polymorphism were prone to gain body fat if they had a sedentary lifestyle.

In this study, significantly higher insulin and HOMA-R together with significantly lower fasting plasma glucose were shown in the T/T group compared with the M/T group. Recently, Guo et al [36] reported that Mexican Americans with the T allele of AGT Met235Thr polymorphism had significantly higher insulin resistance and insulin sensitivity index. Although we did not use the glucose clamp method to evaluate insulin resistance, our results appear to be in accordance with this report and suggest that there may be a similar relationship between insulin resistance and AGT Met235Thr polymorphism in the Japanese population. Although these results demonstrated significantly higher rates of visceral obesity and insulin resistance for the T/T genotype in obese subjects, we did not find any differences in T-allele frequency between obese and control subjects. We suppose that genotype-specific differences begin to appear during the process of gaining excess body weight. Genotype-specific differences among obese subjects regarding phenotypes such as visceral obesity, energy expenditure, and the effect of weight reduction therapy without differences in genotype frequency between obese and control subjects were also reported in Refs [37-39].

Some obesity-related gene polymorphisms such as Trp64Arg substitution of β_3-adrenergic receptor [26] and A-3826G substitution of UCP1 [38,40] induce genotype-dependent differences in energy expenditure and thereby influence the outcome of weight reduction therapy. As AGT is considered to be one of the most important obesity-related proteins, we had considerable interest in the linkage between AGT Met235Thr gene polymorphism and energy expenditure. However, we did not find a positive association between them, nor any significant differences in the reduction of body weight, waist circumference, or VWAT obtained by diet and exercise therapy.

With regard to hypertension, we did not find any genotype-dependent differences in systolic or diastolic blood pressure either before or after the diet and exercise therapy, in contrast to previous reports [14-19]. Hypertensive individuals with the AA genotype of the AGT A(−6)G polymorphism, which is in tight linkage with the T/T genotype of AGT Met235Thr polymorphism, showed a significant drop in blood pressure after weight reduction therapy [20,21] and after low-fat, fruit-and-vegetable–rich diet therapy [22]. In our study, after the weight reduction therapy, body weight, waist circumference, and VWAT were reduced in obese Japanese women and blood pressure was also improved. However, just as we failed to demonstrate a genotype-dependent difference in the reduction of body fat, we could not find any difference in improvement of blood pressure dependent on AGT Met235Thr gene polymorphism.

In conclusion, the T/T genotype of AGT Met235Thr gene polymorphism is linked to visceral obesity and insulin resistance in obese Japanese women, and this genotype is regarded to have greater risk for obesity-related diseases in obese Japanese women. Further studies on the association between obesity and this gene polymorphism are warranted.

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