Peroxisome Proliferator-Activated Receptor γ Gene Polymorphism is Associated With Serum Triglyceride Levels and Body Mass Index in Japanese Type 2 Diabetic Patients

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Peroxisome proliferator-activated receptor γ (PPAR γ) controls adipocyte differentiation and regulates lipid and glucose homeostasis. Therefore, the PPAR γ gene may affect insulin sensitivity and resistance. We analyzed the relationship between C/T exon 6 polymorphism of the PPAR γ gene and various clinical parameters in type 2 diabetic patients. There were no significant differences in the frequencies of genotype and allele between diabetic patients with and without nephropathy. Diabetic patients were divided into two groups: patients bearing at least one T allele (CT/TT), and patients with no T allele (CC). Levels of serum triglyceride and body mass index (BMI) were significantly higher in the CT/TT genotype group than in the CC genotype

group. Since obesity affects insulin resistance, the diabetic patients were also divided into two groups: those with a BMI of <23, and those with a BMI of >23. In patients with a BMI of <23, there was no significant change in the levels of glycosylated hemoglobin A1c (HbA1c) between the CC and CT/TT genotype groups. However, in patients with a BMI of >23, HbA1c levels were significantly higher in the CT/TT genotype group than in the CC genotype group. It appears that the CT/TT genotype with PPAR γ gene polymorphism may contribute to higher BMI and higher serum triglyceride and HbA1c levels in Japanese type 2 diabetic patients. *J. Clin. Lab. Anal.* 18:317–321, 2004. © 2004 Wiley-Liss, Inc.

Key words: PPAR γ ; polymorphism; BMI; diabetes

INTRODUCTION

Diabetic nephropathy is the major determinant of morbidity and mortality in patients with type 1 and type 2 diabetes (1). Since diabetic nephropathy occurs in familial clusters, and not all patients with poor metabolic control develop nephropathy, genetic factors may contribute to a person's susceptibility to this disease (2–4).

The peroxisome proliferator activated receptors (PPARs) are members of the nuclear hormone receptor superfamily and consist of three subtypes: PPAR α , PPAR δ , and PPAR γ (5). PPAR γ has a higher affinity for fat-specific peroxisome proliferator response elements (PPREs) compared to PPAR α or PPAR δ (5). There are three PPAR γ isoforms of the gene (PPAR γ 1, PPAR γ 2, and PPAR γ 3) that result from alternative promoters and differential splicing (6,7). PPAR γ 1 is expressed at low levels in diverse tissues, including adipose, skeletal muscle, heart, and liver tissue. PPAR γ 2 is expressed almost exclusively in adipose tissue, whereas

PPAR γ 3 appears to be expressed in colon and adipose tissue (6–8). Recently, thiazolidinediones (such as troglitazone) were shown to bind to PPAR γ with high affinity. In vitro, these agents stimulate adipocyte differentiation and improve insulin sensitivity (9). In humans, thiazolidinediones decrease insulin resistance and decrease hypertriglyceridemia (10). Therefore, we hypothesized that PPAR γ gene polymorphism may be a genetic marker of diabetic nephropathy. Recently, Meirhaeghe et al. (11) identified PPAR γ polymorphism of a silent C→T substitution in exon 6. The objective of the present study was to investigate whether PPAR γ

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Received 18 May 2002; Accepted 1 September 2004

DOI 10.1002/jcla.20045

Published online in Wiley InterScience (www.interscience.wiley.com).

gene polymorphism is associated with the development or progression of type 2 diabetes in Japanese subjects.

MATERIALS AND METHODS

Patients and Biochemical Measurements

In this study, 140 Japanese type 2 diabetic patients (93 men and 47 women; mean age = 64.0 ± 12.7 years) were analyzed. The diabetic patients were divided into two groups as follows: 1) diabetic patients without nephropathy (normoalbuminuric patients with a diabetic duration of >15 years, and urinary albumin excretion rate (AER) of <20 $\mu\text{g}/\text{min}$ or 30 mg/g creatinine (s-Cr)), and 2) diabetic patients with nephropathy (hemodialysis patients). Since obesity affects insulin resistance, the diabetic patients were also divided into two groups: patients with a BMI of <23, and patients with a BMI of >23. All patients gave their informed consent to participate in this study. The BMI was calculated as weight/height² (kg/m^2). Blood samples were obtained from hemodialysis patients at the start of hemodialysis, and from the other patients after they fasted overnight. Serum creatinine (s-Cr) was assayed by the kinetic Jaffe method. Serum total cholesterol (T-cho), triglyceride (TG), and high-density lipoprotein-cholesterol (HDL-C) were measured by a conventional laboratory technique. The mean Q1glycosylated hemoglobin A1c (HbA1c) levels at three different times were assayed by high-performance liquid chromatography (Adams HA-8131; Arkray, Tokyo, Japan). The normal range of HbA1c is 4.3–5.8% in our laboratory.

Genomic DNA Analysis

Genomic DNA from each patient was prepared from peripheral leukocytes in 200- μL blood samples by means of a DNA-selective preparation method (Qiagen, Hilden, Germany). A DNA fragment was amplified by polymerase chain reaction (PCR) with the following primers: forward primer 5'-GCC CAG GTT TGC TGA ATG -3', and reverse primer 5'-TGA AGA CTC ATG TCT GTC-3'. PCR was performed in 35 cycles by the following steps: denaturation at 94°C for 30 sec, annealing at 59°C for 30 sec, and extension at 72°C for 50 sec. After amplification, the PCR product was digested with restriction endonuclease Pml I (New England Biolabs, Inc., Beverly, MA), electrophoresed in 3% agarose gel, and visualized by staining with ethidium bromide. Digestion of the PCR product with Pml I produced three distinct patterns: a single 208-bp product was derived from T/T homozygotes; 208-, 158-, and 50-bp products were derived from

C/T heterozygotes; and 158- and 50-bp products were derived from C/C homozygotes.

Statistical Analysis

All data were expressed as mean $\pm$ standard deviation (SD). Data were analyzed by means of the Chi-square test or Mann-Whitney's U-test. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Relationship Between PPAR γ Gene Polymorphism and Diabetic Nephropathy

Since frequencies of the TT genotype were very low in diabetic patients and controls, the patients were divided into two groups: a CC genotype group, and a CT/TT genotype group. Frequencies of the PPAR γ gene genotype did not differ between diabetic patients with and without nephropathy, as shown in Table 1. The genotype distribution observed in diabetic patients was in accordance with the Hardy-Weinberg equilibrium.

Relationship Between PPAR γ Gene Polymorphism and Clinical Parameters

Serum triglyceride levels and BMI were significantly higher in the CT/TT genotype group than in the CC genotype group (Table 2). After diabetic nephropathy was excluded, serum triglyceride levels and BMI were significantly higher in the CT/TT genotype group than in the CC genotype group (Table 3). In patients with diabetic nephropathy, there was no significant difference in the BMI; however, serum triglyceride levels tended to be slightly higher in the CC genotype group compared to the CT/TT genotype group (Table 4). The other clinical parameters, including gender, age, sex, BMI, duration of diabetes, and HbA1c levels, did not show significant differences between the CC and CT/TT genotype groups. The proportion of patients receiving insulin, oral hypoglycemic agents, and anti-hyperlipidemic agents did not significantly differ between the CC and CT/TT genotype groups.

TABLE 1. Frequencies of PPAR γ genotypes in diabetic patients with or without nephropathy*

Genotype	DMN (–) (n = 79)	DMN (+) (n = 61)
CC	55	46
CT/TT	24	15

* $P = 0.57$; χ^2 test.

DMN, diabetic nephropathy.

TABLE 2. Comparison of clinical parameters in diabetic patients with different genotypes*

Genotype	CC (n = 101)	CT/TT (n = 39)
Sex (male/female)	67/34	26/13
Patients (nonHD/HD)	55/46	24/15
Duration of diabetes (years)	19.8 $\pm$ 8.2	17.3 $\pm$ 6.3
Age (years old)	63.9 $\pm$ 10.5	66.7 $\pm$ 13.8
BMI	21.4 $\pm$ 2.6	22.5 $\pm$ 3.8 ^a
HbA1c (%)	6.8 $\pm$ 1.2	7.1 $\pm$ 1.5
s-Cr (mg/dL)	4.8 $\pm$ 5.0	4.9 $\pm$ 5.4
T-cho (mg/dL)	169.7 $\pm$ 33.4	179.5 $\pm$ 49.7
TG (mg/dL)	118.7 $\pm$ 60.2	165.5 $\pm$ 123.4 ^b
HDL-C (mg/dL)	51.6 $\pm$ 19.2	47.4 $\pm$ 14.4

*Values presented as mean $\pm$ standard deviation (SD).

^a $P < 0.05$ vs. CC.

^b $P < 0.005$ vs. CC.

HD, hemodialysis; BMI, body mass index; HbA1c, glycosylated hemoglobin A1c; s-Cr, serum creatinine; T-cho, serum total cholesterol; TG, serum triglyceride; HDL-C, high density lipoprotein-cholesterol.

TABLE 3. Comparison of clinical parameters in diabetic patients without nephropathy with different genotypes*

Genotype	CC (n = 55)	CT/TT (n = 24)
Sex (male/female)	36/19	16/8
Duration of diabetes (years)	19.5 $\pm$ 7.2	16.5 $\pm$ 4.7
Age (years old)	62.7 $\pm$ 11.6	61.8 $\pm$ 14.1
BMI	21.4 $\pm$ 2.4	23.3 $\pm$ 3.3 ^a
HbA1c (%)	7.0 $\pm$ 1.2	7.3 $\pm$ 1.7
s-Cr (mg/dL)	0.7 $\pm$ 0.2	0.8 $\pm$ 0.2
T-cho (mg/dL)	179.1 $\pm$ 34.6	195.2 $\pm$ 51.2
TG (mg/dL)	119.0 $\pm$ 56.3	174.7 $\pm$ 130.1 ^b
HDL-C (mg/dL)	58.7 $\pm$ 19.3	51.4 $\pm$ 14.1

*Values presented as mean $\pm$ standard deviation (SD).

^a $P < 0.005$ vs. CC.

^b $P < 0.01$ vs. CC.

BMI, body mass index; HbA1c, glycosylated hemoglobin A1c; s-Cr, serum creatinine; T-cho, serum total cholesterol; TG, serum triglyceride; HDL-C, high density lipoprotein-cholesterol.

TABLE 4. Comparison of characteristics of diabetic patients on HD with different genotypes*

Genotype	CC (n = 46)	CT/TT (n = 15)
Sex (male/female)	31/15	10/5
Duration of diabetes (years)	20.1 $\pm$ 9.4	18.6 $\pm$ 8.2
Age (years old)	65.3 $\pm$ 11.6	66.7 $\pm$ 12.4
BMI	21.4 $\pm$ 2.8	21.3 $\pm$ 4.2
HbA1c (%)	6.6 $\pm$ 1.1	6.7 $\pm$ 1.0
s-Cr (mg/dL)	9.8 $\pm$ 3.0	11.4 $\pm$ 2.3
T-cho (mg/dL)	158.5 $\pm$ 30.8	154.4 $\pm$ 36.0
TG (mg/dL)	118.3 $\pm$ 57.7	150.8 $\pm$ 144.5
HDL-C (mg/dL)	43.1 $\pm$ 15.4	40.9 $\pm$ 12.7

*Values presented as mean $\pm$ standard deviation (SD).

HD, hemodialysis; BMI, body mass index; HbA1c, glycosylated hemoglobin A1c; s-Cr, serum creatinine; T-cho, serum total cholesterol; TG, serum triglyceride; HDL-C, high density lipoprotein-cholesterol.

In patients with a BMI of <23 , there was no significant change in the levels of HbA1c between the CC and CT/TT genotype groups. However, in patients

with a BMI of >23 , the levels of HbA1c in the CT/TT genotype group were significantly higher than those in the CC genotype group (Fig. 1).

Fig. 1. Influence of PPAR γ gene polymorphism on HbA1c levels. Diabetic patients were divided into two groups according to their BMI. N.S., not significant.

DISCUSSION

The allele frequencies of the C→T substitution in exon 6 of the PPAR γ gene in Japanese type 2 diabetes were in Hardy-Weinberg equilibrium, and were similar to those observed in northern French type 2 diabetic populations (11). We demonstrated that there were no differences in the frequencies of PPAR γ gene genotype between diabetic patients with and without nephropathy. However, Japanese type 2 diabetes patients who had at least one T allele (CT/TT genotype) showed significantly higher BMI and serum triglyceride levels than those with the CC genotype. In hemodialysis patients, there were no significant differences in BMI and serum triglyceride levels between the CC and CT/TT genotypes. Optimal dry weight may be regulated by hemodialysis.

Several studies have reported inconsistent results regarding the Pro12Ala polymorphism in the PPAR γ 2 gene (one of the PPAR γ gene variants) and its association with type 2 diabetes, obesity, and insulin

sensitivity (12–15). These conflicting results are most likely due to differences in selection criteria for study patients, ethnicity, or sample size. It was recently reported that heterozygous PPAR γ -deficient mice are partially protected from high-fat diet-induced obesity and insulin resistance (16). This result suggests that PPAR γ may mediate high-fat diet-induced obesity and insulin resistance. In such mice, food intake is decreased and body temperature is higher compared to wild-type mice (16). Therefore, PPAR γ is viewed as a thrifty gene.

It is still obscure why BMI and serum triglyceride levels are affected by silent single nucleotide polymorphism of the PPAR γ gene. This polymorphism may be linked with another, unidentified gene mutation adjacent to the PPAR γ gene, which regulates BMI. Changes in the activity and/or structure of the PPAR γ gene may change the activity of other transcription factors and the expression of target genes.

Insulin stimulates activation of the PPAR γ gene, and may promote adipocyte differentiation (17). Insulin resistance develops in parallel with increasing body

weight. In our study, HbA_{1c} levels in CT/TT genotype patients with a BMI of >23 were significantly higher than those in CC genotype patients with the same BMI. Although we did not measure serum insulin levels, this result suggests that the CC genotype of PPAR γ gene polymorphism may protect against insulin resistance, except patients with BMI >23. It is possible that genetic or environmental factors that cause obesity interact with the PPAR γ gene, leading to differences in insulin sensitivity between these genotypes in lean and normal to obese patients.

In conclusion, we have demonstrated a significant association between PPAR γ gene polymorphism and serum triglyceride levels or BMI in Japanese type 2 diabetes. In particular, in patients without a tendency toward leanness, HbA_{1c} levels were significantly higher in the CT/TT genotype group than in the CC genotype group. It appears that PPAR γ gene polymorphism may affect glucose and lipid metabolism. It is possible that the mutation of PPAR γ gene modulates the increased risk of obesity and subsequent insulin resistance. Further studies are required to clarify the functional and clinical roles of the PPAR γ gene.

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