Brief Genetics Report

The 3′–Untranslated Region Polymorphism of the Gene for Skeletal Muscle–Specific Glycogen-Targeting Subunit of Protein Phosphatase 1 in the Type 2 Diabetic Japanese Population

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A newly identified 3′–untranslated region (UTR) polymorphism of the gene for skeletal muscle–specific glycogen-targeting subunit of protein phosphatase 1 (*PPP1R3*) was associated with insulin resistance and type 2 diabetes in Pima Indians (Xia J, Scherers W, Cohen PTW, Majer M, Xi T, Norman RA, Knowler WC, Bogardus C, Prochazka M: A common variant in PP1R3 associated with insulin resistance and type 2 diabetes. *Diabetes* 47:1519–1524, 1998). Thus, we investigated the frequency of polymorphism of the adenine- and thymine-rich element (ARE-1 and its variant ARE-2) in 426 Japanese type 2 diabetic and 380 nondiabetic subjects using a polymerase chain reaction (PCR)-restriction enzyme fragment length polymorphism (RFLP) method. The allele frequency of the ARE-2 variant in diabetic subjects was higher than that in nondiabetic subjects (0.34 vs. 0.29; $P < 0.05$), even though its frequency in Japanese subjects was lower ($P < 0.001$) than the reported value in Pima Indians (0.56). An aspartate polymorphism at codon 905 was 100% coupled to the ARE-2 allele, and its allele frequency was higher also in diabetic subjects. Although a serine substitution at codon 883 was partially linked with the ARE-2 allele, there was no difference between diabetic and nondiabetic subjects. These results indicate that the frequency of polymorphism of the *PPP1R3* gene (ARE-2 and Asp905) is different between two ethnic groups and is increased in Japanese people with type 2 diabetes, suggesting that these variants may be a possible marker for searching for diabetogenic genes. *Diabetes* 48:1469–1472, 1999

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ARE, adenine- and thymine-rich element; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction; PP, protein phosphatase; RFLP, restriction enzyme fragment length polymorphism; UTR, untranslated region.

Type 2 diabetes is characterized by insulin resistance in insulin-sensitive peripheral tissues, particularly in the skeletal muscle (1). The insulin-stimulated glucose storage rate is impaired in insulin-resistant subjects, including type 2 diabetic subjects, and the impairment is originated from the insufficient activation of muscle glycogen synthase (2–4). Concerning the molecular mechanism for the impairment of glycogen synthase activation by insulin, the impaired mRNA expression and genetic abnormalities in the muscle glycogen synthase gene (*GYS1*) have been reported (5–7), but this is opposite to its genetic association with insulin resistance (8). Protein phosphatase 1 (PP-1) activates glycogen synthase through its dephosphorylation in response to insulin (9), and the insulin-stimulated activation of PP-1 is impaired in skeletal muscles in insulin-resistant states (10). Regarding the genetic defects in the *PP-1* gene, it has been reported that there is no abnormality of the catalytic subunit of the *PP-1* gene (11,12). However, an amino acid polymorphism involving codon 905 (Asp905Tyr) of the glycogen-targeting subunit of PP-1 (*PPP1R3*) was described to be associated with insulin resistance in Danish Caucasians (13). However, in Japanese subjects, this polymorphism of the *PPP1R3* gene was not associated with type 2 diabetes (14). Recently, a newly identified 3′–untranslated region (UTR) polymorphism of the *PPP1R3* gene was reported to be associated with insulin resistance and prevalence of type 2 diabetes in Pima Indians (15). Interestingly, the specific polymorphism of the *PPP1R3* gene is associated with the half-life of its mRNA, which might be correlated with enzyme concentration in vivo in the study by Xia et al. (15). Thus, to clarify the contribution of this polymorphism of the *PPP1R3* gene in pathogenesis of Japanese people with type 2 diabetes, we investigated the UTR polymorphism of the *PPP1R3* gene in 426 type 2 diabetic and 380 nondiabetic subjects using the polymerase chain reaction (PCR)-restriction enzyme fragment length polymorphism (RFLP) method.

TABLE 1
Genotype and allele frequencies of the *PPP1R3* gene polymorphism ARE in diabetic and nondiabetic subjects

	Japanese			
	Nondiabetic	Diabetic	Total	Pima Indians
Genotype				
n	380	426	806	930
ARE-1/1	191 (50.3)	196 (46.0)	387 (48.0)	182 (19.6)
ARE-1/2	159 (42.0)	172 (40.4)	331 (41.1)	450 (48.4)
ARE-2/2	30 (7.9)	58 (13.6)*†	88 (10.9)	298 (32.0)
Allele				
n	760	852	1,612	1,860
ARE-1	541 (71.2)	564 (66.2)	1,105 (68.5)	814 (43.8)
ARE-2	219 (28.8)	288 (33.8)*	507 (31.5)	1,046 (56.2)

Data are *n* (%). *$P < 0.05$ vs. nondiabetic by the χ^2 test for 3 × 2 and 2 × 2 contingency tables; †$P < 0.05$ vs. nondiabetic by Bonferroni inequality method. Data for Pima Indians are from Xia et al. (15).

We found that the allele frequency of the adenine- and thymine-rich element (ARE)-2 variant in Japanese subjects (both diabetic and nondiabetic subjects) in the present study was similar to that in Aboriginal Canadians (16), but was significantly lower compared with that in Pima Indians (15). Furthermore, the percentage of the Japanese subjects who were homozygous for the ARE-2 allele was significantly lower, compared with that in Pima Indians (10.9 vs. 32.0%, respectively, $P < 0.001$) (Table 1). The difference in distribution of the genotypes of *PPP1R3* may reflect the differences in the genetic backgrounds of the two populations. Nevertheless, this ARE polymorphism was reported to be correlated to its mRNA and protein levels in Pima Indians. Furthermore, in the current study, the allele frequency of ARE-2 in Japanese subjects with type 2 diabetes was higher than that in nondiabetic subjects (0.338 vs. 0.288; $P < 0.05$) (Table 1). Moreover, the percentage of the individuals homozygous for the ARE-2 allele in diabetic subjects was significantly higher than that in nondiabetic subjects (13.6 vs. 7.9%; $P < 0.05$). Because there was an age difference between diabetic and nondiabetic subjects in the current study, we subanalyzed these data in the age-matched subjects with 40- to 60-year-old subjects (105 nondiabetic [age 52.4 ± 5.5 years] and 178 diabetic subjects [age 52.9 ± 5.7 years]); we found that the allele frequency of the ARE-2 genotype in nondiabetic subjects was significantly lower than that in diabetic subjects (0.267 vs. 0.354; $P < 0.05$). These results suggest that the difference of the allele frequency of ARE-2 between nondiabetic and diabetic subjects was not affected by the selection of subjects.

Among these three groups of type 2 diabetic subjects with different PPP1R3 genotypes, most of the clinical parameters, such as type 2 diabetes onset, were comparable (Table 2). Only maximal BMI in subjects with ARE-2/2 was significantly lower than other groups. Obesity is usually associated with insulin resistance, which is thought to be a major risk factor for developing diabetes. However, it is possible that the subjects with ARE-2/2 genotype of the *PPP1R3* gene are associated with the development of diabetes without the effect of obesity. Regarding the direct evidence of the ARE-2 variant on insulin resistance, we observed no significant difference in the fasting insulin levels among three groups in nondiabetic

TABLE 2
Clinical characteristics of diabetic subjects with different genotypes of the ARE polymorphism in the *PPP1R3* gene

	ARE-1/1	ARE-1/2	ARE-2/2
n	196	172	58
M/F	106/90	96/76	34/24
Age (years)	61.1 ± 10.7	59.8 ± 10.6	62.4 ± 10.5
Age at onset of diabetes (years)	47.2 ± 12.3	46.4 ± 12.1	50.4 ± 9.8
BMI (kg/m^2)	23.7 ± 3.5	23.8 ± 3.7	24.0 ± 3.1
Maximum BMI (kg/m^2)	27.1 ± 4.0	27.0 ± 3.9	25.8 ± 3.8*
Family history of diabetes (%)	49.5	45.9	55.2
Fasting plasma glucose (mmol/l)	8.5 ± 2.5	8.4 ± 2.4	8.7 ± 2.5
HbA_{1c} (%)	7.3 ± 1.3	7.3 ± 1.1	7.3 ± 1.1
Urinary C-peptide secretion (μg/day)	92.0 ± 58.8	92.4 ± 67.9	100.6 ± 52.7
Insulin therapy (%)	26.8	35.5	22.4
Systolic blood pressure (mmHg)	139.9 ± 20.8	136.2 ± 20.6	130.2 ± 25.3
Diastolic blood pressure (mmHg)	75.6 ± 10.5	73.5 ± 10.7	74.2 ± 13.9
Hypertension (%)	15.3	23.3	17.2

Data are *n*, %, or means ± SD. *$P < 0.05$ vs. ARE-1/1 by Kruskal-Wallis test. Each maximal BMI was obtained from each medical record or was estimated by interview record. Since an aspartate substitution at codon 905 was 100% coupled to the ARE-2 allele, clinical characteristics of diabetic subjects with different genotypes of the Ser905Tyr polymorphism were identical to those in this table.

TABLE 3
Genotype and allele frequencies of the *PPP1R3* gene polymorphism at codon 883 in diabetic and nondiabetic subjects

	Japanese			
	Nondiabetic	Diabetic	Total	Pima Indians
Genotype				
n	363	416	779	930
Arg/Arg	287 (79.1)	325 (78.1)	612 (78.6)	479 (51.5)
Arg/Ser	71 (19.6)	89 (21.4)	160 (20.5)	382 (41.1)
Ser/Ser	5 (1.4)	2 (0.5)	7 (0.9)	69 (7.4)
Allele				
n	726	832	1,558	1,860
Arg	645 (88.8)	739 (88.8)	1,384 (88.8)	1,340 (72.0)
Ser	81 (11.1)	93 (11.2)	174 (11.2)	520 (28.0)

Data are *n* (%). Data on Pima Indians are from Xia et al. (15).

subjects. Furthermore, there was no difference in 2-h plasma glucose levels during the oral glucose tolerance test (OGTT) among these groups in Japanese nondiabetic subjects, whereas diabetic and IGT subjects homozygous for the ARE-2 allele were reported to have significantly lower 2-h plasma glucose levels than did Aboriginal Canadians (16). Thus, a more sensitive method to determine insulin sensitivity is required to clarify the role of this polymorphism on insulin resistance.

Regarding the other genetic variant of *PPP1R3* gene, a tyrosine substitution of an aspartate at codon 905 was reported to be associated with insulin resistance in Danish Caucasians (13). However, it has been reported that subjects with this Tyr allele are more insulin-sensitive, compared with the Asp allele in Pima Indians (15), and that this Tyr allele is not associated with type 2 diabetes in Japanese subjects (14). In the latter report, the allele frequency of tyrosine substitution (0.684) was higher in Japanese people, compared with Danish Caucasians and Pima Indians (0.079 and 0.438, respectively). In our study, a tyrosine substitution was observed with a frequency similar to that in the report by Xia et al. (15) and as 100% in linkage disequilibrium with the ARE-1 allele in randomly selected diabetic (n = 200) and nondiabetic (n = 200) subjects. As reported in Pima Indians, we found that the ARE-2 allele was coupled to the Asp allele at codon 905, and its allele frequency in diabetic subjects was significantly higher than that in nondiabetic subjects (0.338 vs. 0.255, respectively; $P < 0.02$). Thus, at least the ARE-2 and Asp alleles at codon 905 are possible candidates of susceptible genes of type 2 diabetes in our population and may be different in other ethnic groups. Nevertheless, there was no association of the PPP1R3 genotype with the presence of type 2 diabetes and impaired glucose tolerance in Aboriginal Canadians (16). Furthermore, in the current study, there was a small difference of frequency of either the ARE-2 or Asp allele at codon 905 between diabetic and nondiabetic subjects in Japanese people, respectively. Thus, a further study, which covers a large number of subjects and another ethnic population, will be necessary to evaluate the contribution of ARE-2 and Asp905Tyr on the pathogenesis of insulin resistance and type 2 diabetes.

Concerning Ser883Arg polymorphism, an Arg allele was reported to be in linkage disequilibrium with the ARE-2 allele and was correlated to decreased insulin sensitivity in Pima Indians. However, in Japanese people, an Arg allele was in linkage disequilibrium with the ARE-1 allele, and its allele frequency was much higher than that in Pima Indians (0.888 vs. 0.720, respectively) (Table 3). Furthermore, we did not observe a significant difference of its allele frequency between diabetic and nondiabetic subjects. Moreover, all clinical parameters were comparable among the three groups (data not shown). Thus, the contribution of Ser883Arg polymorphism for pathogenesis of diabetes is unlikely.

In conclusion, the present study indicates that the frequency of polymorphisms of the *PPP1R3* gene (ARE-2 and Asp905) is different among ethnic groups and is increased in Japanese people with type 2 diabetes, suggesting that these variants of the *PPP1R3* gene may be a possible marker for searching for diabetogenic genes.

RESEARCH DESIGN AND METHODS

A total of 426 type 2 diabetic subjects (236 men, 190 women) who were regularly followed in the outpatient clinic of the Third Department of Medicine, Shiga University of Medical Science, were selected and enrolled in this study. After an overnight fast, all patients were interviewed and given a physical examination, and blood samples were taken for biochemical and DNA analysis. Daily urinary C-peptide secretion rates were also determined in 24-h urine samples measured by radioimmunoassay. A total of 380 nondiabetic subjects (197 men, 183 women) were selected based on the 75-g OGTT by the World Health Organization criteria and/or by both fasting plasma glucose (<6.1 mmol/l) and HbA_{1c} (<5.6%) levels. In nondiabetic subjects, 201 subjects were healthy volunteers including our university students, and the others ($n = 179$) were nondiabetic patients who were recruited from the Shiga University Hospital. All nondiabetic subjects had no family history of type 2 diabetes. The age and BMI of the diabetic and nondiabetic subjects were 60.8 ± 10.6 vs. 47.5 ± 16.1 years ($P < 0.01$) and 23.6 ± 3.5 vs. 22.0 ± 2.9 kg/m^2 ($P < 0.01$), respectively. The levels of fasting plasma glucose and HbA_{1c} of the diabetic and nondiabetic subjects were 8.5 ± 2.5 vs. 5.0 ± 0.6 mmol/l ($P < 0.01$) and 7.3 ± 1.2 vs. 5.4 ± 0.3% ($P < 0.01$), respectively. The present study protocol was approved by the ethical committee of Shiga University of Medical Science, and a written informed consent for DNA analysis was obtained. Genomic DNA was extracted from white blood cells, and genotyping of polymorphism at AREs was determined by the modified method of Xia et al. (15). PCR were performed using primers 5′-TAGGTATTTGTAATGTACGTGTA-3′ and the reverse primer 5′-GTAACTGCATTCTCTACAGCAA-3′ as provided by J. Xia (Phoenix, AZ). PCR reaction was carried out as follows: annealing at 57°C for 1 min, extension at 72°C for 1 min, and denaturation at 95°C for 30 s for 30 cycles in a thermal cycler (GeneAmp PCR system 9700; Perkin-Elmer Japan, Chiba, Japan). Because the sizes of PCR products for ARE-1 and ARE-2 variants were 110 and 105 bp, respectively, it was difficult to separate allele difference by standard gel electrophoresis. We found that the PCR products of ARE-1 variant had the restriction site of *Mse*I, whereas those of ARE-2 variant lost this cleavage site. Thus, the PCR products were digested with *Mse*I and were separated on 10% polyacrylamide gel for genotype determination. We also genotyped another polymorphisms of the *PPP1R3* gene (Ser883Arg and Tyr905Asp) in most of our subjects by PCR-RFLP method as reported by Xia et al. (15).

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