Association of Muscle Glycogen Synthase Polymorphism With Insulin Resistance in Type 2 Diabetic Patients

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The aim of the present study is to investigate whether Met416Val (M416V) polymorphism of glycogen synthase (GYS1) gene is associated with insulin resistance in type 2 diabetes. In 100 type 2 diabetic subjects (66 men and 34 women), the M416V polymorphism of GYS1 gene was analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as previously reported, and insulin resistance was assessed by euglycemic hyperinsulinemic clamp represented as M/I value, the mean of glucose infusion rate (M value) adjusted by steady state plasma insulin level. The means of age and body mass index (BMI) of the subjects were 53.1 ± 11.6 (SD) years and 23.3 ± 3.5 kg/m^2. The allele frequencies of M416V polymorphism were 82.0% for MM, 16.0% for MV, and 2.0% for VV, and subjects were subsequently divided into V(+) group (n = 18) and V(-) group (n = 82) according to the presence or absence of V allele. There were no significant differences in age, BMI, blood pressure, fasting plasma glucose or insulin levels or glycosylated hemoglobin (HbA_{1c}) levels between the V(+) and V(-) groups. No significant differences in either M or M/I value were found between the V(+) and V(-) groups (M value, 5.06 ± 2.20 *v* 5.12 ± 2.04 mg · kg^{-1} · min^{-1}, *P* = .841; M/I value, 5.24 ± 3.07 *v* 5.39 ± 2.87 mg · kg^{-1} · min^{-1} · mU^{-1} · L, *P* = .576). BMI showed the strongest independent contribution to M/I value, but the presence of V allele did not in multiple regression analysis. In conclusion, the M416V polymorphism of GYS1 gene is not associated with insulin resistance in type 2 diabetes.

INSULIN RESISTANCE is pathognomonic in type 2 diabetes.[1] Research on candidate genes for insulin resistance is now emerging to clarify the genetic factors involved in diabetes. Among these candidate genes, the glycogen synthase gene (GYS1) has been focused on, because glycogen synthase (GS) is a key regulator enzyme of glucose storage pathway, which contributes to insulin-mediated glucose uptake in skeletal muscle.[2,3] Type 2 diabetes exhibits decreased mRNA expression of GYS1 gene[4,5] and, consequently, GS activity[5,6] in skeletal muscle.

The *Xba*I polymorphisms and simple tandem repeat DNA polymorphism of GYS1 gene are reported to be associated with type 2 diabetes in Finnish, French, and Japanese populations.[7-10] The 4 mutations involving substitutions of amino acid of GS have been identified as mutations modifying the encoding Gln71His, Gly464Ser, Pro442Ala, and Met416Val.[11,12] The Met416Val (M416V) polymorphism, most common among 4 mutations, is reported to be associated with insulin resistance in Japanese type 2 diabetes,[12] but another study demonstrated no association with insulin resistance in Finnish healthy populations.[11,13] Therefore, the association of the M416V polymorphism with insulin resistance in vivo remains to be clarified in the search of candidate genes for diabetes. It is a crucial problem to resolve this controversy of whether insulin sensitivity in vivo is measured accurately in a large number of patients. The aim of the present study was to clarify whether the M416V polymorphism of GYS1 gene is associated with insulin resistance assessed by euglycemic hyperinsulinemic clamp, the current gold standard technique, in type 2 diabetic patients.

SUBJECTS AND METHODS

Subjects

One-hundred unrelated Japanese type 2 diabetic subjects (66 men and 34 women) were randomly recruited from patients participating in diabetes education programs among those attending our diabetic outpatient clinic at Osaka City University Hospital. The diagnosis of type 2 diabetes was based on a previous history of diabetes or the American Diabetes Association criteria.[14] Clinical characteristics of the type 2 diabetic subjects are shown in Table 1. Fifty-seven diabetic subjects were treated with sulfonylureas (SU), 17 with α-glucosidase inhibitors (GI), 11 with the combination of SU and GI, and 24 with diet therapy alone. After admission, all subjects were under the medical nutrition therapy (energy, 25 to 30 kcal/kg ideal body weight), and euglycemic hyperinsulinemic clamp was performed within 1 to 2 weeks after admission as described below.[15,16] Subjects with chronic renal failure were excluded, and none of subjects had any clinical evidence of active hepatic, renal, endocrine, or inflammatory diseases. Informed consent was obtained from all participants, and the study was approved by the Ethics Committee.

Determination of GYS1 Genotype

DNA was extracted from peripheral-blood leukocytes by standard methods.[17] The M416V genotype of the GYS1 gene was determined according to the previously described polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.[11,12] The wild-type and mutated alleles were determined by the presence or absence of the *Nla*III restriction site that occurs as the result of a nucleotide substitution of changing methionine to valine in the amino acid sequence of the GS protein. The M416V mutation destroys the recognition site of restriction enzyme *Nla*III. Briefly, DNA (50 to 100 ng) was amplified by PCR. The PCR products of exon 10 were ethanol-precipitated and subsequently digested for 3 hours at 37°C with the restriction endonuclease *Nla*III. The resulting fragments were separated by electrophoresis on a 2.5% agarose gel and visualized by ethidium bromide staining.

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Table 1. Clinical Characteristics of Type 2 Diabetic Subjects With and Without M416V Mutation of Glycogen Synthase Gene

	All Subjects	M416V(+) (n = 18)	M416V(−) (n = 82)	P Value
Age (yr)	53.1 ± 11.6	52.4 ± 12.2	53.3 ± 11.5	.754
BMI (kg/m^2)	23.3 ± 3.5	23.9 ± 2.9	23.2 ± 3.6	.441
Duration of diabetes (yr)	8.6 ± 6.9	10.8 ± 8.8	8.1 ± 6.3	.129
Systolic BP (mm Hg)	125 ± 20	130 ± 21	124 ± 20	.224
Diastolic BP (mm Hg)	72 ± 11	72 ± 11	72 ± 10	.925
HbA_{1c} (%)	9.0 ± 2.0	9.1 ± 1.9	9.1 ± 2.0	.984
Fasting glucose (mmol/L)	8.1 ± 2.4	7.9 ± 1.6	8.1 ± 2.6	.746
Fasting insulin (pmol/L)	38.4 ± 35.4	31.2 ± 16.2	40.2 ± 38.4	.358
Cholesterol (mmol/L)	5.02 ± 1.16	5.15 ± 1.27	4.99 ± 1.16	.622
HDL cholesterol (mmol/L)	1.16 ± 0.36	1.19 ± 0.47	1.16 ± 0.36	.709
Triglycerides (mmol/L)	1.35 ± 0.87	1.90 ± 1.65	1.23 ± 0.53	.003*
Free fatty acid (mEq/L)	0.55 ± 0.25	0.48 ± 0.19	0.56 ± 0.26	.201

NOTE. All values are mean ± SD. M and SSPI, glucose infusion rate and steady-state plasma insulin levels during hyperinsulinemic clamp; M/I, GIR divided by SSPI and multiplied by 100 for convenience. Comparisons between subjects with and without M416V polymorphism, M416V(+) and M416V(-), were tested by Student's t test.

*$P < .05$.

Insulin Resistance in Type 2 Diabetic Subjects

Insulin resistance in all type 2 diabetic subjects was evaluated by euglycemic hyperinsulinemic glucose clamp (clamp) according to the method of DeFronzo et al[18] using an artificial pancreas (model STG-22, Nikkiso, Tokyo, Japan). After an overnight fast, venous blood sampling and measurement of blood pressure were performed in the supine position, and the clamp protocol was begun as previously described.[15,16] In brief, insulin (humulin, Eli Lilly, Indianapolis, IN) was infused in a continuous fashion at a rate of 1.25 mU · kg^{-1} · min^{-1} after priming insulin infusion during the first 10 minutes of the clamp at the same doses as reported previously. Blood glucose levels were determined every 5 minutes during the 120-minute clamp study, and euglycemia (5.0 mmol/L) was maintained by infusion of variable amounts of 20% glucose solution. The mean coefficient of variance of blood glucose in maintaining euglycemia was 1.29% and ranged from 0.4% to 2.9%. The total-body glucose disposal rate was evaluated as the mean of the glucose infusion rate (GIR) during the last 30 minutes of the clamp and defined as M value in units of mg · kg^{-1} · min^{-1}. The mean plasma insulin level during the steady state (SSPI) was 633.6 ± 186.0 (SD) pmol/L in all diabetic subjects. The insulin resistance index, M/I value, adjusted by insulin levels, was calculated by dividing the mean GIR by SSPI levels during the last 30 minutes of the clamp and multiplying by 100 for convenience.

Biochemical Analysis

Plasma glucose levels were measured by the glucose oxidase method, glycated hemoglobin (HbA_{1c}) levels by high-performance liquid chromatography (reference range, 4.0% to 5.5%), and plasma insulin levels by immunoradiometric assay (Insulin RIA Bead II kit; Dainabot, Tokyo, Japan). Serum total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, and free fatty acid levels were measured by enzymatic methods adapted to an autoanalyzer (Hitachi 7450; Hitachi, Tokyo, Japan).

Statistical Analysis

Statistical analyses were performed with the Stat View 5 system (SAS, Institute, Cary, NC) for Windows computers. All values are expressed as means ± SD, unless otherwise indicated. Student's t-tests were appropriately used for comparisons of the 2 groups. Simple linear regression analyses and multiple regression analyses were performed to examine the relationships between insulin resistance index and clinical variables. P values less than .05 were regarded as statistically significant.

RESULTS

Allele Frequency of M416V Polymorphism of GYS1 Gene

The allele frequencies of M416V polymorphism in all type 2 diabetic subjects were 82.0% (n = 82) for MM, 16.0% (n = 16) for MV, and 2.0% (n = 2) for VV. The genotype frequencies were found to be comparable with those in previous reports on Japanese and Finnish subjects (Japanese, $\chi^2 = 1.663$, $P = .197$; Finnish, $\chi^2 = .027$, $P = .870$).[16,17] To explore the effects of the presence of V allele on insulin resistance, the subjects were divided into the V(+) group (n = 18) and V(-) group (n = 82) according to the presence or absence of the V allele. Clinical characteristics of the 2 groups are shown in Table 1. There were no significant differences in age, duration of diabetes, BMI, blood pressure, fasting plasma glucose, or insulin levels, HbA_{1c}, or total or HDL cholesterol levels between the 2 groups. The serum triglyceride level in the V(+) group was significantly higher than that in the V(-) group.

Insulin Resistance and M416V Genotype in Diabetic Subjects

In all diabetic subjects, M value ranged from 1.11 to 12.05, with a mean of 5.11 ± 2.06 mg · kg^{-1} · min^{-1} and M/I value 1.19 to 14.92, with a mean of 5.36 ± 2.89 mg · kg^{-1} · min^{-1} · mU^{-1} · L. No significant differences were found in M values and M/I values among MM, MV, and VV allele group (M, 5.12 ± 2.04, 5.04 ± 2.25, 5.20 ± 2.60 mg · kg^{-1} · min^{-1}, $P = .954$, respectively; M/I, 5.39 ± 2.87, 5.22 ± 3.81, 5.38 ± 2.83 mg · kg^{-1} · min^{-1} · mU^{-1} · L, $P = .992$, respectively). There were no significant differences in either steady-state blood glucose (5.03 ± 0.16 v 5.03 ± 0.18 mmol/L, $P = .863$) or insulin (SSPI) level between the V(+) and V(-) groups (648 ± 186 v 630 ± 186 pmol/L, $P = .707$, respectively). Neither M nor M/I value in the V(+) group differed significantly from that in the V(-) groups as shown in Fig 1 (M, 5.06 ± 2.20 v 5.12 ± 2.04 mg · kg^{-1} · min^{-1}, $P = .903$; M/I, 5.24 ± 3.07 v 5.39 ± 2.87 mg · kg^{-1} · min^{-1} · mU^{-1} · L, $P = .841$, respectively). In simple regression analysis, BMI and fasting insulin and free fatty acid levels significantly showed inverse correlation with the M/I value for the group of all subjects (Table 2). Because

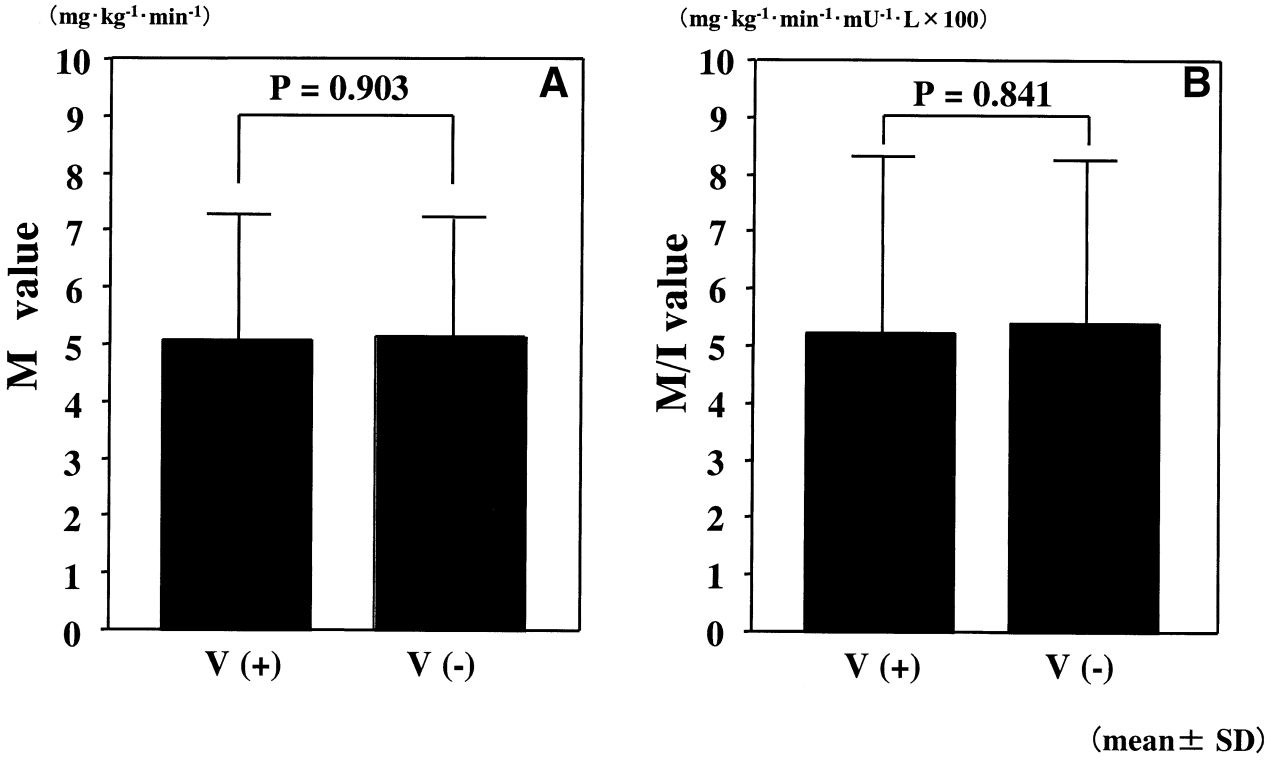

Fig 1. Insulin resistance in 100 type 2 diabetic subjects with and without the M416V allele of glycogen synthase gene. The V(+) group included 18 type 2 diabetic subjects with the M416V allele and the V(-) group included 82 without the M416V allele. Insulin resistance was assessed by euglycemic hyperinsulinemic clamp. (A) M value was determined by GIR during the steady state and (B) M/I value by GIR adjusted by steady state plasma insulin level, multiplied by 100. There was no significant difference in M or M/I value between V(+) and V(-) groups in type 2 diabetic subjects.

insulin resistance in type 2 diabetes is expected to be affected by various clinical factors, we performed multiple regression analyses to explore the impact of the presence of V allele on insulin resistance. The model used for analysis included M/I value as a dependent variable and the presence of V allele entered as 1 as well as age, gender (male entered as 1 and female as 0), BMI, HbA_{1c}, serum triglyceride, and free fatty acid levels as independent variables. BMI ($\beta = -0.462$, $P < .001$) showed the strongest independent contribution to M/I value, but the presence of V allele ($\beta = 0.045$, $P = .642$) did not in multiple regression analysis, as shown in Table 3 ($R^2 = .320$, $P < .001$).

Table 2. Simple Regression Analyses of Clinical Factors Possibly Affecting Insulin Resistance Index, M/I, Assessed by Euglycemic Hyperinsulinemic Clamp, in all Type 2 Diabetic Subjects

	r	*P*
Age	−.119	.238
BMI	−.501	<.001*
Systolic BP	−.122	.238
Diastolic BP	−.110	.288
HbA_{1c}	.185	.066
Fasting glucose	.046	.650
Fasting insulin	−.490	<.001*
Cholesterol	−.142	.159
HDL cholesterol	.0004	.997
Triglycerides	−.098	.332
Free fatty acid	−.226	.025*

*$P < .05$.

DISCUSSION

The present study provided no evidence that M416V mutation of the GYS1 gene is associated with insulin resistance in type 2 diabetes. We used a euglycemic hyperinsulinemic glucose clamp technique, the gold standard technique for measur-

Table 3. Multiple Regression Analyses of Clinical Factors Possibly Affecting Insulin Resistance Index, M/I, Assessed by Euglycemic Hyperinsulinemic Clamp, in all Type 2 Diabetic Subjects

	β	*P*
BMI	−.446	<.001
Male gender	.185	.050
Age	−.101	.304
Triglycerides	−.096	.346
HbA_{1c}	.084	.377
Free fatty acid	−.069	.478
Presence of V allele	.045	.642
R^2	.320	<.0001

Abbreviations: β, standard correlation coefficient; R^2, multiple coefficients of determination.

ing insulin sensitivity in humans, in all 100 type 2 diabetic patients examined. Type 2 diabetic subjects with V allele mutation showed clinical characteristics comparable to those of subjects without V allele mutation, except for serum triglyceride level. We found no difference in insulin resistance between type 2 diabetic subjects with and without V allele. Furthermore, the presence of V allele did not have a significant impact on insulin resistance index in analyses adjusting for clinical factors possibly affecting insulin resistance.

M416V is the most common in both diabetic and healthy subjects among the Finnish and Japanese populations.[11,12] Rissanen et al[11] have investigated the association between Gln71His (exon2) and Met416Val (exon10) polymorphisms of the GYS1 gene and insulin resistance assessed by euglycemic hyperinsulinemic clamp in 82 Finnish healthy subjects and insulin-modified minimal model technique in another 295 healthy subjects. They demonstrated that there were no significant differences in insulin resistance assessed by either euglycemic hyperinsulinemic clamp or minimal model technique between subjects with and without Met416Val polymorphism. Another study found no association of M416V polymorphism with insulin resistance assessed by euglycemic hyperinsulinemic glucose clamp in 137 Finnish healthy subjects, although data were not presented in detail.[13] Regarding the association of M416V polymorphism with insulin resistance in type 2 diabetic subjects, there is one report by Shimomura et al[12] that demonstrated 82 type 2 diabetic patients with M416V had lower insulin sensitivity index than those without this mutation assessed by an insulin-modified minimal model technique. Our finding of a lack of association with insulin resistance and M416V polymorphism is compatible with that for Finnish healthy subjects,[11,13] but not with for Japanese diabetic subjects.[12] The frequency of distribution of M416V polymorphism in our study is comparable to those in previous Japanese[12] and Finnish[11] studies. It appears that age, BMI, and glycemic control of our diabetic subjects were comparable to those in previous studies. Thus, it is likely that the subjects of our study have a clinical profile possibly affecting insulin resistance similar to that in previous studies. One explanation may be possible for discordance in type 2 diabetic patients of our findings with previous studies, that is, the different methods measuring insulin sensitivity in humans. Although the index assessed by insulin-modified minimal model technique is correlated with the index by euglycemic hyperinsulinemic clamp,[19-21] it is also reported that there are systematic errors in minimal models estimates of glucose effectiveness and insulin sensitivity.[22,23] Our study, using the current gold standard method for insulin resistance in humans, can exclude such possibility of limitations as in the minimal model technique.

Type 2 diabetic patients have various clinical factors possibly influencing insulin resistance, that is, age, gender, obesity, glucose, and lipid toxicity, as well as genetic factors.[1] In fact, in simple regression analyses of our subjects, insulin resistance index was significantly correlated with BMI, fasting insulin, and free fatty acid levels, as expected. When multiple regression analysis was performed to resolve the complexity and mutual interactions of these clinical factors, only degree of obesity was found to be an independent factor for insulin resistance, but the presence of V allele was not. However, this result does not rule out the possibility of a causative impact of another genetic factor or polymorphism on insulin resistance, because the coefficient of determinant (R^2) was only 0.32 and the remainder of the variability in insulin resistance, 68%, cannot be explained by the factors included in this analytic model.

In conclusion, it is unlikely that M416V polymorphism of the GYS1 gene makes a major contribution to insulin resistance in vivo in type 2 diabetes. Further studies are needed in search of candidate genes and their polymorphism for insulin resistance in type 2 diabetes.

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