Brief Genetics Report

Expression of Naturally Occurring Variants in the Muscle Glycogen Synthase Gene

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On the basis of accumulating biochemical and metabolic evidence, the muscle glycogen synthase (GS) gene (*GYS1*) can be considered an important candidate for skeletal muscle insulin resistance. In support of this hypothesis, an association between markers in the *GYS1* gene on chromosome 19q13.3 (1) and type 2 diabetes has been found in Finnish, French, Japanese, and Pima Indian populations (2–5). Mutation screening of the coding regions of the *GYS1* gene has revealed four amino acid variants: Gln71His, Met416Val, Pro442Ala, and Gly464Ser (6–9). To obtain information on the functional importance of these naturally occurring variants in the *GYS1* gene, we expressed the mutated enzymes in vitro in COS7 cells, measured their catalytic efficiency, and estimated the kinetic parameters. Furthermore, the allelic frequencies and biological consequences of the variants were studied in Scandinavian populations.

The association and phenotypic studies were performed in Finnish, Swedish, and Danish type 2 diabetic patients and nondiabetic healthy control subjects. For association studies, the Met416Val polymorphism in the *GYS1* gene (9) was analyzed in 688 unrelated type 2 diabetic patients (340 Finnish, 103 Swedish, and 245 Danish) and 633 unrelated control subjects (295 Finnish, 98 Swedish, and 240 Danish) without a family history of type 2 diabetes. The Gly464Ser mutation in exon 11 of the *GYS1* gene (6) was screened in 598 type 2 diabetic patients (359 Finnish and 239 Swedish) and in 255 control subjects (125 Finnish and 130 Swedish), and the Pro442Ala mutation in exon 11 (9) was analyzed in 342 type 2 diabetic patients and 300 control subjects from Finland. Description of the clinical characteristics of subjects screened for the different *GYS1* variants and research design and methods is available in the online appendix at www.diabetes.org/diabetes/appendix.asp. Briefly, in the Finnish and Swedish study groups, the Met416Val, Pro442Ala, and Gly464Ser variants were screened by polymerase chain reaction, followed by restriction fragment length analysis on an agarose gel (6,7). In the Danish population, the Met416Val variant was screened by single-strand conformational polymorphism. The clinical characteristics of different genotype carriers were compared with estimates of the metabolic and clinical consequences of the *GYS1* gene variants. Seven different mutated *GYS1* cDNAs were created by site-directed mutagenesis and expressed transiently in COS7 cells: GYS1-71His (Gln71 His), GYS1-416Val (Met416 Val), GYS1-442Ala (Pro442 Ala), and GYS1-464Ser (Gly464 Ser) and three control mutations: GYS1-8Ala (Ser8 Ala), GYS1-38Ser (Asn38 Ser), and GYS1-39Gln (Lys39 Gln). The control mutations were created to test the sensitivity of the expression system and the activity assay. The Ser8Ala mutation was created as a positive control, since changing the phosphorylation site 2 (Ser8) to alanine should increase the GS activity at low glucose-6-phosphate (G-6-P) concentrations (10,11). The two other control mutations, Asn38Ser and Lys39Gln, were created as negative controls designed to decrease the activity of GS, since they are located near the proposed uridine diphosphate glucose (UDPG) binding site (12,13). GS activity was determined from cell homogenates at 0.3 and 7.1 mmol/l UDPG with 0.1 and 10 mmol/l G-6-P using a fluorometric method (14). To obtain a dose-response curve for the allosteric activation by G-6-P, the GS activity was determined at 0, 0.03, 0.5, 2.0, and 10.0 mmol/l G-6-P, while the UDPG concentration was fixed at 0.3 mmol/l. To obtain a dose-response curve for the UDPG substrate activation, the GS activity was measured at 0, 0.1, 0.3, 0.6, 1.2, and 7.1 mmol/l UDPG, while G-6-P concentration was fixed at 0.1 mmol/l. GS activity at physiological concentrations of G-6-P (0.1 mmol/l) is called GS activity, whereas GS activity at 10 mmol/l G-6-P is referred to as total GS activity. Fractional velocity (FV) is defined as GS activity at 0.1 mmol/l G-6-P (or at another given G-6-P concentration) divided by total GS activity. Expression of the GYS1-WT and the different mutated muscle GSs was analyzed by Western blot with a rabbit polyclonal antibody against human muscle GS (2).

Prevalence of different GS variants in type 2 diabetic patients and control subjects. The Met416Val polymorphism was more common in Finnish than in Swedish or Danish control subjects (6.4 vs. 0 and 0.4%; $P < 0.05$ and 0.0005, respectively). There was, however, no difference in the fre-

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Additional information can be found in an on-line appendix at www.diabetes.org/diabetes.appendix.asp.

FV, fractional velocity; G-6-P, glucose-6-phosphate; GS, glycogen synthase; UDPG, uridine diphosphate glucose.

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quency of the Val allele carriers between type 2 diabetic patients and control subjects, either in Finland (6.8 vs. 6.4%; $P = 0.87$), Sweden (1.0 vs. 0.0%; $P = 0.32$), or Denmark (0.8 vs. 0.4%; $P = 0.57$). In this new series of 598 type 2 diabetic patients and 225 control subjects from Finland and Sweden, we identified the Gly464Ser variant in only one female type 2 diabetic patient from Finland. The variant was also found in her sister with type 2 diabetes and in two of five nondiabetic subjects in the family. The Pro442Ala variant was not detected in the 342 type 2 diabetic patients and 300 control subjects from Finland.

Expression of the mutated GS enzymes. The expression of the wild-type muscle GS in COS7 cells resulted in ~20 times higher total GS activity compared with the normal intracellular GS activity in COS cells (272 ± 36 vs. 13 ± 1 nmol · min^{-1} · mg^{-1} protein). All the expressed GSs were detected as single 84 kDa bands with similar electrophoretic mobilities and level of expression in the Western blot analysis (data not shown). Neither the GS activity at physiological concentrations of G-6-P and UDPG, nor the total GS activity or fractional velocities of the GYS1-71His, GYS1-416Val, or GYS1-464Ser differed significantly from those of the wild-type enzyme yielding normal dose-response curves for the activation by both UDPG and G-6-P (Fig. 1). The calculated K_m constants at 0.1 mmol/l G-6-P were 1.2 ± 0.3, 1.4 ± 0.3, 1.4 ± 0.4, and 1.1 ± 0.1 for GYS1-WT, GYS1-71His, GYS1-416Val, and GYS1-464Ser, respectively (Fig. 1). In contrast, GYS1-442Ala resulted in significantly decreased activation by both UDPG (K_m 2.5 ± 0.7; $P < 0.05$) and G-6-P (K_a 1.8 ± 0.1 vs. 1.0 ± 0.2; $P < 0.05$) compared with the wild type. GS activity of the GYS1-442Ala at low UDPG and low G-6-P concentrations resulted in 90% decreased activity compared with the wild type (0.9 ± 1.5 vs. 9.5 ± 2.3 nmol · min^{-1} · mg^{-1} protein; $P < 0.002$), whereas the GS activity at high UDPG and low G-6-P concentrations was decreased by 61% (19 ± 11 vs. 49 ± 5 nmol · min^{-1} · mg^{-1} protein; $P < 0.01$), and the total GS activity by 24% (207 ± 14 vs. 272 ± 36 nmol · min^{-1} · mg^{-1} protein; $P < 0.05$). The dose-response curve for UDPG activation indicated decreased ability to catalyze glycogen synthesis at low G-6-P concentrations, yet the effect of this mutation cannot be exclusively explained by the decreased allosteric activation by G-6-P, since the activity at high G-6-P concentrations was also decreased. It is possible that the change from the helix-breaking amino acid proline into another aliphatic amino acid (alanine) has a significant influence on the protein conformation.

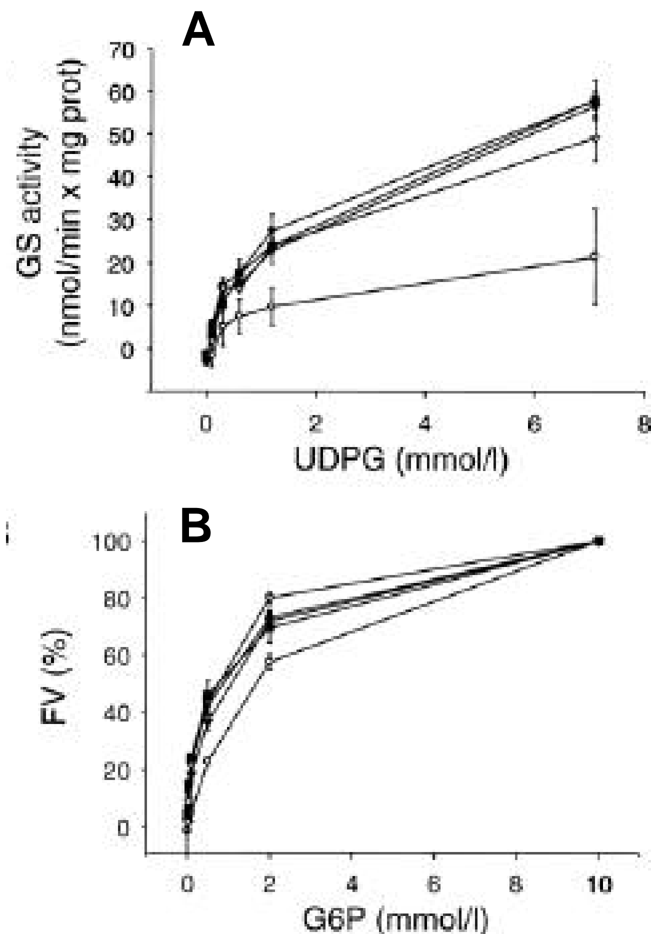

FIG. 1. The dose-response curves for UDPG activation of the expressed GSs with 0.1 mmol/l G-6-P (*A*) and for G-6-P activation at 0.3 mmol/l UDPG (*B*). ●, GYS1-WT; ■, GYS1-71His; ▲, GYS1-416Val; ○, GYS1-442Ala; △, GYS1-464Ser. Values are presented as means ± SD.

Three control mutations were expressed to estimate the sensitivity of the expression system used. As expected, the Ser8Ala mutation resulted in increased FV at 7.1 mmol/l UDPG compared with the GYS1-WT (34 ± 10 vs. 18 ± 2%; $P < 0.002$). Replacing an asparagine at codon 38 by a serine was assumed to dramatically affect the glycogen synthesis rate. In fact, the mutation resulted in a 88% decrease in total GS activity (34 ± 21 vs. 272 ± 36 nmol · min^{-1} · mg^{-1} protein; $P < 0.01$), while the GS activity at low G-6-P and high UDPG concentration was unmeasurable. The third control mutation, Lys39Gln, which was supposed to moderately decrease the glycogen synthesis rate at low substrate concentrations resulted in 56% decreased FV at 0.3 mmol/l UDPG compared with the wild-type enzyme (10 ± 3 vs. 23 ± 3%; $P < 0.05$).

Metabolic and clinical consequences of GS variants. The type 2 diabetic patients and control subjects with different Met416Val genotypes did not significantly differ in age, BMI, blood glucose, HbA_{1c}, fasting plasma insulin, or serum lipid concentrations (data not shown). In keeping with the expression data, the glucose disposal rate measured during a hyperinsulinemic clamp did not differ between normoglycemic Met/Met homozygotes ($n = 128$) and Met/Val heterozygotes ($n = 9$) (6.5 ± 2.6 vs. 5.9 ± 2.1 mg · kg^{-1} · min^{-1}). These results are in accordance with measurements in 52 normoglycemic Met416Val carriers from Finland (7), but in contrast to the findings of decreased insulin sensitivity in Japanese type 2 diabetic patients with this variant (9). The metabolic results are consistent with the results from the present expression study, where the properties of GYS1-416Val could not be distinguished from those of the wild-type enzyme. Viewed in context with the expression data, it is unlikely that this relatively common variant has functional importance for insulin-stimulated glycogen synthesis or the development of insulin resistance in type 2 diabetes.

The Gly464Ser variant was identified in one Finnish type 2 diabetic family. Carriers of Gly464Ser did not significantly differ from the nondiabetic family members without the variant with respect to BMI, waist-to-hip ratio, fasting blood glucose, and fasting serum insulin concentrations (data not shown). In a recent Finnish study, the Gly464Ser was identified in

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three type 2 diabetic families and in two families with familial combined hyperlipidemia from eastern Finland. In contrast to our previous results (6), the mutation was not associated with insulin resistance in these families (7). Interestingly, it seems as if the Gly464Ser originates in eastern Finland, since we could not identify this variant in western Finland (6) or in Sweden, nor has it been found in Japan or England (9,15). The expression data, however, challenge the view that this new serine would be of importance for inactivation of the enzyme by phosphorylation or other mechanisms.

The Japanese patient carrying the Pro442Ala mutation (9) was a 77-year-old female with BMI 21.9 kg/m^2, HbA_{1c} 6.3%, fasting serum insulin 5.6 mU/l, and a fasting C-peptide concentration of 0.32 nmol/l. She had type 2 diabetes for 19 years, and she had been treated with diet alone for several years up until the last 2 years, during which time she had received tolbutamide (200 mg/day). She also had hypertension and mild ischemic heart disease. If the Pro442Ala mutation in the *GYS1* gene is the cause of diabetes in this patient, it would indicate that this relatively severe impairment in muscle glycogen synthesis is not sufficient to cause severe diabetes, at least not when the mutation presents in its heterozygous form.

In conclusion, the common Met416Val variant in the *GYS1* gene is not of functional importance for glycogen synthesis in skeletal muscle, nor is the variant associated with in vivo impairment of insulin sensitivity in nondiabetic individuals. In contrast, the rare Pro442Ala mutation resulted in significantly decreased capability to synthesize glycogen, and represents the first mutation in the *GYS1* gene with functional consequences. It remains to be shown whether this impairment in skeletal muscle glycogen synthesis is sufficient to cause the mild diabetes found in the mutation carrier.

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