

Brief Genetics Report

Polymorphism in the Glycogen-Associated Regulatory Subunit of Type 1 Protein Phosphatase (PPP1R3) Gene and Insulin Sensitivity

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A polymorphism (PP1ARE) in the 3'-untranslated region of the gene encoding the glycogen-associated regulatory subunit of type 1 protein phosphatase PPP1R3 is associated with insulin resistance in Pima Indians. The aim of this study was to investigate whether two common variants in the PPP1R3 gene, Asp905Tyr and PP1ARE, are associated with reduced insulin sensitivity or can predict the development of impaired glucose tolerance (IGT) or type 2 diabetes during a 20-year follow-up period in 696 50-year-old Caucasian men. The allelic frequency of Tyr905 was 0.11 (95% CI 0.09–0.13) and of PP1ARE 0.34 (0.31–0.37) and the two polymorphisms were in linkage disequilibrium ($\chi^2 = 46$, $P < 0.0001$, Fisher's exact test). None of the polymorphisms was associated with the development of IGT or type 2 diabetes, but the PP1ARE polymorphism was weakly correlated to whole-body insulin sensitivity ($r = -0.08$, $P = 0.04$). In conclusion, we found no evidence in Swedish men that the PP1ARE or the Asp905Tyr variants over a 20-year period predict the development of IGT or type 2 diabetes, but the PP1ARE polymorphism could have a higher penetrance in other populations. *Diabetes* 49:298–301, 2000

The finding of reduced insulin-stimulated whole-body glucose disposal rate under hyperinsulinemic-euglycemic clamp conditions in both type 2 diabetic patients and their glucose-tolerant first-degree relatives (1) has suggested that insulin resistance, in part, may be an inherited element in the etiology of type 2 diabetes. Since the major routing of insulin-dependent whole-body glucose uptake is the nonoxidative glycogen synthesis in skeletal muscle (2), several genes encoding key enzymes in this pathway have been evaluated as candidate genes for

the impaired insulin-regulated glycogen synthesis associated with type 2 diabetic patients and their glucose-tolerant relatives (3–8). One of these genes, PPP1R3, which encodes the glycogen-associated regulatory subunit of type 1 protein phosphatase (9), harbors two common amino acid variants: Asp905Tyr and Arg883Ser. In glucose-tolerant Danish Caucasians, Tyr905 is not associated with type 2 diabetes or decreased insulin-stimulated whole-body glucose uptake, but it is associated with decreased insulin-stimulated non-oxidative glucose metabolism, i.e., glycogen synthesis (10). The allelic frequencies of Tyr905 vary from 0.10 in Caucasians and >0.44 in Pima Indians to 0.68 in the Japanese population (10–12). Arg883Ser was identified in Pima Indians together with a five-base pair polymorphism (PP1ARE2) in the 3'-untranslated region (11). In the Pima Indians, the three gene variants are in linkage disequilibrium so that the wild-type allele Asp905 and the Arg883 are 100% coupled with the PP1ARE five-base pair polymorphism (PP1ARE2) and therefore all three variants are associated with a 35–22% reduction in insulin-stimulated whole-body glucose disposal under hyperinsulinemic-euglycemic clamp conditions. In contrast, Tyr905 and Ser883 always occur with the wild-type PP1ARE (PP1ARE1) in this ethnic group, and they are all associated with a 28–56% higher insulin sensitivity (11).

To investigate the potential impact of the Arg883Ser, Asp905Tyr, and PP1ARE variants of PPP1R3 as predictors of impaired glucose tolerance (IGT) or overt type 2 diabetes in a prospective setting and their potential influence on insulin sensitivity in a cross-sectional setting in the Caucasian population, we genotyped 696 men participating in the Uppsala Health Survey Study.

The Arg883Ser variant was not found in 82 random volunteers and was not further investigated. The allelic frequency of Tyr905 was 0.11 (95% CI 0.09–0.13) and of PP1ARE2 0.34 (0.31–0.37). Table 1 shows the genotypic variation of the Tyr905 and PP1ARE alleles in the study cohort. Both variants were in Hardy-Weinberg equilibrium, but the two polymorphisms were in linkage disequilibrium ($\chi^2 = 46$, $P < 0.0001$, Fisher's exact test).

Since this coupling in Caucasians is not 100% as in the Pima Indians, we could evaluate the physiological impact of the PP1ARE variant regardless of the genotype on the other polymorphic site, codon 905 (Table 2), or in combination with the Asp905 genotype (Table 3). First, analyzing for the

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IGT, impaired glucose tolerance; IMGU, insulin-mediated glucose uptake; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test; OHA, oral hypoglycemic agents.

TABLE 1

Genotypes for the codon 905 and PP1ARE variants of the glycogen-targeting subunit of type 1 protein phosphatase (PPP1R3) in 696 70-year-old Swedish Caucasian men

Genotypes	PP1ARE11	PP1ARE12	PP1ARE22
Asp905Asp*	217	248	91
Asp905Tyr	84	47	0
Tyr905Tyr	8	1	0

Wild-type, heterozygote, and homozygote of PP1ARE are designated 11, 12, and 22, respectively. The average age of study participants was 70 years. *Asp905Asp and PP1ARE are in linkage disequilibrium (3×3 contingency table, $\chi^2 = 46$, $P < 0.0001$, Fisher's exact test).

PP1ARE variant alone, the three groups (PP1ARE11, PP1ARE12, and PP1ARE22) showed a significant but weak trend when ranked in a correlation analysis ($r = -0.08$, $P = 0.04$ after correction for BMI) and regardless of the other polymorphic site on codon 905 (Table 2). However, this correlation was not strong enough for the PP1ARE polymorphism to have a significant effect on the insulin-mediated glucose uptake (IMGU) (Table 2, $P = 0.33$) when analyzed with the general linear model also used in the study of this polymorphism in the Pima Indians (11). Next, we included an interaction term (BMI*PP1ARE) with BMI as a continuous variable in the regression analysis. There was no significant interaction of the PP1ARE variant with BMI on insulin sensitivity (Table 2, $P = 0.08$).

Also, the combination of homozygous Asp905/PP1ARE1 ($n = 202$) was not associated with significant differences, as evaluated by a Student's *t* test, in IMGU during a clamp (5.2 ± 2.5 vs. 4.9 ± 2.4 mg glucose \cdot min $^{-1}$ \cdot [mU/I] $^{-1}$ \cdot 100, $P = 0.08$) when compared with any other combination of PP1ARE and codon 905 variants: PP1ARE12/Asp905, PP1ARE22/Asp905, PP1ARE12/Tyr905, or PP1ARE22/Tyr905 carriers ($n = 453$).

In the Pima Indians, the PP1ARE2 allele is always seen together with the Asp905 allele (11) and, therefore, we also subanalyzed our data according to this genotype on codon 905 (Table 3). Thus, when eliminating the Tyr905 variant, the combination Asp905/PP1ARE2 was still weakly correlated to IMGU ($r = -0.09$, $P = 0.04$, Table 3) in these 70-year-old Swedish men, but in contrast to what was reported for these genotypes in the Pima Indians (11), the Asp905/PP1ARE1 carriers were not more insulin sensitive than the Asp905/

PP1ARE2 (5.2 ± 2.5 vs. 4.8 ± 2.4 mg glucose \cdot min $^{-1}$ \cdot [mU/I] $^{-1}$ \cdot 100, $P = 0.07$; $P = 0.10$, corrected for BMI). Together, these weak correlations between genotypes and IMGU in our study ($\text{IMGU}_{\text{PP1ARE11}} > \text{IMGU}_{\text{PP1ARE12}} > \text{IMGU}_{\text{PP1ARE22}}$), which were not evident in Pima Indians (11), however, might suggest a subtle gene-dosage effect of the PP1ARE2 variant on insulin resistance. But, in view of the multiple tests performed on different polymorphisms within the same gene and in the same cohort, a *P* value of 0.04 can hardly be considered significant. We found no correlation between the PP1ARE variants and fasting serum insulin ($r = 0.05$, $P = 0.21$), even though fasting serum insulin and IMGU are highly correlated ($r = 0.50$) in the study population.

Regarding the prediction of type 2 diabetes during the 20 years of follow-up, the prevalence of type 2 diabetes at age 70 years was similar between the Asp905/PP1ARE11 ($n = 208$), Asp905/PP1ARE12 ($n = 239$), and Asp905/PP1ARE22 ($n = 90$) genotypes: 0.17 vs. 0.15 vs. 0.16, respectively, and comparable to the overall diabetes prevalence in the cohort of 0.15. In addition, the prevalence of glucose intolerance after the 20 years was similar between Asp905/PP1ARE11 ($n = 208$), Asp905/PP1ARE12 ($n = 239$), and Asp905/PP1ARE22 ($n = 90$) genotypes: 0.11 vs. 0.12 vs. 0.14, respectively (prevalence in cohort: 0.13). In the Pima Indians, the prevalence of early-onset type 2 diabetes (<45 years of age) among Asp905/PP1ARE12 and Asp905/PP1ARE22 carriers was reported to be higher compared with Tyr905/PP1ARE11 carriers (11).

The Tyr905 allele of PP1G has previously been examined in a cross-sectional study from the Danish Caucasian population (10) and showed no associations to either type 2 diabetes or alterations in insulin-mediated whole-body glucose uptake during a hyperinsulinemic-euglycemic clamp. These data were confirmed by the present study, since the Asp905/PP1ARE1 carriers were not different from the Tyr905/PP1ARE1 carriers regarding whole-body insulin sensitivity and there was no significant correlation between the Tyr905 and IMGU (Table 3). We have previously found preliminary evidence of increased insulin sensitivity in obese carriers of the Tyr905 variant (13) when analyzing by linear regression, including a dichotomous obesity (BMI) variable in the interaction term ([BMI, 80th percentile] *Tyr905), but this effect could not be seen if the more correct interaction term (BMI*Tyr905) with BMI as a continuous variable was included in the analysis and consequently relied on a statistical artifact.

TABLE 2

BMI, fasting serum insulin, and IMGU of the 666 Swedish men according to either PP1ARE genotypes or to codon 905 genotypes of PPP1R3

	PP1ARE11	PP1ARE12	PP1ARE22	Asp905Asp	Asp905Tyr	Tyr905Tyr
n	290	285	91	525	121	9
BMI (kg/m 2)	26.2 \pm 3.2	26.2 \pm 3.5	26.3 \pm 3.1	26.2 \pm 3.4	26.3 \pm 2.9	26.0 \pm 2.9
Fasting serum insulin (mU/I)	13.3 \pm 7.0	13.2 \pm 6.8	15.1 \pm 14.7	13.5 \pm 8.7	13.6 \pm 7.6	14.2 \pm 4.7
IMGU (mg \cdot kg $^{-1}$ \cdot min $^{-1}$ \cdot [mU/I] $^{-1}$ \cdot 100)	5.11 \pm 2.42*	4.86 \pm 2.31*	4.78 \pm 2.57*	4.97 \pm 2.43	4.93 \pm 2.31	4.86 \pm 2.1

Data are means \pm SD. Serum insulin and plasma glucose were measured as previously described (19). IMGU is measured from 60 to 120 min during a 2-h, 56 mU \cdot min $^{-1}$ \cdot m $^{-2}$ body surface hyperinsulinemic-euglycemic clamp. The average age of study participants was 70 years. * $P = 0.33$ using the general linear modeling program and $P = 0.08$ including the interaction term BMI*PP1ARE in the analysis. Using trend test and Spearman correlation analysis, $P = 0.04$ after correction for BMI (SAS Institute, Cary, NC).

TABLE 3

BMI, fasting serum insulin, and IMGU of the 525 Swedish male Asp905Asp carriers according to PP1ARE genotypes and of the 285 Swedish male PP1ARE11 carriers according to codon 905 genotypes of PPP1R3

	PP1ARE11	PP1ARE12	PP1ARE22	Asp905Asp	Asp905Tyr	Tyr905Tyr
n	202	236	87	202	75	8
BMI (kg/m ²)	26.1 ± 3.3	26.2 ± 3.6	26.4 ± 3.1	26.2 ± 2.9	26.3 ± 2.9	26.0 ± 2.9
Fasting serum insulin (mU/l)	13.0 ± 6.4*	13.4 ± 7.1*	15.2 ± 15.0*	13.5 ± 8.7	13.6 ± 7.6	14.2 ± 4.7
IMGU (mg · kg ⁻¹ · min ⁻¹ · [mU/l] ⁻¹ · 100)	5.21 ± 2.46†	4.83 ± 2.33†	4.78 ± 2.60†	5.21 ± 2.46	4.92 ± 2.37	4.86 ± 2.24‡

Data are means ± SD. Serum insulin and plasma glucose were measured as previously described (19). IMGU is measured from 60 to 120 min during a 2-h, 56 mU · min⁻¹ · m⁻² body surface hyperinsulinemic-euglycemic clamp. The average age of study participants was 70 years. *P = 0.21, †P = 0.05, and ‡P = 0.48 using trend test and Spearman correlation analysis, after correction for BMI (SAS Institute, Cary, NC).

Also, in accordance with previous reports (10,12), Tyr905 did not predict diabetes, since the prevalence of diabetes was similar in each of the groups: Asp905Asp/PP1ARE11 (0.17), Asp905Tyr/PP1ARE11 (0.14), and Tyr905Tyr (0.13).

Few *in vivo* data of the actual role of PP1G in mediating the effect of insulin on glycogen synthesis and glucose transport exist, and somewhat conflicting evidence has been reported on this issue. Stable overexpression of PP1G in L6 myoblasts/myotubes does not affect basal glucose uptake and glycogen synthesis, but in response to maximal doses of insulin, both are increased compared with untransfected cells (14). However, studies with transient overexpression of PP1G in L6 myoblasts/myotubes using an adenovirus-based system show both an increase in basal and insulin-stimulated glycogen synthesis, but glucose uptake remains unaffected (15). Studies in humans have shown a positive correlation between the PP1G protein content in skeletal muscle and IMGU (11) but also that a change in insulin-stimulated glycogen synthesis may not necessarily be reflected in IMGU (10). These studies raise the question as to whether changes in glycogen synthesis on the cellular level are reflected by corresponding changes in glucose uptake through a possible "pull" mechanism or rather that increased glycogen synthesis will concomitantly activate glycogen phosphorylase and hence lead to compensatory glycogenolysis. Accordingly, transgenic mice overexpressing the glycogen synthase gene in skeletal muscle show both increased glycogen synthesis and glycogenolysis but unaffected glucose uptake (16).

The findings in this study do not support the view that PP1ARE being a marker for decreased PP1G mRNA and protein content is associated with changes in insulin-mediated whole-body glucose uptake in Caucasians to the same extent as has been reported in the Pima Indians (11). In the Pima Indians, association between the PP1ARE variant and insulin-resistant phenotype was shown at three different levels: low-dose and high-dose hyperinsulinemic clamp and fasting serum insulin levels (11, Table 1). In our study, we could demonstrate only a weak trend for the association of the PP1ARE variant with an insulin-resistant phenotype at one level: when estimated by IMGU and not when estimated by fasting serum insulin.

That analyses of genotype-phenotype interactions in humans (i.e., quantitative traits) are difficult to interpretate is further evidenced by two recent studies: one in aboriginal Canadians with IGT or overt type 2 diabetes in which the PP1ARE2 allele was associated with 34% lower values of 2-h post-oral glucose tolerance test (OGTT) plasma glucose lev-

els (17), hence suggesting an increase in insulin sensitivity and an opposite effect of the variant in that specific population compared with the findings in Pima Indians. A second study in the Japanese population showed the PP1ARE22 genotype and the overall allelic frequency of the PP1ARE2 allele to be associated with type 2 diabetes but not associated with any insulin-resistant phenotype among nondiabetic subjects (18).

Finally, the effect on insulin-stimulated glycogen synthesis could not be addressed in our study because the clamp methodology did not include indirect calorimetry. Therefore, both the Tyr905 and PP1ARE2 alleles need to be expressed in suitable cell systems or, alternatively, living carriers of these variants should be examined by nuclear magnetic resonance spectroscopy during hyperinsulinemic-hyperglycemic clamp conditions (2) to obtain a more direct answer to the question as to whether genetic variability of PP1G is directly involved in insulin resistance of glycogen synthesis and glucose intolerance.

RESEARCH DESIGN AND METHODS

Subjects. In total, 696 males from the Uppsala Health Survey Study participated in the present study. Briefly, in 1970–1973, all 50-year-old men (n = 2,841) living in the municipality of Uppsala who were born in 1920–1924 were invited to take part in a health survey that included estimation of glucose tolerance by an intravenous glucose tolerance test (IVGTT). Subjects with treated diabetes or with fasting plasma glucose > 6.7 mmol/l or a disappearance rate of glucose (K value) 0.9 during the IVGTT were excluded as described (19). The participation rate was 82% (n = 2,322). For the second survey, those participants with a fasting glucose > 5.7 mmol/l underwent a standard 75-g OGTT. In 1992–1994, the 1,680 surviving men who had been examined in 1970–1973 and who were still living in Uppsala were invited for a third examination that included an estimation of the insulin sensitivity by a 120-min hyperinsulinemic-euglycemic clamp (56 mU · min⁻¹ · m⁻²). With a mean steady-state level of ~600 pmol/l of serum insulin concentration reached during the 60–120 min period in which the IMGU was measured, this hyperinsulinemic-euglycemic clamp applied in our study (56 mU · min⁻¹ · m⁻² body surface) is well within normal physiological ranges of hyperinsulinemia and is comparable to the low-dose clamp applied in the Pima Indian study (11). Finally, a 75-g OGTT according to 1985 WHO criteria was applied for the diagnosis of diabetes or glucose intolerance (20). The participation rate was 73% (n = 1,221), and 17% had a first-degree relative with type 2 diabetes by questionnaire. Of the 696 participants from whom DNA was available for genotyping in this study, 72% remained glucose tolerant, whereas 12 and 16% had developed IGT or overt diabetes, respectively, during the 20 years of follow-up. All diabetic subjects were classified as having type 2 diabetes because all were treated with diet alone or oral hypoglycemic agents (OHA). Only 10% were treated with OHA in combination with insulin. Of the 696 participants, 666 subjects underwent a 56-mU hyperinsulinemic-euglycemic clamp. The study was approved by the ethics committee of the Faculty of Medicine at the University of Uppsala. Informed consent was obtained from all participants.

Genetic analyses. Genotyping was carried out by polymerase chain reaction–restriction fragment length polymorphism for the Asp905Tyr (10) and the Arg883Ser (11) variants. The PP1ARE variant was determined by denaturing gel electrophoresis as described (11).

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