Brief Genetics Report

The Gly972Arg Polymorphism in the Insulin Receptor Substrate-1 Gene Contributes to the Variation in insulin Secretion in Normal Glucose-Tolerant Humans

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The Gly972Arg polymorphism in the insulin receptor substrate (IRS)-1 was found in some studies to have a higher prevalence in type 2 diabetic subjects than in control subjects. Previously, transfection of IRS-1 with this polymorphism into insulin-secreting cells resulted in a marked reduction of glucose-stimulated insulin secretion compared with the wild-type transfected cells. In the present study, we compared insulin secretion in well-matched normal glucose-tolerant subjects with and without this polymorphism. Several validated indexes of β-cell function from the oral glucose tolerance test were significantly lower in X/Arg (n = 31) compared with Gly/Gly (n = 181) (P between 0.002 and 0.05), whereas insulin sensitivity (measured with a euglycemic clamp) was not different. During a modified hyperglycemic clamp, insulin secretion rates were significantly lower in Gly/Arg (n = 8) compared with Gly/Gly (n = 36) during the first phase (1,711 ± 142 vs. 3,014 ± 328 pmol/min, P = 0.05) and after maximal stimulation with arginine (5,340 ± 639 vs. 9,075 ± 722 pmol/min, P = 0.03). In summary, our results suggest that the Gly972Arg polymorphism in IRS-1 is associated with decreased insulin secretion in response to glucose but not with insulin sensitivity. It is possible that this polymorphism causes insulin resistance at the level of the β-cell and contributes to the polygenic etiology of type 2 diabetes. *Diabetes* 50:882–885, 2001

The pathogenesis of type 2 diabetes involves a combination of impaired insulin secretion and insulin resistance (1). The insulin receptor substrate (IRS)-1 represented a prime candidate for genetic variants potentially impairing insulin signaling, and the Gly972Arg polymorphism was found with higher frequency in subjects with type 2 diabetes in some (2,3) but not all studies (4). In vitro, this mutation resulted in reduced insulin signaling along the phosphatidylinositol 3-kinase (PI3-K) pathway (5,6) and a decrease in insulin-stimulated GLUT4 translocation, glucose uptake, and glycogen synthesis (6,7). In vivo, an association with insulin resistance was observed (8), but this association was not confirmed by several other studies (9–14).

Recently, insulin-secreting cells overexpressing the IRS-1 Gly972Arg variant had a decreased sulfonylurea- and glucose-stimulated insulin secretion compared with cells overexpressing the wild-type IRS-1 (15). It was consequently suggested that this polymorphism might represent a genetic variant unifying insulin resistance and β-cell dysfunction. Alternatively, the IRS-1 Gly972Arg variant might prevent the physiological compensatory increase in insulin secretion that occurs when insulin sensitivity deteriorates.

To specifically address the question of whether the IRS-1 972Arg variant contributes to the variation of insulin secretion in humans, we analyzed oral glucose tolerance tests (OGTTs) of 212 subjects (31 with and 181 without the mutation). We used a number of recently validated indexes to estimate β-cell function from insulin (and C-peptide) concentrations obtained during an OGTT. In addition, we studied 8 subjects with and 36 subjects without the polymorphism using a sophisticated hyperglycemic clamp method with the secretagogues glucagon-like peptide 1 (GLP-1) and arginine in addition to hyperglycemia.

We recruited 212 unrelated volunteers who were healthy and glucose-tolerant according to World Health Organization criteria (16). We studied these subjects using OGTTs and euglycemic hyperinsulinemic clamps (1 mU · kg^{-1} · min^{-1}) (11), after first obtaining local ethical committee approval and the patients' consent. In addition, a subset of 44 subjects (unknown genotype at time of recruitment) were studied using a modified hyperglycemic clamp (17). The allelic frequency of the Arg allele was 7.8% and the genotype distribution was in Hardy-Weinberg equilibrium (P = 0.8, χ^2 test). The IRS-1 Gly972Arg polymorphism was determined by polymerase chain reaction and subsequent restriction enzyme analysis with *Mva*I as previously described (11).

Blood glucose, plasma insulin, and C-peptide concentradions during the OGTT are shown in Fig. 1. On average, the subjects with the Arg allele had a 20–30% lower insulin

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CIR_{30}, corrected insulin response after 30 min; CP_{AUC}, C-peptide area under the curve; G_{AUC}, glucose area under the curve; GLP-1, glucagon-like peptide 1; I_{AUC}, insulin area under the curve, IRS, insulin receptor substrate; ISI, insulin sensitivity index; IVGTT, intravenous glucose tolerance test; MCR, metabolic clearance rate of glucose; OGTT, oral glucose tolerance tests; PI3-K, phosphatidylinositol 3-kinase.

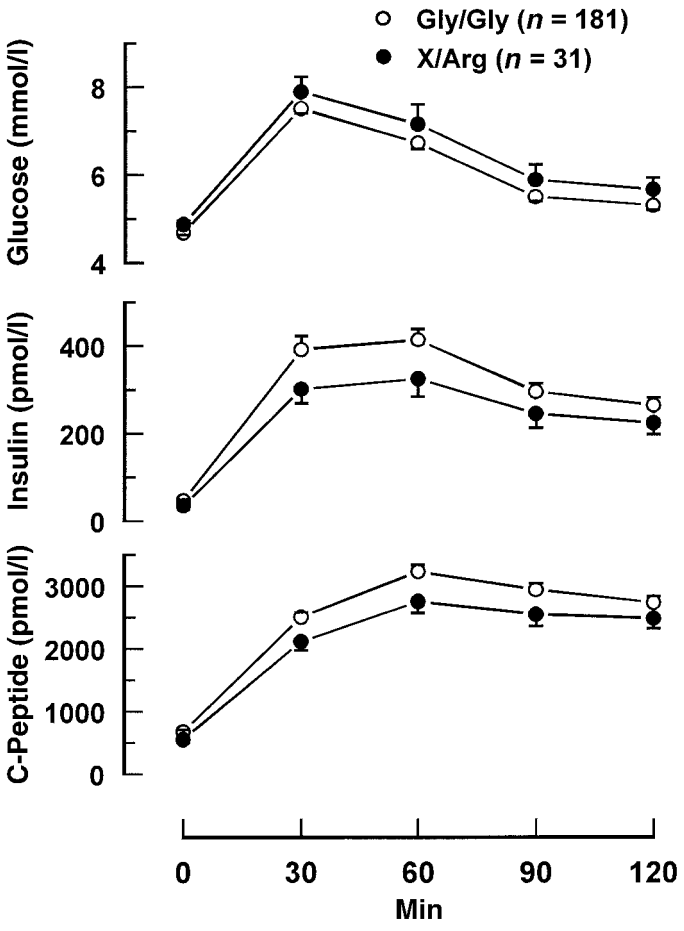

FIG. 1. Blood glucose, plasma insulin and C-peptide in subjects without (Gly/Gly) and with (X/Arg) the Gly972Arg polymorphism in IRS-1 during an OGTT.

secretion compared with the wild-type group. This difference was statistically significant in all seven indexes (Figs. 1 and 2, Table 1).

Insulin secretion in the X/Arg group was clearly lower throughout the entire hyperglycemic clamp. The mean

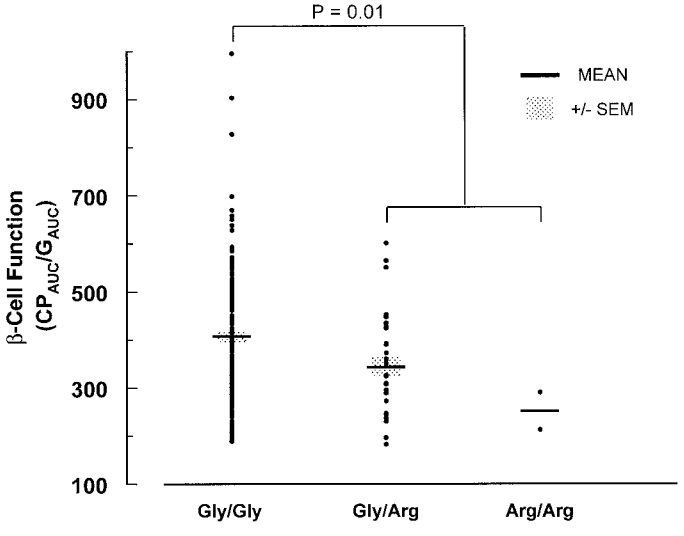

FIG. 2. β-cell function (expressed as CP_{AUC}/G_{AUC} during an OGTT) in subjects without (Gly/Gly) and with (X/Arg) the Gly972Arg polymorphism in IRS-1.

TABLE 1
Different indexes for insulin secretion from subjects without (Gly/Gly) and with (X/Arg) the Gly972Arg polymorphism in IRS-1

	Gly/Gly	X/Arg	*P*
n	181	31	
Estimated first phase	1,134 ± 61	872 ± 80	0.01
Estimated second phase	298 ± 14	239 ± 17	0.007
I_{AUC}/G_{AUC}	45 ± 10	36 ± 3	0.02
CP_{AUC}/G_{AUC}	406 ± 10	341 ± 19	0.002
CIR_{30}	0.0090 ± 0.0009	0.0056 ± 0.0006	0.03
Insulinogenic index (I)	0.43 ± 0.04	0.30 ± 0.03	0.03
Insulinogenic index (CP)	2.28 ± 0.08	1.96 ± 0.20	0.05

Data are means ± SE. CIR was calculated as $Ins_{30}/(Gluc_{30} \times (Glu_{30}–70)$; the insulinogenic index for insulin was calculated as $(Ins_{30}–Ins_0)/Gluc_{30}$ and analogously for C-peptide (see RESEARCH DESIGN AND METHODS for details).

insulin and C-peptide concentrations during the different phases of insulin secretion are shown in Table 2. The differences consistently achieved statistical significance during first phase and maximal insulin secretion. During second phase insulin secretion, they approached statistical significance; however, during the GLP-1 phases, the differences were not significant (Fig. 3, Table 2). We were unable to demonstrate a difference in insulin sensitivity between the two groups.

In the present study we found decreased glucose-stimulated insulin secretion during an OGTT and a hyperglycemic clamp in subjects with the Gly972Arg polymorphism in IRS-1 (Tables 3 and 4). Our results thus confirm in humans the data by Porzio et al. (15) showing diminished glucose-stimulated insulin secretion in insulin-secreting cells (RIN cells) transfected with the Arg972 variant of IRS-1. Moreover, our findings are also consistent with the report on a young healthy lean man homozygous for the Arg allele who was shown to have a 50% reduced fasting C-peptide and a 28% reduced acute C-peptide response to intravenous glucose compared with lean wild-type men (7). However, our data are at variance with the acute insulin response in the heterozygous carriers of the Arg allele in the same study (7), which was not different from that of the wild type. Differences in ethnic background

TABLE 2
Plasma C-peptide and insulin concentrations during the different phases of the modified hyperglycemic clamp

	Gly/Gly	X/Arg	*P*
C-peptide (pmol/l)			
First phase	1,548 ± 112	949 ± 96	0.001
Second phase	2,722 ± 162	2,021 ± 212	0.08
GLP-1, first phase	6,089 ± 387	4,614 ± 463	0.11
GLP-1, second phase	9,487 ± 549	7,570 ± 832	0.13
Max	11,678 ± 668	8,978 ± 1,033	0.06
Insulin (pmol/l)			
First phase	320 ± 42	146 ± 23	0.001
Second phase	380 ± 53	184 ± 34	0.03
GLP-1, first phase	1,857 ± 199	1,066 ± 165	0.12
GLP-1, second phase	4,187 ± 431	2,409 ± 359	0.12
Max	6,573 ± 633	3,646 ± 508	0.05

Data are means ± SE. first phase, mean of 2.5–10 min; second phase, mean of 80–120 min; GLP first phase, mean of 125–130 min; GLP second phase, mean of 160–180 min; maximal insulin secretion (Max), mean of 182.5–190 min.

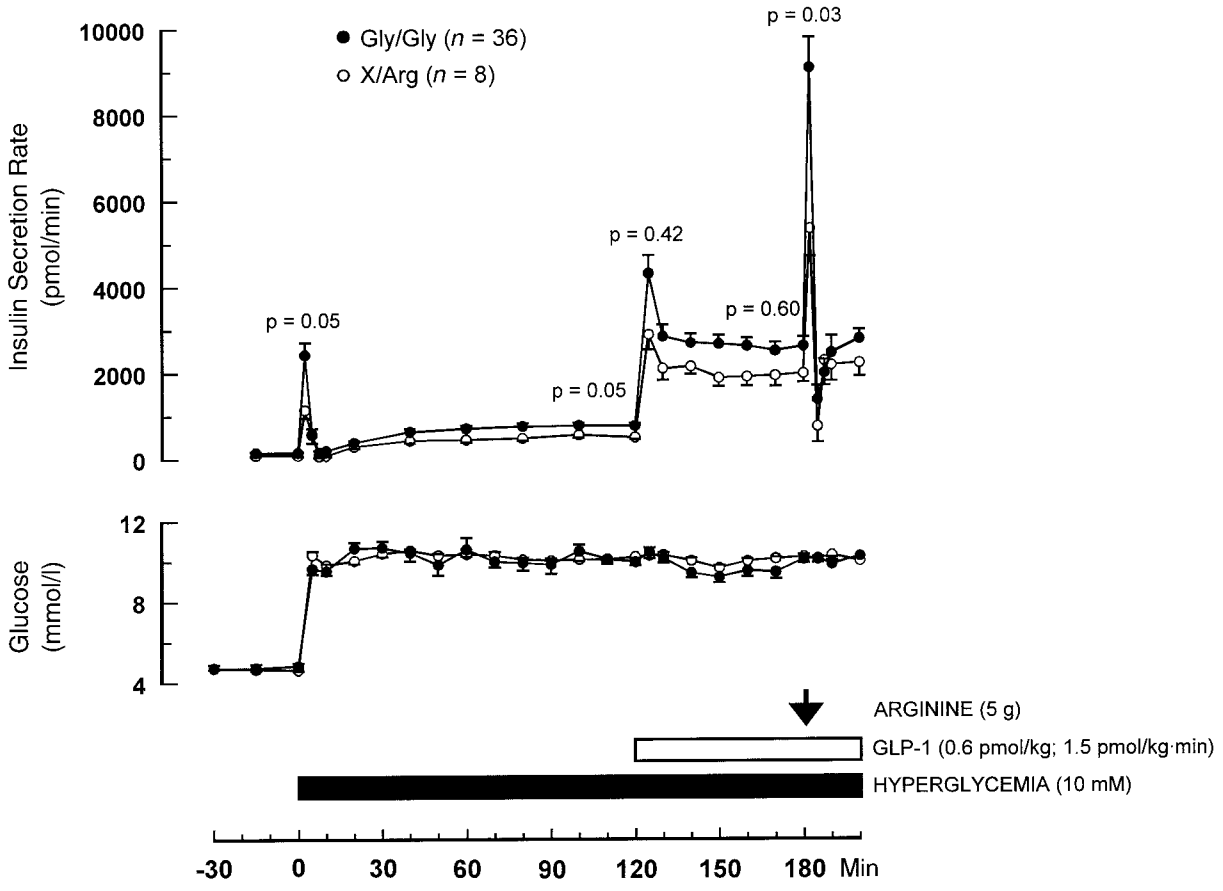

FIG. 3. Blood glucose and insulin secretion rates (ISR) during a modified hyperglycemic clamp in subjects without (Gly/Gly) and with (X/Arg) the Gly972Arg polymorphism in IRS-1. The indicated *P* values were calculated at 2.5 min, 80–120 min, 125 min, 160–180 min, and 182.5 min.

(Danes versus Germans) and the technique used to assess β-cell function (intravenous glucose tolerance test [IVGTT] versus hyperglycemic clamp and OGTT) may explain this discrepancy.

Although the OGTT has known limitations for assessing β-cell function (e.g., poor reproducibility and interindividual variation of glucose concentration), some indexes derived from the OGTT have recently been validated against the hyperglycemic clamp or the IVGTT. All of the indexes used expressed insulin (or C-peptide) concentrations relative to the absolute or incremental glucose concentration during the OGTT. The findings from the OGTTs and hyperglycemic clamps were essentially consistent.

There is growing evidence for the important role of intact signaling through the IRS family in the regulation of glucose-stimulated insulin secretion (IRS-1) and growth and survival of β-cells (IRS-2) (18). The markedly reduced glucose- and sulfonylurea-stimulated insulin secretion in RIN cells expressing the Arg972 variant of IRS-1 was accompanied by a decreased binding of the p85 subunit of PI3-K to IRS-1 (15), and PI3-K has been implicated in the trafficking of vesicles via its association with dynamin (19). Autocrine activation of the release of intracellular calcium stores was shown to be mediated by IRS-1 and PI3-K in various types of β-cells (20). Thus, the evidence available from in vitro work supports the concept that the

TABLE 3
Characteristics of subjects without (Gly/Gly) and with (X/Arg) the Gly972Arg polymorphism in IRS-1 undergoing the OGTT

	Gly/Gly	X/Arg	*P*
n (M/F)	181 (79/92)	31 (12/19)	—
Age (years)	32 ± 1	34 ± 2	0.56
BMI (kg/m^2)	24.8 ± 0.3	24.1 ± 0.8	0.51
Waist-to-hip ratio	0.83 ± 0.01	0.81 ± 0.01	0.12
Fasting plasma glucose (mmol/l)	4.69 ± 0.05	4.88 ± 0.08	0.10
Fasting plasma insulin (pmol/l)	47 ± 2	36 ± 3	0.03
MCR (ml/kg · min)*	7.9 ± 0.3	7.7 ± 0.4	0.94
ISI (μmol · kg^{-1} · min^{-1} · pmol/l^{-1})*	0.075 ± 0.003	0.084 ± 0.008	0.33

Data are means ± SE. Higher values indicate greater insulin sensitivity.

TABLE 4
Characteristics of subjects without (Gly/Gly) and with (X/Arg) the Gly972Arg polymorphism in IRS-1 undergoing the hyperglycemic clamp

	Gly/Gly	X/Arg	*P*
n (M/F)	36 (16/20)	8 (4/4)	—
Age (years)	33 ± 2	36 ± 5	0.62
BMI (kg/m^2)	24.9 ± 0.9	23.0 ± 0.9	0.56
Waist-to-hip ratio	0.82 ± 0.03	0.81 ± 0.01	0.43
Fasting plasma glucose (mmol/l)	4.67 ± 0.08	4.83 ± 0.13	0.48
Fasting plasma insulin (pmol/l)	48 ± 6	45 ± 14	0.31

Data are means ± SEM.

Gly972Arg polymorphism in IRS-1 may be of functional significance in insulin secretion.

Finally, in a subanalysis, we compared subjects with a BMI greater or smaller than 25 kg/m^2. The C-peptide area under the curve (CP_{AUC})/glucose area under the curve (G_{AUC}) was significantly greater in the Gly/Gly obese compared with the Gly/Gly lean subjects (461 ± 17 vs. 373 ± 10, $P < 0.001$). In contrast, the CP_{AUC}/G_{AUC} was virtually identical between the lean and obese subjects in the X/Arg group (336 ± 25 vs. 341 ± 27, $P = 0.91$; $P = 0.078$ ANOVA). Although this analysis is limited by the relatively small number of subjects in the X/Arg group (19 lean and 12 obese), the cross-sectional analysis, and the failure to reach statistical significance with ANOVA, the results suggest that subjects carrying the Arg allele, unlike the wild type, are unable to respond to an increase in body weight (and thus insulin resistance) with an adequate compensatory increase in insulin secretion.

In conclusion, taking together the results from the OGTT and the hyperglycemic clamp, our findings strongly suggest that the Gly972Arg polymorphism in IRS-1 is associated with β-cell dysfunction in response to glucose in our population of normal glucose-tolerant subjects. In contrast, there was no measurable effect on insulin sensitivity of glucose disposal. Although our data are somewhat discrepant with a recent analysis finding no significantly increased risk of type 2 diabetes in carriers of the IRS-1 972 polymorphism (4), the IRS-1 Gly972Arg polymorphism may well contribute to the biological variation of β-cell function.

RESEARCH DESIGN AND METHODS

The different phases of insulin secretion during the hyperglycemic clamp were calculated as described in Table 2. β-cell function during the OGTT was assessed using various estimates: estimated 1st and 2nd phase (21); the ratios of insulin area under the curve (I_{AUC}/G_{AUC} or CP_{AUC}/G_{AUC} (21); the corrected insulin response after 30 min (CIR_{30}) (22); and the insulinogenic index for insulin and C-peptide as $(CP_{30}–CP_0)/Gluc_{30}$, with CP_0 defined as C-peptide level at baseline (21,22). Insulin sensitivity during the euglycemic clamp was expressed as insulin sensitivity index (ISI) and metabolic clearance rate of glucose (MCR) (11).

For statistical comparisons with the wild-type (Gly/Gly), subjects heterozygous (Gly/Arg) and homozygous (Arg/Arg) for the mutation were combined and referred to as X/Arg. For statistical analysis, the secretion indexes were log transformed and linearly adjusted for BMI and age. Comparisons among genotypes were made using the unpaired Student's *t* test or Wilcoxon rank test where appropriate. A P value of <0.05 was considered to be statistically significant.

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