

Brief Genetics Report

Variations in Insulin Secretion in Carriers of Gene Variants in IRS-1 and -2

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Associations between type 2 diabetes (and/or parameters contributing to glucose homeostasis) and genetic variation in the genes encoding insulin receptor substrate (IRS)-1 and -2 have been reported in several populations. Recently, it has been reported that the Gly⁹⁷²Arg variant in IRS-1 was associated with reduced insulin secretion during hyperglycemic clamps in German subjects with normal glucose tolerance. We have examined glucose-stimulated insulin secretion in relation to gene variants in the IRS-1 (Gly⁹⁷²Arg) and IRS-2 (Gly¹⁰⁵⁷Asp) genes in two Dutch cohorts. Subjects with normal ($n = 64$) or impaired ($n = 94$) glucose tolerance underwent 3-h hyperglycemic clamps at 10 mmol/l glucose. All subjects were genotyped for the IRS-1 and IRS-2 variants by PCR-RFLP-based methods. We did not observe any significant difference in both first- and second-phase insulin secretion between carriers and noncarriers of both gene variants, nor was there evidence for an association with other diabetes-related parameters. We conclude that the common gene variants in IRS-1 and IRS-2 are not associated with altered glucose-stimulated insulin secretion in two populations from the Netherlands. *Diabetes* 51:884–887, 2002

It has been proposed that genetic variation in the insulin receptor substrate (IRS)-1 and -2 genes is associated with the development of insulin resistance and type 2 diabetes in some populations (1–5). Recent evidence suggests that these mutations in IRS-1 and -2 are involved in the development of insulin resistance in humans (6,7). Insulin resistance at the level of the β -cell may result in reduced insulin secretion (8–11). In line with these observations, Stumvoll et al. (12) recently reported reduced insulin secretion in carriers of the Gly⁹⁷²Arg variant in IRS-1. It was shown that normal glucose tolerant (NGT) carriers of the variant had a

reduced first- and second-phase insulin secretion during a modified hyperglycemic clamp.

Genetic association studies are vulnerable for false-positive results (13). On the other hand, they have a greater power than linkage-based approaches to detect common variants that have a small effect on the phenotype (4). Therefore, the replication of association data in other populations remains essential for correct interpretation of the data.

In this study, we have analyzed insulin secretion by hyperglycemic clamps in NGT individuals in relation to the Gly⁹⁷²Arg variant in the IRS-1 gene and the Gly¹⁰⁵⁷Asp variant in the IRS-2 gene. Furthermore, we have expanded the study by the inclusion of a cohort of individuals with impaired glucose tolerance (IGT group). A large fraction of this cohort may eventually convert to overt type 2 diabetes (14).

The clinical characteristics of both study groups are shown in Table 1. The distribution of the different genotypes in each subject group is shown in Tables 2 and 3. All genotype distributions were in Hardy-Weinberg equilibrium (data not shown).

The observed IRS-1 genotype distribution in both groups is comparable to the observed frequencies in the Dutch population (3). When we examined first- and second-phase insulin secretion in NGT IRS-1 X/Arg carriers compared with wild-type Gly/Gly carriers, there were no significant differences (Table 2, Fig. 1A). Furthermore, we did not find any significant differences in glucose and insulin levels during a 2-h oral glucose tolerance test (OGTT) (data not shown). Separate testing of the individuals with and without a first-degree relative with type 2 diabetes did not alter our findings. Inclusion of 12 additional subjects with IGT into the NGT group (sampled from the same popula-

TABLE 1
Clinical characteristics of the study groups

	NGT	IGT
<i>n</i> (M/F)	64 (16/48)	94 (45/49)
Age (years)	45.8 \pm 6.5	57.2 \pm 7.3
BMI (kg/m ²)	25.8 \pm 3.8	28.4 \pm 3.8
Waist-to-hip ratio	0.81 \pm 0.07	0.93 \pm 0.09
Fasting plasma glucose (mmol/l)	4.6 \pm 0.5	6.6 \pm 0.6
Fasting plasma insulin (pmol/l)	32 (24–42)	66 (47–97)

Data are means \pm SD or median (interquartile range)

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IRS, insulin receptor substrate; NGT, normal glucose tolerant; OGTT, oral glucose tolerance test.

TABLE 2
Hyperglycemic clamp results in NGT and IGT subjects according to IRS-1 972 genotype

	NGT		IGT	
	Gly/Gly	X/Arg	Gly/Gly	X/Arg
<i>n</i>	55	9	82	12
Fasting plasma glucose (mmol/l)	4.6 ± 0.1	4.7 ± 0.1	6.6 ± 0.1	6.5 ± 0.2
Fasting plasma insulin (pmol/l)	34 (24–42)	30 (24–43)	67 (48–101)	69 (49–93)
First-phase insulin secretion (pmol/l)	1,698 (1,110–2,280)	1,512 (1,257–3,825)	576 (231–1,084)	923 (431–1,171)
Second-phase insulin secretion (pmol/l)	300 (234–421)	332 (238–446)	213 (138–382)	250 (211–323)
Glucose infusion rate (mg/kg · min ⁻¹)	9.2 (6.6–12.3)	8.7 (7.1–17.2)	5.5 (4.1–6.7)	5.4 (3.6–6.5)
Insulin sensitivity index (100 · mg/kg · min ⁻¹ /pmol/l)	3.2 (2.0–4.5)	2.9 (1.7–4.1)	1.4 (1.0–2.2)	1.6 (0.8–1.9)
Total cholesterol (mmol/l)	4.97 ± 0.12	4.98 ± 0.33	5.77 ± 0.10	6.24 ± 0.36
HDL cholesterol (mmol/l)	1.36 ± 0.05	1.46 ± 0.09	1.04 ± 0.03	1.04 ± 0.06
LDL cholesterol (mmol/l)	3.10 ± 0.11	3.04 ± 0.28	3.69 ± 0.10	4.09 ± 0.23
Triglycerides (mmol/l)	1.11 ± 0.07	1.05 ± 0.12	2.19 ± 0.15	2.38 ± 0.38

Data are means ± SE or median (interquartile range); X = Gly or Arg.

tion as the NGT subjects) also did not alter the results (data not shown). Moreover, in IGT subjects we also did not observe a difference in insulin secretion in carriers of the X/Arg genotype (Table 2, Fig. 1B). The insulin sensitivity index, as calculated from the hyperglycemic clamp, was also not significantly different between carriers and noncarriers, nor was there any evidence for an interaction with BMI for each of the variables tested.

The frequency of the IRS-2 1057 variant was similar to that reported in other populations (2,7). Both the first and the second phase of glucose-stimulated insulin secretion were not significantly different between the three genotypes in either group (Table 3, Fig. 2A and B). Analyzing the data, assuming a dominant effect of the mutation, also did not alter the results. There was no evidence for an interaction between the gene variant and BMI in these studies. Data from OGTTs were also comparable between

the three different genotypes (not shown). Carriers of the combination of both mutations (NGT *n* = 6, IGT *n* = 7) did not have altered insulin secretion or insulin sensitivity index compared with the rest of the study group (data not shown). Mice with disruptions of the IRS genes also have abnormal lipid profiles (15,16). In our study we found no significant differences in lipid profiles between carriers and noncarriers, although this might be due to the sample size used (Tables 2 and 3).

In this study we have shown that two gene variants in the genes for IRS-1 and -2 are not associated with a detectable difference in the biological variation in glucose-stimulated insulin secretion in two Dutch populations. Both in NGT (partly first-degree relatives of type 2 diabetic subjects) and in IGT subjects, we observed no significant differences in insulin secretion. Our results are in contrast with those recently published for a German population of

TABLE 3
Hyperglycemic clamp results in NGT and IGT subjects according to IRS-2 1057 genotype

	NGT			IGT		
	Gly/Gly	Gly/Asp	Asp/Asp	Gly/Gly	Gly/Asp	Asp/Asp
<i>n</i>	33	25	6	38	44	12
Fasting plasma glucose (mmol/l)	4.4 ± 0.1	4.7 ± 0.1	4.5 ± 0.2	6.6 ± 0.1	6.6 ± 0.1	6.4 ± 0.1
Fasting plasma insulin (pmol/l)	28 (24–42)	34 (28–43)	28 (24–37)	62 (44–105)	68 (51–102)	75 (46–93)
First-phase insulin secretion (pmol/l)	1,569 (1,101–2,535)	1,758 (1,254–2,253)	1,740 (1,235–2,153)	551 (300–1,244)	570 (208–1,060)	723 (269–1,636)
Second-phase insulin secretion (pmol/l)	274 (190–430)	332 (278–572)	267 (213–345)	197 (111–327)	220 (166–420)	277 (181–453)
Glucose infusion rate (mg/kg · min ⁻¹)	8.1 (6.3–11.0)	9.6 (7.9–12.7)	12.5 (9.0–16.9)	4.9 (3.8–6.5)	5.5 (3.9–6.7)	5.9 (4.6–7.0)
Insulin sensitivity index (mg/kg · min ⁻¹ /pmol/l)	3.3 (1.8–4.5)	2.9 (2.0–3.7)	5.4 (2.9–8.3)	1.4 (1.0–2.2)	1.5 (0.8–2.3)	1.6 (0.8–2.0)
Total cholesterol (mmol/l)	5.06 ± 0.15	4.97 ± 0.18	4.83 ± 0.44	5.77 ± 0.14	5.91 ± 0.16	5.67 ± 0.24
HDL cholesterol (mmol/l)	1.44 ± 0.06	1.30 ± 0.08	1.54 ± 0.22	1.01 ± 0.04	1.07 ± 0.03	1.10 ± 0.08
LDL cholesterol (mmol/l)	3.15 ± 0.14	3.13 ± 0.16	2.88 ± 0.37	3.58 ± 0.13	3.94 ± 0.14	3.46 ± 0.24
Triglycerides (mmol/l)	1.04 ± 0.06	1.21 ± 0.13	0.92 ± 0.13	2.49 ± 0.25	1.91 ± 0.15	2.39 ± 0.41

Data are means ± SE or median (interquartile range).

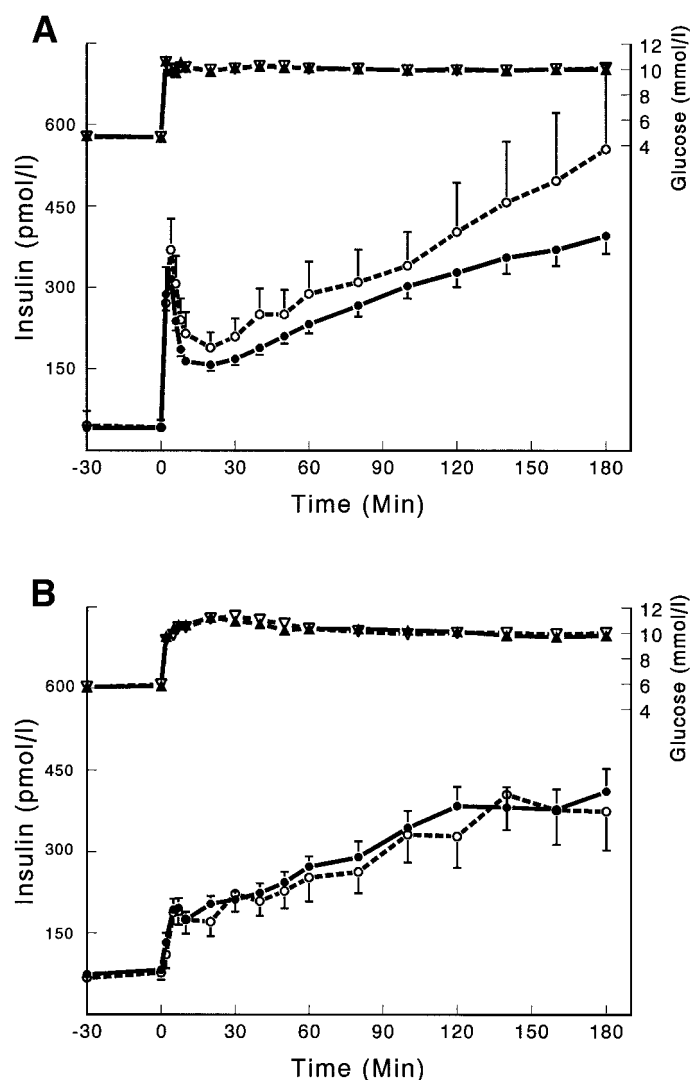


FIG. 1. Glucose and insulin profiles during a 3-h hyperglycemic clamp at 10 mmol/l glucose according to the IRS-1 972 genotype. Data are expressed as the means \pm SE. **A:** Subjects with NGT ($n = 64$). **B:** Subjects with IGT ($n = 94$). Filled symbols represent the Gly/Gly carriers and open symbols represent the X/Arg carriers (X represents either Gly or Arg: NGT group, $n = 9$; IGT group, $n = 12$).

NGT subjects in which a reduced insulin secretion in IRS-1 X/Arg carriers was reported (12). It might be that these discrepancies were due to the different populations studied. We have studied a larger group of NGT first-degree relatives and IGT subjects, whereas Stumvoll et al. (12) examined healthy volunteers at a younger age category than the subjects we examined. However, by studying first-degree relatives of type 2 diabetic subjects and IGT subjects, these groups are known to have decreased insulin secretion during hyperglycemic glucose clamps (14,17,18), one would expect that if the IRS-1 972 variant would affect insulin secretion this would most likely become apparent in these at-risk subjects.

The IRS-2 Gly¹⁰⁵⁷ Asp mutation was recently implicated in the pathogenesis of insulin resistance and type 2 diabetes in Italians but not in the Danish population (5,7). Homozygous mice with two null alleles of the IRS-2 gene have diabetes and altered β -cell properties (19,20). We hypothesized that this mutation might impair insulin secretion by impairing signal transduction in the pancreatic

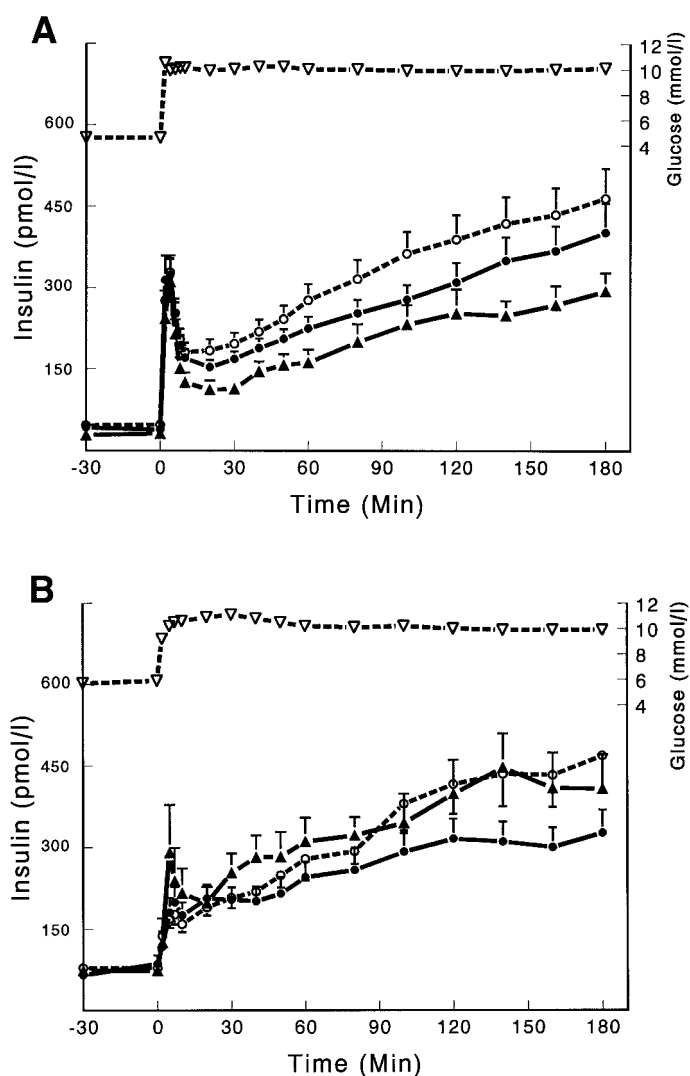


FIG. 2. Glucose and insulin profiles during a 3-h hyperglycemic clamp at 10 mmol/l glucose according to the IRS-2 1057 genotype. Data are expressed as means \pm SE. **A:** Subjects with NGT ($n = 64$). **B:** Subjects with IGT ($n = 94$). Δ , Mean glucose levels in each subject group; \bullet , insulin levels in homozygous wild-type subjects; \circ , heterozygotes; \blacktriangle , homozygous mutant subjects.

β -cell. Our data on both normal and IGT subjects, however, do not support this hypothesis. This suggests that variation at this position in the gene is not associated with glucose-induced insulin secretion in the two Dutch study populations. Our data further corroborate the data previously reported in Danish, Scandinavian, and German populations (5,21). Also, in these studies there was no consistent association between the IRS-2 1057 variant and glucose-induced insulin secretion.

We conclude that association between gene variants in IRS-1 and -2 and variation in glucose-stimulated insulin secretion during hyperglycemic clamps was not detectable in two different populations from the Netherlands.

RESEARCH DESIGN AND METHODS

NGT ($n = 64$) and IGT ($n = 94$) subjects were participants of two separate study cohorts in the Netherlands. Subjects with normal glucose tolerance were partially selected as first-degree relatives of type 2 diabetic subjects ($n = 44$). The other part of this cohort consists of matched control subjects without a family member with known diabetes (22). Glucose tolerance status was

confirmed by OGTT in all subjects according to 1985 World Health Organization criteria (23).

Subjects with IGT were selected based on two separate OGTTs (measurements at baseline and after 2 h). They were included in this study if the mean postload glucose level was between 8.6 and 11.1 mmol/l; details of both study groups are described elsewhere (22,24). Informed consent was obtained from all participants in the studies, and the local medical ethics committee approved the protocol. Participants were analyzed for the presence of the IRS-1 972 and IRS-2 1057 variants as described previously (1,2).

Hyperglycemic clamp. All subjects had undergone 3-h hyperglycemic clamps at 10 mmol/l glucose before genotyping. First-phase insulin secretion was calculated as area under the curve during the first 10 min of the clamp. Second-phase insulin secretion was calculated as the average insulin level during the third hour minus basal. Details of the clamp procedure were as described previously (22,24). We assessed the insulin sensitivity index with the hyperglycemic clamps as the glucose infusion rate (expressed as $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) divided by average plasma insulin level of the third hour (expressed as pmol/l). In previous studies, it has been shown that this manner of assessing insulin sensitivity gives almost equivalent results as assessments with the gold standard, the hyperinsulinemic-euglycemic clamp (25). A priori power calculations showed that the design used in this study would allow the detection of a difference in the first or second phase of insulin secretion between 25 and 50%, with 90% power ($P \leq 0.05$), dependent on the gene variant studied and the model applied (either dominant or recessive, data not shown). This design should thus provide enough power to at least detect the differences in insulin secretion as reported by Stumvoll et al. (12).

Statistical analyses. All analyses were performed with SPSS version 10.0 software (SPSS, Chicago). Data are presented as the means \pm SE or median with interquartile range. ANOVA or the Mann-Whitney *U* test was used for general comparisons between the different genotypes. Variables were log transformed before analysis if necessary. Adjustments for age, sex, and BMI were done in separate analyses for all parameters. Results were regarded as significant at $P \leq 0.05$.

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