Brief Genetics Report

Association Testing in 9,000 People Fails to Confirm the Association of the Insulin Receptor Substrate-1 G972R Polymorphism With Type 2 Diabetes

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The insulin receptor substrate (IRS)-1 is an important component of the insulin signal transduction cascade. Several reports suggest that a Gly→Arg change in codon 972 is associated with type 2 diabetes and related traits, and a recent meta-analysis reported a modest but nominally significant association with type 2 diabetes (odds ratio [OR] 1.25 in favor of carriers of the Arg allele [95% CI 1.05–1.48). To test the reproducibility of the model in a recent meta-analysis, we examined genotype-phenotype correlation in three large Caucasian samples (not previously reported for this variant) totaling 9,000 individuals (estimated to have >95% power to obtain a $P < 0.05$ for the OR of 1.25 estimated in the meta-analysis). In our combined sample, comprising 4,279 case and 3,532 control subjects, as well as 1,189 siblings discordant for type 2 diabetes, G972R was not associated with type 2 diabetes (OR 0.96 [0.84–1.10], $P = 0.60$). Genotype at G972R had no significant effect on various measures of insulin secretion or insulin resistance in a set of Scandinavian samples in whom we had detailed phenotypic data. In contrast, the well-documented associations of peroxisome proliferator–activated receptor γ P12A and Kir6.2 E23K with type 2 diabetes are both robustly observed in these 9,000 subjects, including an additional (previously unpublished) confirmation of Kir6.2 E23K and type 2 diabetes in the Polish and North American samples (combined OR 1.15 [1.05–1.26], $P = 0.001$). Despite genotyping 9,000 people and >95% power to reproduce the estimated OR from the recent meta-analysis, we were unable to replicate the association of the IRS-1 G972R polymorphism with type 2 diabetes. *Diabetes* 53: 3313–3318, 2004

Insulin receptor substrate (IRS)-1 is one of multiple proteins that mediate signal transduction of the activated insulin receptor (1). When phosphorylated on tyrosine residues, IRS proteins bind effector molecules that contain the src homology 2 domain, including phosphatidylinositol 3-kinase (PI3K). Binding of PI3K by IRS proteins activates a phosphorylation cascade that ultimately leads to various downstream effects of insulin in specific tissues (2). Based on evidence obtained from tissue-specific knockout experiments, IRS-1 is thought to be a necessary component of insulin action in skeletal muscle, adipose tissue, and pancreatic β-cells (3).

IRS-1 is therefore an attractive candidate gene to harbor genetic variation that might influence insulin resistance and/or type 2 diabetes in humans (4). In particular, a common coding variant of IRS-1 (the substitution of a glycine residue for arginine at position 972, G972R) has been associated with type 2 diabetes in a number of studies, although not in others (rev. in 5). A recent meta-analysis examined 27 studies comprising 8,827 subjects and estimated an odds ratio (OR) of 1.25 for type 2 diabetes in carriers of the minor Arg allele; the statistical significance of this result was modest ($P \sim 0.03$). In vitro, the G972R variant causes a decrease in PI3K activity in myeloid cells that express the insulin receptor (6), and overexpression of the mutant allele leads to decreased glucose transport in skeletal muscle cells (7). Interestingly, one study claims that insulin secretion is decreased in human islets isolated from carriers of the Arg polymorphism (8).

In general, genetic association studies have been plagued

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HOMA, homeostasis model assessment; HOMA-β, HOMA of β-cell function; HOMA-IR, HOMA of insulin resistance; IRS, insulin receptor substrate; ISI, insulin sensitivity index; OGTT, oral glucose tolerance test; PI3K, phosphatidylinositol 3-kinase; PPAR, peroxisome proliferator–activated receptor.

TABLE 1
Clinical characteristics of patient samples

Sample	Sex (M/F)	Age (years)	BMI (kg/m^2)	Fasting plasma glucose (mmol/l)	Plasma glucose at 2-h OGTT (mmol/l)* or HbA_{1c} (%)†
Sibships					
Diabetes/severe IGT sib	280/329	65 ± 10	29 ± 5	9.3 ± 3.3	14.3 ± 5.6*
NGT sib	275/305	62 ± 10	26 ± 3	5.4 ± 0.4	6.0 ± 1.1*
Scandinavia case/control					
Diabetes/severe IGT	252/219	60 ± 10	28 ± 5	9.8 ± 3.4	15.0 ± 5.3*
NGT	254/217	60 ± 10	27 ± 4	6.2 ± 1.8	6.8 ± 2.8*
Sweden case/control					
Diabetes/severe IGT	267/247	66 ± 12	28 ± 4	8.5 ± 2.5	15.5 ± 4.0*
NGT	267/247	66 ± 12	28 ± 4	4.8 ± 0.6	ND
Botnia case/control					
Diabetes/severe IGT	425/507	68 ± 12	27 ± 8	9.1 ± 3.2	14.3 ± 6.0*
NGT	60/125	48 ± 11	24 ± 5	5.3 ± 0.4	5.5 ± 1.1*
Canada case/control					
Diabetes	70/57	53 ± 8	29 ± 5	6.4 ± 1.8	12.8 ± 2.1*
NGT	70/57	52 ± 8	29 ± 4	5.1 ± 0.6	6.1 ± 1.1*
Genomics Collaborative U.S. case/control					
Diabetes	644/582	63 ± 11	33 ± 7	9.8 ± 3.0	8.0 ± 3.1†
NGT	644/582	61 ± 10	27 ± 5	5.1 ± 0.9	ND
Genomics Collaborative Poland case/control					
Diabetes	422/587	62 ± 10	30 ± 5	8.9 ± 4.0	7.9 ± 1.3†
NGT	422/587	59 ± 7	26 ± 4	4.8 ± 1.2	ND

Data are means ± SD. Plasma glucose was measured at baseline (fasting) and 2 h after an OGTT. IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

by irreproducibility (9), with nonreplication thought to be due to a combination of inappropriately low thresholds for declaring association and inadequate statistical power in replication attempts to distinguish the few true signals from background noise (10). The few cases in which association to type 2 diabetes and other diseases has been confirmed have required very large sample sizes to obtain convincing signals. In type 2 diabetes, robust and consistently reproducible associations have been obtained for the P12A polymorphism in peroxisome proliferator–activated receptor (PPAR)-γ (11–15), the E23K polymorphism in the ATP-sensitive potassium channel Kir6.2 (16–19), and more recently, SNP-44 in CAPN10 (20,21). In light of the recent meta-analysis indicating that IRS-1 G972R might truly be associated with type 2 diabetes, we set out to test whether we could reproduce this finding in an adequately powered large collection of diabetic case and control subjects.

RESEARCH DESIGN AND METHODS

The Scandinavian/Canadian diabetic subsamples studied herein have been described elsewhere (19). Briefly, they comprise 1,189 Scandinavian siblings discordant for type 2 diabetes; a Scandinavian case-control sample totaling 942 subjects individually matched for age, BMI, and geographic region; a case-control sample from Sweden totaling 1,028 subjects who were individually matched for sex, age, and BMI; and another individually matched case-control sample totaling 254 subjects from the Saguenay Lac-St. Jean region in Quebec. The current study also includes a case-control sample totaling 1,117 individuals from the Botnia region of Finland, jointly analyzed with the above Scandinavian samples. In addition, this study also includes two large samples obtained via collaboration with Genomics Collaborative: a case-control sample of 2,452 subjects from the U.S. and a case-control sample of 2,018 subjects from Poland. The discordant sibpairs were included in this study to increase sample size, assess the potential role of population stratification in the event that an association was found, and perform an additional phenotypic comparison in genetically related individuals. The phenotypic characteristics of all patient subsamples are presented in Table 1.

Genotyping and clinical analysis. Genotyping and biochemical measurements were performed as previously described (19). Our genotyping failure rate was <1% and consensus rate 100% (based on 4,470 duplicate genotypes). The G972R polymorphism is rs1801278 in the dbSNP database. Although dbSNP lists it at position 971, in order to be consistent with the literature, we have used the previously published nomenclature, which is based on an original sequence that contains an extra glycine at position 135 (22). A 75-g oral glucose tolerance test (OGTT) was performed in a subset of the Scandinavian subjects. The insulinogenic index was calculated from the OGTT data as ([insulin at 30 min] – [insulin at 0 min])/(glucose at 30 min) (23). Percent homeostasis model assessment of β-cell function (HOMA-β) was estimated as (20 × fasting serum insulin/[fasting plasma glucose − 3.5]) (24). An estimate of insulin resistance was derived by HOMA of insulin resistance (HOMA-IR) as ([fasting serum insulin × fasting plasma glucose]/22.5) (24). The insulin sensitivity index (ISI) was calculated as previously described (25). The insulin disposition index was calculated as both (insulinogenic index/HOMA-IR) and (insulinogenic index × ISI)/100. Genotype counts for the various samples tested in this study are posted on our website (http://genetics.mgh.harvard.edu/AltshulerWeb/publicationdata/Florez_IRS1.html).

Statistical analysis. Power calculations were performed with the program of Purcell et al. (26) (available at http://statgen.iop.kcl.ac.uk/gpc). To examine the association of IRS-1 G972R with type 2 diabetes, we used simple χ^2 analysis in the case-control samples and the discordant allele test (27) in the sibpairs. Results from the various subsamples were combined by Mantel-Haenszel meta-analysis of the ORs (28). Homogeneity among studies was tested using a Pearson χ^2 goodness-of-fit test, as previously described (28).

Phenotype comparisons. We obtained the insulinogenic index, percent HOMA-β, HOMA-IR, the ISI, and the insulin disposition index (calculated by two different methods, see above) in our Scandinavian control subjects. We then compared these parameters between carriers of the G972R variant (Arg/Arg and Arg/Gly) and noncarriers (Gly/Gly) by *t* test. As an independent test, we also examined pairs of nondiabetic Scandinavian siblings who were discordant for the G972R genotype; within each pair, the relevant phenotypic measurement was compared with the corresponding variable in the respective sibling by paired *t* test. In cases where there were multiple sibs from which to choose, the two discordant sibs who were closest in age were selected.

RESULTS

Power calculations. To calculate the sample size required to replicate the association of IRS-1 G972R with type 2 diabetes, we assumed a minor allele frequency of 10% and an OR of 1.25 in G972R carriers versus noncarri-

TABLE 2
Association study of G972R

Allele R versus allele G	OR	95% CI	*P*
Scandinavian/Canadian samples	1.01	0.82–1.24	0.91
Genomics Collaborative U.S. sample	0.81	0.64–1.02	0.07
Genomics Collaborative Poland sample	1.13	0.86–1.48	0.39
Meta-analysis	0.96	0.84–1.10	0.60

Results from the different Scandinavian/Canadian subsamples shown in Table 1 are analyzed jointly in the first row. Results from the Scandinavian/Canadian and two Genomics Collaborative samples are further combined by Mantel-Haenszel meta-analysis. A formal test for heterogeneity was not significant ($P = 0.50$).

ers, as reported in the recent meta-analysis by Jellema et al. (5). We further assumed a type 2 diabetes disease prevalence of ~10%. Under these parameters, we estimated that ~3,000 case and ~3,000 control subjects would provide >95% power to reject the null hypothesis at $P < 0.05$ under the genetic model proposed by Jellema et al. Our combined sample of ~4,200 case and ~3,500 control subjects provides 98.9% power to reject the null hypothesis of no association at $P < 0.05$. Under the assumption that the minor allele frequency is 7% (as observed in the U.S. and Polish samples), our combined sample size is estimated to provide >96% power to confirm or reject the reported association.

Association study. The results of the association study for each of the diabetic subsamples are presented in Table 2. In the Scandinavian/Canadian sample, comprising 2,044 case subjects, 1,297 control subjects, and 1,189 siblings discordant for type 2 diabetes, G972R was not associated with type 2 diabetes. A second sample of North-American Caucasians, totaling 1,226 type 2 diabetic case and 1,226 control subjects, also failed to replicate the association and actually trended in the opposite direction. A third sample of 1,009 type 2 diabetic case and 1,009 control subjects from Poland was consistent with published data but did not reach statistical significance. A combined meta-analysis of all three samples fails to replicate the reported association (Table 2). When our data are combined with the diabetic trios reported by Altshuler et al. (11) and all the studies included in Jellema et al. (5), the G972R association is not statistically significant (OR 1.07 [95% CI 0.97–1.18], $P = 0.17$).

Control analyses. One possibility for nonreplication is heterogeneity among studies; that is, that some of the subsamples are inconsistent with the OR of the overall data. A formal test of heterogeneity in the group of samples genotyped in this study was not significant ($P = 0.50$), arguing against this hypothesis.

To document that the samples in question are valid for association testing to type 2 diabetes, we considered the two most widely reproduced associations of common genetic variants to type 2 diabetes, those of PPAR-γ P12A and Kir6.2 E23K (10). The association of PPAR-γ P12A with type 2 diabetes has been previously reproduced in both the Scandinavian/Canadian samples (11) and in an overlapping set of the Genomics Collaborative samples (14). We recently published the validation of the Kir6.2 E23K polymorphism in the Scandinavian and Canadian samples (19), but this polymorphism has not been previously tested in the Genomics Collaborative samples. We therefore typed Kir6.2 E23K in the U.S. and Polish samples from Genomics Collaborative, demonstrating a combined OR of 1.15 (95% CI 1.05–1.26, $P < 0.001$), consistent with previous reports (16–19). This result further strengthens the already convincing Kir6.2 E23K association to type 2 diabetes and serves as a further positive control (with PPAR-γ P12A) that these samples are valid to demonstrate genetic associations with type 2 diabetes.

Age of onset. The analysis by Jellema et al. (5) suggested that the G972R polymorphism might influence the age of onset of type 2 diabetes, with an earlier age of onset correlating with a higher summary OR. We therefore stratified the Scandinavian patients for whom we had precise age of onset of diabetes by Arg carrier status at G972R. The proportion of subjects who developed type 2 diabetes in any given decade did not differ between the two genotypic groups (Fig. 1).

Genotype-phenotype correlations. It has been previously suggested that G972R decreases both insulin sensitivity (29) and insulin secretion (30) in humans. We therefore calculated two measures of insulin secretion (the insulinogenic index and percent HOMA-β), two measures of insulin sensitivity (HOMA-IR and the ISI), and the corresponding insulin disposition indexes in the Scandinavian control subjects who underwent an OGTT. None of these variables were significantly different in carriers of the G972R versus noncarriers (Table 3). When siblings discordant for genotype at G972R were paired and assessed for the above measures, no statistically significant difference was found in any of the parameters tested (Table 3). Even when we restricted our analysis of insulin sensitivity to subjects with BMI >25 kg/m^2 (as done by Clausen et al. [29]), we did not uncover any significant differences among genotypes (data not shown).

DISCUSSION

Our study was designed to test a specific hypothesis proposed by the meta-analysis of Jellema et al. (5): whether the G972R variant in IRS-1 was associated with common type 2 diabetes. Our sample was well powered to detect the estimate from the meta-analysis, with a confidence level that exceeded 95%. Neither our large samples nor an independent large-scale association study by Zeggini et al. (31) could confirm the hypothesized relationship between IRS-1 G972R and type 2 diabetes.

These failures to replicate the findings of the recent meta-analysis can have several possible explanations. First, the initial association may have been a statistical fluctuation, as the meta-analysis was only mildly statistically significant. Despite a growing body of literature suggesting that G972R may alter IRS-1 function in vitro and in vivo (4,6–8,29,30,32), the variant (while affecting elements of glucose homeostasis) may not influence overall type 2 diabetes risk in the population.

A second possibility is that the association signal reported by others was valid, but there is heterogeneity among populations, either in the extent of linkage disequilibrium in this region or in gene-gene or gene-environment interaction. In regard to linkage disequilibrium, it is possible that the association signal reported by others may not be due to G972R itself, but to another nearby genetic variant; elucidation of this possibility requires a thorough

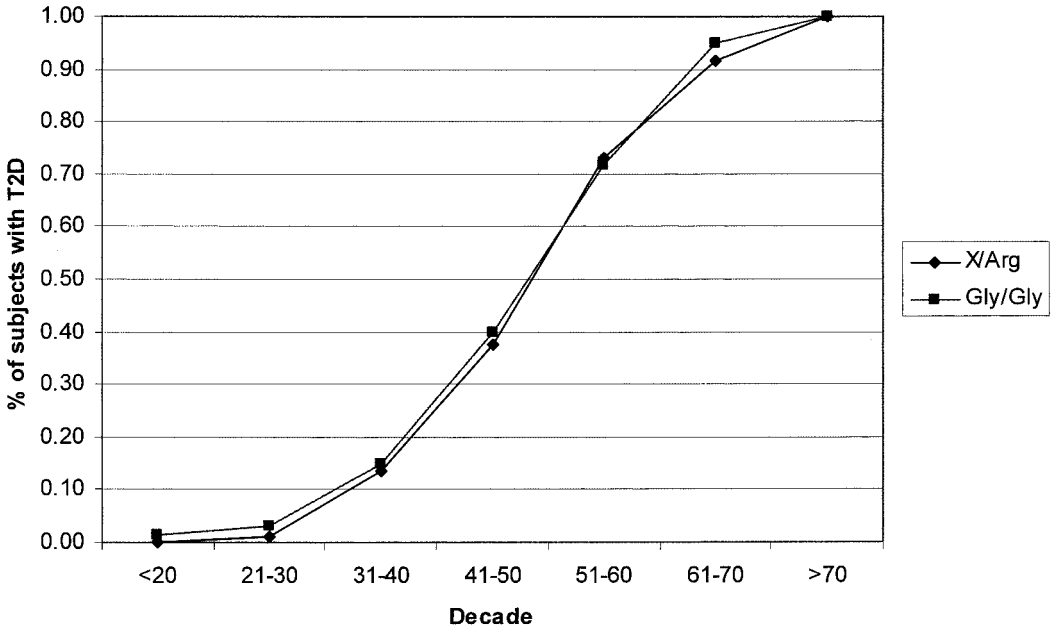

FIG. 1. Age of onset of type 2 diabetes depending on genotype at IRS-1 G972R. The 709 Scandinavian subjects for whom we had precise age of onset of type 2 diabetes were stratified by genotype (Arg carriers versus Gly/Gly homozygotes), and the proportion developing type 2 diabetes by each decade was plotted over time. There is no difference between genotypic groups in the proportion of patients that develop type 2 diabetes at any given time.

understanding of the haplotype structure of the IRS-1 region and a systematic assessment of its common genetic variation in large diabetic patient samples. Nevertheless, the proposed model has been that G972R itself is the functional polymorphism and not a proxy for some other undiscovered variant. With respect to genetic heterogeneity, we note that formal tests for heterogeneity were not positive. While it will never be possible to rule out heterogeneity due to an as yet unmeasured exposure, it is our opinion that in general, given the low prior probabilities in genetic association studies, heterogeneity for a true effect is a less likely explanation than a statistical fluctuation.

Third, our power to reproduce the association may be less than estimated if the OR proposed by Jellema et al. (5) is an overestimate due to the "winner's curse" or publication bias. However, in light of our results and the independent negative results of Zeggini et al. (31), even quite modest estimates of the OR become inconsistent with these follow-up and unbiased studies.

Our study was designed to address the specific hypothesis of the putative IRS-1 G972R association with type 2 diabetes and not to test intermediate measures of insulin secretion and action. For example, we note that our sample of 131 G972R carriers who underwent OGTT only has a ~40% power to detect the difference in insulinogenic index found by Stumvoll et al. (30); furthermore, other studies have used parameters of insulin secretion that are slightly different from ours. Thus, our power to detect genotype-phenotype correlation for these intermediate traits is less than for the association to type 2 diabetes itself.

In mice, complete absence of IRS-1 only leads to a mild increase in insulin resistance and an impairment in insulin secretion, which are not sufficient to cause diabetes (rev.

TABLE 3
Genotype-phenotype correlations

	Case/control			Discordant sibpairs		
	X/Arg	Gly/Gly	*P*	Arg	Gly	*P*
n	131	887		18	18	
Fasting insulin (pmol/l)	7.27 ± 0.32	7.85 ± 0.16	0.18	8.36 ± 1.00	7.71 ± 0.79	0.51
Insulinogenic index	7.78 ± 0.52	7.32 ± 0.19	0.42	10.54 ± 2.17	9.51 ± 2.14	0.48
HOMA-β	79.70 ± 3.84	87.74 ± 2.05	0.15	83.37 ± 10.89	76.73 ± 11.37	0.50
HOMA-IR	1.78 ± 0.08	1.90 ± 0.04	0.25	2.04 ± 0.25	1.86 ± 0.19	0.46
ISI	102.72 ± 4.29	107.22 ± 2.31	0.50	89.27 ± 8.74	105.19 ± 17.03	0.25
Disposition 1	48.21 ± 2.10	49.27 ± 0.79	0.63	43.16 ± 2.34	47.01 ± 2.55	0.26
Disposition 2	6.74 ± 0.38	6.30 ± 0.13	0.26	7.60 ± 1.32	6.69 ± 0.51	0.47

Data are means ± SE. Various parameters of insulin secretion and sensitivity were calculated as described and compared across genotypes in all Scandinavian nondiabetic subjects and nondiabetic siblings discordant for G972R for whom we had OGTT data by simple two-tailed and paired *t* tests, respectively. ISI was calculated from the OGTT. Disposition 1, insulin disposition index derived from insulinogenic index and HOMA-IR; disposition 2, insulin disposition index derived from insulinogenic index and ISI (see text for details).

in 3). On the other hand, mice, which are compound heterozygotes for the null alleles of IRS-1 and the insulin receptor, become frankly hyperinsulinemic, and a high proportion of them develop overt diabetes (3). Similarly, it is possible that in humans, impaired IRS-1 function may influence various components of glucose metabolism but only cause overt type 2 diabetes when present with other genetic defects. Evaluation of this possibility requires a more thorough dissection of the genetic structure of type 2 diabetes (including other genes in the insulin signaling pathway), as well as large enough samples to assess gene-gene interactions.

Genetic association studies with large sample size provide additional statistical power to test previous hypotheses of genetic associations. Given that essentially every report of sufficient sample size has consistently reproduced the associations of PPAR-γ P12A and Kir6.2 E23K with type 2 diabetes in multiple populations, there is reason to hope that large studies can become a reliable approach to distinguishing valid associations from false-positives; in this regard, it is a useful step forward to have validated polymorphisms that can serve as positive controls for large patient samples used to study type 2 diabetes. Similarly, SNP-44 of CAPN10, recently confirmed in two overlapping meta-analyses (20,21) and a follow-up test (20), may also serve as a valuable positive control, as will future confirmed associations. In the future, continued collaboration among investigators to assemble well-powered studies should advance our understanding of the genetic architecture of common type 2 diabetes.

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