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IRS1 G972R Missense Polymorphism Is Associated With Failure to Oral Antidiabetes Drugs in White Patients With Type 2 Diabetes From Italy

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This study tried to replicate in a large sample of white patients with type 2 diabetes (T2D) from Italy a previously reported association of the *IRS1* G972R polymorphism with failure to oral antidiabetes drugs (OAD). A total of 2,409 patients from four independent studies were investigated. Case subjects (*n* = 1,193) were patients in whom, because of uncontrolled diabetes (i.e., HbA_{1c} >8%), insulin therapy had been added either on, or instead of, maximal or near-maximal doses of OAD, mostly metformin and sulfonylureas; control subjects (*n* = 1,216) were patients with HbA_{1c} <8% in the absence of insulin therapy. The *IRS1* G972R polymorphism was typed by TaqMan allele discrimination. In all samples, individuals carrying the *IRS1* R972 risk variant tended to be more frequent among case than control subjects, though reaching statistical significance only in one case. As no *IRS1* G972R-by-study sample interaction was observed, data from the four samples were analyzed together; a significant association was observed (allelic odds ratio [OR] 1.30, 95% CI 1.03–1.63). When our present data were meta-analyzed with those obtained in a previous study, an overall R972 allelic OR of 1.37 (1.12–1.69) was observed. This study confirms in a large and ethnically homogeneous sample that *IRS1* G972R polymorphism is associated with failure to OAD among patients with T2D.

Type 2 diabetes (T2D), which is the leading cause of chronic kidney disease, blindness, and limb amputation and a major cardiovascular risk factor, is becoming epidemic, thus imposing a tremendous burden on the health care systems (1). Although several strategies are available to treat hyperglycemia (2), good glycemic control is achieved in approximately only half of patients with T2D (3). In order to decrease the deleterious effect of hyperglycemia on both diabetes morbidity and mortality (4), a better understanding of how best to use the several therapeutic options in each individual patient has become a real clinical need (3,5). Among oral antidiabetes drugs (OAD), the insulin sensitizer metformin and sulfonylureas are the most widely used, especially in combination (2). When no good glycemic control is achieved, the addition of insulin therapy either on or instead of OAD is the most effective choice (6). Thus, in the routine clinical setting, the initiation of insulin therapy in patients who are already treated with OAD may be considered

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a consequence, and therefore a proxy, of secondary failure to these agents.

As for any drugs, the efficacy of OAD varies substantially across individuals and is supposed to be under the control of both environmental and genetic factors (7). Among the few genetic markers associated with the need to add insulin in patients treated with OAD is the *IRS1* G972R missense single nucleotide polymorphism (SNP) (rs1801278) (8). Such an interesting finding has been reported in a relatively small sample and, so far, has not been replicated in independent studies. The aim of our present investigation was to try replicate this previously reported association in a large sample of white patients with T2D living in a geographically and culturally homogenous area (i.e., southern and central Italy).

RESEARCH DESIGN AND METHODS

Study Samples

Four independent samples of patients from Italy with T2D (defined according to American Diabetes Association 2003 criteria), whose general clinical features have been previously reported (9), were studied. Briefly, the first cohort consisted of 402 patients (236 case and 166 control subjects), consecutively recruited at the Endocrine Unit of the University of Foggia (Apulia). The second sample comprised 778 patients (267 case and 511 control subjects) consecutively recruited at the University Hospital of Pisa (Tuscany). The last two cohorts of patients were recruited at Scientific Institute Casa Sollievo della Sofferenza, San Giovanni Rotondo (Apulia) in different periods: the first consisted of 709 patients (422 case and 287 control subjects) consecutively recruited between November 2000 and September 2005; the second comprised 520 patients (268 case and 252 control subjects) recruited between September 2008 and October 2010. The study protocol was approved by local institutional review boards and was conducted according to the Helsinki Declaration. Written informed consent was obtained from each participant.

Status Ascertainment

In all samples, case subjects (a total of 1,193 individuals) were patients requiring insulin therapy added either on or instead of maximal or near-maximal doses of OAD (mostly sulfonylureas and metformin, with only 2–4% also being on thiazolidinediones and/or gliptins at the time of recruitment) due to uncontrolled diabetes (i.e., HbA_{1c} >8%), in the absence of known conditions predisposing to poor glycemic control (i.e., endocrine and infectious diseases, cancers, and glucocorticoid treatment). Conversely, control subjects (a total of 1,216 individuals) were all patients in acceptable glycemic control (i.e., HbA_{1c} <8%) in the absence of insulin therapy (i.e., 263 with diet only and 953 with diet plus OAD). All patients had no clinical signs of autoimmune diseases.

Genotyping

DNA was extracted from whole blood using a standard method. All study participants were typed for the *IRS1* missense variant G972R (rs1801278) by means of TaqMan assay on a 7900HT platform (Applied Biosystems, Foster City, CA). Genotyping quality was tested by including six blinded duplicate samples in each 96-well assay. The average agreement rate of duplicate samples was greater than 99%. All samples were in Hardy-Weinberg equilibrium (HWE).

Statistical Analysis and Power Calculation

The clinical characteristics of the study participants are reported as mean and SD for continuous variables and frequencies and percentages for categorical variables. Deviation from HWE of the rs1801278 was investigated by exact χ^2 test.

Logistic regression analysis (univariate or multivariate when disease duration was considered as a covariate) was used to model the effect of the *IRS1* G972R polymorphism on dichotomous outcomes and results were reported as odds ratios (ORs) with 95% CIs. Log transformation was used when analyzing non-normally distributed variables (e.g., disease duration).

A triangulation experiment approach (10) was followed to determine whether the association between polymorphisms of *IRS1* G972R and failure to OAD might be mediated by disease duration. Generalized linear models were used to estimate the effect size (i.e., β) per minor allele of *IRS1* G972R polymorphisms for disease duration (β1) and for failure to OAD (β2) and the effect size of disease duration for failure to OAD (β3). We tested the difference between β2 and β3 using a bivariate hierarchical linear model, which allowed us to account for potential between outcomes correlation.

An unbiased association between *IRS1* G972R polymorphisms and failure to OAD also was studied using propensity score methodology (11). Propensity score logistic regression models were built to predict the probability to be a case according to disease duration. Therefore, the association between *IRS1* G972R polymorphisms and failure to OAD was adjusted for propensity score quintiles using a multivariable logistic model. One-way ANOVA was used for the association between genotype and disease duration.

Pooled data analyses of this study were performed in an individual patient data meta-analysis fashion (i.e., adjusting for study sample) after excluding the presence of a statistically significant variant-by-sample interaction, according to a fixed-effects model. Between-study heterogeneity was assessed by Cochran *Q* test. In the absence of heterogeneity ($P > 0.10$), fixed-effects meta-analysis was used to estimate summary OR and the corresponding 95% CI between this study and the previous one (8). In the four pooled samples, we had >99% power with an α error of 0.05 to detect the same effect reported by Sesti et al. (8) that we aimed to try replicate in this study.

A P value < 0.05 was considered significant. All analyses were performed using SPSS version 15.0 (Chicago, IL) and SAS release 9.1 (SAS Institute, Cary, NC).

RESULTS

A total of 2,409 white subjects with T2D from four independent Italian samples, whose clinical features are shown in Table 1, were studied. As expected, and partly according to status ascertainment, in all samples case subjects had higher HbA_{1c} levels than control subjects (Table 1). Also expected (12) was that in all samples, duration of T2D was much longer in case than in control subjects.

IRS1 G972R genotype frequencies were in HWE in all study samples. Disease duration (log-transformed) across genotype groups tended to be different, though not significantly, both in control (β = 0.16, P = 0.10) and case subjects (β = 0.10, P = 0.12) and was significantly different in the pooled sample (i.e., control plus case subjects; β = 0.12, P = 0.03, after adjusting for case-control status). All other clinical features were not different across genotype groups in both case and control subjects (data not shown).

In all four samples, individuals carrying the *IRS1* R972 risk variant tended to be more frequent among case than control subjects, though reaching statistical significance only in the Pisa sample (Fig. 1). Given that no *IRS1* G972R-by-study sample interaction was observed (P = 0.43), data from the four samples were pooled and analyzed together. In the whole sample comprising a total of 2,409 individuals, R972 carriers showed a 30% increased risk of being case subjects (Fig. 1).

In order to test the robustness of our finding, we performed several sensitivity analyses as follows. First, we excluded from control subjects those who were treated with diet only (n = 263) and found that not much change was observed (OR 1.32, 95% CI 1.03–1.68; P = 0.027). Second, we excluded from case subjects those who with insulin treatment had HbA_{1c} <8% (n = 396) and found the *IRS1* R972 allele was more frequent among case subjects (OR 1.40, 95% CI 1.09–1.80; P = 0.009). Finally, when both the above-mentioned 263 control and 396 case subjects were excluded from our analysis, an overall OR of 1.43 (95% CI 1.09–1.87) was observed (P = 0.009).

When the association between *IRS1* G972R and failure to OAD was adjusted for disease duration, an OR of 1.17 (95% CI 0.91–1.50) was obtained. A similar result was obtained when a propensity score quintiles adjustment was carried out (OR 1.15, 95% CI 0.89–1.48). Finally, when performing a triangulation experiment, the expected OR of *IRS1* G972R if its effect was entirely mediated by disease duration was 1.19 (95% CI 1.17–1.22).

When data from the present four study samples were meta-analyzed with those obtained by Sesti et al. (8), an overall R972 allelic OR of 1.37 (95% CI 1.12–1.69, P = 0.003) was observed in a total of 2,886 individuals (Fig. 1).

DISCUSSION

We report that in a pooled analysis of four different case-control studies the *IRS1* G972R polymorphism is associated with a 30% increased risk of failure to OAD. It is of note that, though statistical significance was reached only in one of the four studies, the trend of association was in the same direction in all of them, so that no heterogeneity was observable. In addition, our present data replicate those previously reported by Sesti et al. (8), thus further increasing the consistency of the observed association.

The mechanism through which *IRS1* G972R affects the efficacy of OAD is not known and cannot be unraveled by association studies, which allow only for speculation. Of note, given that T2D is a progressive disease in which severity increases over time, in some circumstances failing to OAD rather than being determined by a reduced response to drug-specific molecular mechanisms refers to the progression of disease severity and/or duration, mainly linked to the progressive deterioration of insulin secretion and sensitivity (3). This, in turn, reduces the response to many antihyperglycemic treatments, regardless of their different mechanisms of action. Along the same line, so far pharmacogenetic studies of OAD have pointed genetic variants either specifically affecting drug metabolism, transport, and targeting or involved, more in general, in causal pathways of hyperglycemia, which are targeted by the drug of interest (13). This is likely to be the case of *IRS1* R972, which has been repeatedly

Table 1—Clinical characteristics of 2,409 white patients with T2D from four independent study samples

	Foggia		Pisa		SGR1		SGR2	
	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases
N	166	236	511	267	287	422	252	268
Male [*n* (%)]	91 (54.8)	108 (45.8)	321 (62.8)	138 (51.7)	156 (54.4)	196 (46.4)	141 (56.0)	157 (58.6)
Age (years)	63.4 ± 12.4	64.6 ± 10.0	59.5 ± 7.2	58.7 ± 8.2	60.6 ± 9.9	64.3 ± 9.2	62.1 ± 8.7	63.0 ± 9.5
BMI (kg/m^2)	29.9 ± 6.4	30.2 ± 6.2	29.6 ± 5.5	29.0 ± 5.4	30.9 ± 5.5	30.8 ± 6.0	30.5 ± 6.3	31.0 ± 6.2
Duration of diabetes (years)	9.4 ± 8.8	16.9 ± 10.0	7.4 ± 6.9	16.4 ± 10.2	6.4 ± 6.7	15.5 ± 9.2	6.7 ± 6.8	15.9 ± 9.0
HbA_{1c} (%)	6.7 ± 0.7	9.5 ± 2.0	6.9 ± 0.6	8.5 ± 4.6	6.8 ± 0.7	9.3 ± 1.9	6.8 ± 0.7	8.9 ± 2.0
Antihypertensive therapy [*n* (%)]	121 (73.0)	190 (80.5)	280 (54.8)	136 (50.9)	147 (54.9)	255 (66.9)	186 (74.1)	213 (79.8)
Statin therapy [*n* (%)]	45 (27.1)	109 (46.2)	129 (25.2)	77 (28.8)	74 (26.0)	160 (37.9)	178 (70.6)	190 (71.2)

Data are presented as *n* (%) or mean ± SD. SGR, San Giovanni Rotondo.

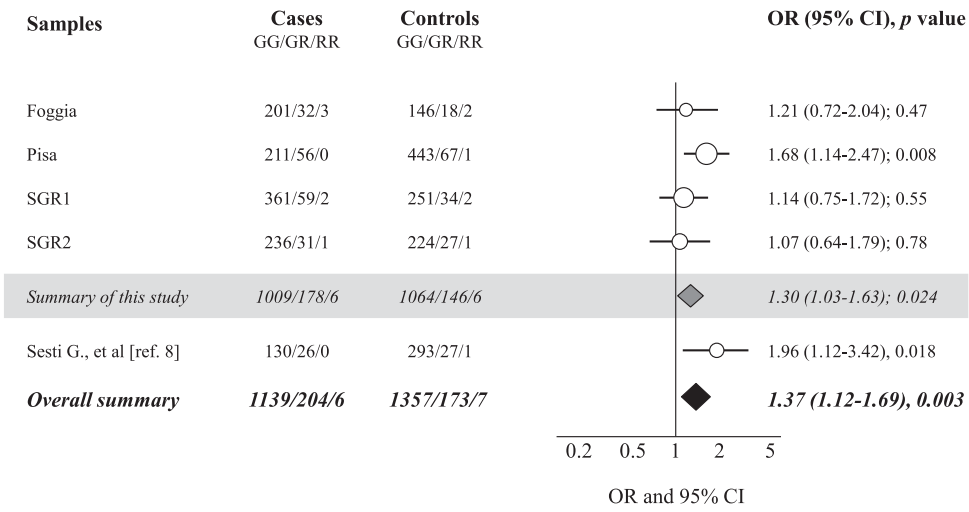

Figure 1—Meta-analysis of five case-control studies. The cumulative effect of the five studies on the association between *IRS1* G972R polymorphism and the failure to OAD was tested by a random-effects model. ORs and 95% CIs for additive genetic model are shown. Sizes of OR symbols are proportional to the study sample size. SGR, San Giovanni Rotondo.

reported to impair glucose homeostasis (14) by affecting both insulin sensitivity (15,16) and secretion (15,17)—two pathways that are, conversely, specifically improved by metformin and sulfonylureas, respectively (2,3,5). In addition, given the previously reported modifying effect of age (18) and age of disease onset (14) of the role of R972 on glucose homeostasis, it cannot be excluded that individuals carrying this variant are affected by a more severe form of T2D due to a longer disease duration and are, therefore, more likely to need insulin therapy, regardless of the specific therapeutic efficacy of OAD across genotype groups. Given that, in fact, in our samples *IRS1* G972R was slightly associated with disease duration, we tried to deeply investigate the above-mentioned possibility by taking into account by several means the role of disease duration on the association between *IRS1* G972R and OAD failure. Altogether, the results we obtained quite consistently suggest that while the association between *IRS1* and failure to OAD is, in fact, partly mediated by disease duration, some of it remains unexplained and therefore might be secondary to direct effects of *IRS1* on metformin and/or sulfonylureas specific mechanism (s) of action.

Our study has some limitations, however. We do acknowledge that defining failure to OAD as the need of adding insulin therapy on, or instead of, OAD in patients with poor glycemic control is just a proxy of this important and frequent (3) clinical condition. In addition, although only patients with no clinical signs of autoimmune diseases were recruited, we acknowledge that the need of insulin treatment and, therefore, status ascertainment may have been affected by the inclusion of patients with latent autoimmune diabetes of the adult. Additionally, metformin and/or sulfonylureas intolerability (i.e., gastrointestinal symptoms and recurrent hypoglycemic episodes) may have also played a role in initiating insulin treatment, thus affecting the ascertainment of failure to OAD.

The risk that the ascertainment strategy we used may have been flawed is further increased by the cross-sectional nature of our study design, which, in contrast to prospective observation, may be biased when assessing drug efficacy. Being aware of this limitation, we carried out several sensitivity analyses. To this end, we re-ran analyses using different subgroups of case and/or control subjects and found that the observed association between *IRS1* G972R polymorphism and failure to OAD did not change much. In addition, a similar association has been previously reported in an independent study, which also used the addition of insulin therapy as a proxy of secondary failure to a combination of metformin plus sulfonylureas (8). Thus, although only a well-powered prospective intervention study will have the last word, present and previous (8) studies do point to *IRS1* G972R as a marker of OAD efficacy.

A further limitation, which is shared with the previous study we wanted to replicate (8), is that most patients were failing to a combination of both metformin and secretagogues, thus making it impossible to draw any conclusion about the role of *IRS1* variability on the efficacy of each specific oral agent. Of note, at the time of study entry, only a small proportion (i.e., 2–4%) of patients were on thiazolidinediones and/or gliptins, making them a very unlikely significant disturbing effect on status ascertainment and overall results.

We also acknowledge that although the *IRS1* locus has been conclusively associated with T2D (19,20) and insulin resistance (21–23), the common variants that reach genome-wide statistical significance are not in tight linkage disequilibrium with G972R. In addition, the initial

reports of the association of G972R with T2D and quantitative glycemic phenotypes were not substantiated in subsequent larger studies (24,25) and by a meta-analysis comprising all published data (14). Thus, although heterogeneity of G972R on the risk of T2D in different individual subgroups has been clearly described and may well explain part of these inconsistencies (14), its real role on glucose homeostasis is not definitively established and needs further efforts. Finally, we recognize that the choice to concentrate on a single genetic polymorphism may be viewed as a limitation.

A strength of our study is the ethnic homogeneity of study samples, with all of them being from southern and central Italy. Conversely, it is not known whether our present finding is generalizable to patients with T2D from other geographical regions with different genetic and/or environmental background.

The pharmacogenetics of OAD in patients with established T2D, which may help personalize and, thus, improve the treatment of hyperglycemia, is still in its infancy, with most previous studies being carried out in small samples of patients with T2D and none of them based on an appropriately designed protocol (7). In this context, while waiting for pharmacogenetic trials specifically designed in the setting of international collaborations, studies such as ours, carried out in large samples and able to replicate previous observations play the important function to prioritize, among several candidate genes, those worthy of further and deeper investigation as possible markers of drug efficacy.

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Author Contributions. S.P. designed the study; acquired, analyzed, and interpreted the data; and wrote the manuscript. E.M. acquired, analyzed, and interpreted the data and reviewed and edited the manuscript. D.L., O.L., D.B., L.M., F.A., L.P., S.F., L.G., M.Ci., G.P., and S.D.C. acquired data and reviewed and edited the manuscript. M.Co. analyzed and interpreted the data and reviewed and edited the manuscript. V.T. designed the study; acquired, analyzed, and interpreted the data; and wrote the manuscript. V.T. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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