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ORIGINAL ARTICLE

Pharmacogenetics of oral antidiabetes drugs: evidence for diverse signals at the *IRS1* locus

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To investigate the role of *IRS1* locus on failure to oral antidiabetes drugs (OADs) we genotyped single-nucleotide polymorphisms (SNPs), rs2943641, rs7578326 (tagging all SNPs genome-wide associated with type 2 diabetes (T2D) and related traits at this locus) and rs1801278 (that is, the loss-of-function *IRS1* G972R amino acid substitution) in 2662 patients with T2D. Although no association with OAD failure was observed for rs2943641 and rs7578326 SNPs (odds ratio (OR): 1.04, 95% confidence interval (CI): 0.93–1.16 and OR: 0.97, 95% CI: 0.87–1.09 respectively), a significant association was observed for rs1801278 (OR: 1.34, 95% CI: 1.08–1.66). When meta-analyzed with previous published data, an allelic OR of 1.41 (1.15–1.72; $P = 0.001$) was obtained, so that homozygous R972R individuals have $>80\%$ higher risk of failing to OADs as compared with their G972G counterparts. In all, though further studies are needed for confirming this finding, our present data point to *IRS1* rs1801278 as a potential biomarker for pursuing the goal of stratified medicine in the field of antihyperglycemic treatment in T2D.

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INTRODUCTION

Type 2 diabetes (T2D) increases the risk of end-stage renal disease, blindness, major adverse cardiovascular events and, eventually, all-cause mortality.[1] All this imposes a severe burden on patients and their relatives as well as on health-care systems; a burden that is likely to increase over the next decades because of the epidemic proportion that T2D is taking on.[2]

Reducing blood glucose plays a critical role for preventing and minimizing the devastating consequences of T2D. Several options are available for treating hyperglycemia, among which oral antidiabetes drugs (OADs) are the most common.

As an average, OADs are reasonably effective; however, when looking at individual level, they achieve adequate glycemic control in approximately only half of patients with T2D.[3] In addition, OADs may cause significant adverse effects,[4,5] and hence it happens that there are diabetic individuals on OADs who pay the cost of suffering of clinically relevant side effects without even benefiting adequately in terms of blood glucose lowering. This is to say that a more precise way (that is, stratified medicine) of using OADs in each single diabetic patient is definitively needed.[3,6]

Studies on the pharmacogenetics of OADs have pointed, among others, genes that play a role on the pathogenic mechanisms of hyperglycemia.[7] Among these, we have recently reported that *IRS1* rs1801278 single-nucleotide polymorphism (SNP) is associated with OAD response in patients with T2D.[8] Of note, rs1801278 encodes for a loss-of-function G972R amino acid substitution, as repeatedly reported by *in vitro*,[9,10] animal[11] and *in vivo*[12,13] studies.

Other common variants at the *IRS1* locus, which are very relevant in the context of T2D and related traits[14–22] and which are not in linkage disequilibrium (LD) with rs1801278, were not investigated in our first study, thus leaving inconclusive our understanding about the role of *IRS1* locus on OAD efficacy.

In this new study, we have verified whether information derived from all SNPs in the *IRS1* locus so far associated at genome-wide level of significance with T2D and related traits[14–22] are also associated with efficacy of OADs, as mainly represented by metformin–sulfonylureas combination. This aim has been pursued by genotyping SNPs rs2943641 and rs7578326 (as Tag SNPs of the 6 SNPs in the *IRS1* locus associated with T2D and related traits) in a case–control study comprising a total of 2662 patients with T2D in whom the initiation of insulin therapy in those already on OADs was considered as a proxy of inefficacy of these agents.

MATERIALS AND METHODS

Study samples

Five independent samples of patients from Italy with T2D (defined according to American Diabetes Association 2003 criteria), whose general clinical features are shown in Table 1, were studied. The five samples were recruited in outpatient diabetic clinics at four different research/academic hospitals from central southern Italy. Briefly, the first two cohorts of patients (that is, SGR1 and SGR2) were recruited at Scientific Institute Casa Sollievo della Sofferenza, San Giovanni Rotondo (Apulia) between November 2000 and September 2005 and between September 2008 and October 2010.[23] Patients of the third cohort (that is, Foggia) were consecutively recruited at the Endocrine Unit of the University of Foggia (Apulia) from January 2002 to September 2008.[8] Patients of the fourth cohort (that is, Pisa) were consecutively recruited between November 2002 and April 2004 at the University Hospital of Pisa (Tuscany).[8] Finally, patients of the last cohort (that is, Rome) were consecutively recruited between

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Table 1. Clinical features of patients with T2D from five independent study samples

	Foggia		*Pisa*		*SGR1*		*SGR2*		*Rome*	
	Controls	*Cases*	*Controls*	*Cases*	*Controls*	*Cases*	*Controls*	*Cases*	*Controls*	*Cases*
N	166	236	511	267	287	422	252	268	181	74
Male (*n* (%))	91 (54.8)	108 (45.2)	321 (62.8)	138 (51.7)	156 (54.4)	196 (46.4)	141 (56.0)	157 (58.6)	125 (69.1)	47 (63.5)
Age (years)	63.4 ± 12.5	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	66.2 ± 8.4	65.4 ± 9.1
BMI (kg m^{-2})	30.0 ± 6.4	30.2 ± 6.2	29.6 ± 5.3	29.1 ± 5.2	30.9 ± 5.5	30.8 ± 6.0	30.6 ± 6.1	31.0 ± 6.2	28.5 ± 4.1	28.5 ± 5.2
Duration of diabetes (years)	9.3 ± 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	8.8 ± 8.5	17.4 ± 10.5
HBA1C (%)	6.7 ± 0.8	9.5 ± 2.0	6.9 ± 0.7	8.3 ± 1.2	6.8 ± 0.7	9.3 ± 1.9	6.8 ± 0.7	8.9 ± 2.0	6.4 ± 0.7	8.0 ± 1.4
Antihypertensive therapy (*n* (%))	121 (73.3)	190 (80.9)	280 (54.8)	136 (50.9)	147 (54.9)	255 (66.9)	186 (74.1)	213 (79.8)	138 (76.2)	58 (78.4)
Statin therapy (*n* (%))	45 (27.3)	109 (46.4)	129 (25.2)	77 (28.8)	74 (25.8)	160 (37.9)	178 (70.6)	190 (70.9)	106 (58.6)	48 (64.9)

Abbreviations: BMI, body mass index; HbA1c, glycated hemoglobin; SGR, San Giovanni Rotondo; T2D, type 2 diabetes. BMI values are mean ± s.d.

2005 and 2012 at the 'Sapienza' University Hospital of Rome.[24] From this cohort only 255 DNA samples were available for the present study.

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.

All patients underwent physical examination and measurements of glycated hemoglobin (HbA1c), lipid levels, blood pressure and body mass index as previously described.[23,24]

Presence of hypertension was defined as a systolic blood pressure of >140 mm Hg or diastolic blood pressure of >90 mm Hg or both, or the presence of antihypertensive treatment.

Status ascertainment

In all samples, 'cases' (a total of 1267 individuals) were patients requiring insulin therapy added either on or instead of maximal or near-maximal doses of OADs (mostly sulfonylureas and metformin, with only 2–4% also being on thiazolidinediones and/or gliptins at the time of recruitment) due to uncontrolled diabetes (that is, HbA1c $>8\%$), in the absence of known conditions predisposing to poor glycemic control (that is, endocrine and infectious diseases, cancers, and glucocorticoid treatment) (Table 1). Conversely, 'controls' (a total of 1397 individuals) were all patients who were in acceptable glycemic control (that is, HbA1c $\leqslant 8\%$) in the absence of insulin therapy (Table 1). All patients had no clinical signs of autoimmune diseases.

Tag SNP selection and genotyping

Six SNPs in the *IRS1* locus have been so far reported associated at genome-wide level of significance with T2D and other cardiometabolic traits (including rs7578326, rs2943634, rs2943640, rs2943641, rs2972146 and rs2943650).[14–22] The extent of LD among these six SNPs and among these six SNPs and rs1801278 (that is, *IRS1* G972R) was evaluated by Haploview[25] based on data from phase 2 of the HapMap (CEU sample). Although all six SNPs were in high LD, two tag SNPs to be further investigated were selected by Tagger.[26]

DNA was extracted from whole blood using standard methods. All study participants were genotyped for Tag SNPs rs2943641 and rs7578326 by means of ready to use TaqMan assays (Life Technologies) on a HT 7900 platform (Applied Biosystems, Foster City, CA, USA). Genotyping quality was tested by including six blinded duplicate samples in each 384-well assay. The average agreement rate of duplicate samples was $>99\%$. All samples were in Hardy–Weinberg equilibrium (HWE) ($P>0.05$).

Genotype data for the rs1801278 SNP were already available for 4 out of 5 samples (that is, SGR1, SGR2, Pisa and Foggia),[8] and hence only samples from Rome were genotyped at this SNP, as described above.

Statistical methods

Patients' baseline characteristics were reported as mean ± s.d. and percentages for continuous and categorical variables, respectively. Log transformation was used when analyzing disease duration that was non-normally distributed. Deviation from HWE of the rs1801278, rs2943641 and rs7578326 was investigated by exact χ^2 test.

Univariate or multivariable logistic regression analysis was used to assess the effect of the *IRS1* SNPs (assuming an additive genetic model of inheritance) on dichotomous outcomes in the pooled sample in a fixed-effects individual participant data meta-analysis manner[27] (adjusting for study sample after having observed no SNP-by-study sample interaction). Results were reported as odds ratios (ORs) with 95% confidence intervals (CIs).

Aggregate data meta-analysis was also performed to combine our results with those previously reported.[28] Between-study heterogeneity was assessed by Cochran's 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.

A *P*-value of <0.05 was considered as statistically significant. All analyses were performed using SPSS version 15.0 (Chicago, IL, USA) and Comprehensive Meta-analysis Software (Biostat, Inc.).

Power study

In the 5 pooled samples, we had $>99\%$ power with a type I error of 5% to detect an OR equal to 1.37 (as reported for G972R)[8] for both rs2943641 (minor allele frequency = 0.368) and rs7578326 (minor allele frequency = 0.362).

RESULTS

Clinical features of all study subjects from 5 independent samples (that is, 2664 individuals) are shown in Table 1. Not surprisingly, many of such features were different between controls and cases, pointing to a more severe disease in the latter individuals (Table 1).

LD extent evaluated across the 6 SNPs previously associated at genome-wide level of significance to T2D and cardiometabolic traits[14–22] shows that all SNPs are in high LD ($r^2>0.9$) (Supplementary Figure 1). Two tag SNPs (that is, rs2943641 and rs7578326) were able to capture the entire genetic variability of all the 6 SNPs, whereas none of them were in LD with rs1801278 SNP (that is, *IRS1* G972R), with *D*' and r^2 values for each SNP being $\leqslant 0.65$ and $\leqslant 0.02$, respectively.

Association between SNP rs2943641 and failure to OADs

Genotype frequencies at rs2943641, available in 1391 controls and 1211 cases, were in HWE in all samples (all *P*-values being >0.05).

Genotype distribution across controls and cases as well as the additive OR (95% CI) for failure to OADs in each single sample are shown in Figure 1a. As no SNP-by-sample heterogeneity on failure to OADs was observed ($P_{het}=0.50$), data from all samples were pooled and analyzed. No association was observed between rs2943641 and failure to OADs in each single sample and in the pooled sample as well (Figure 1a).

No association was observed between rs2943641 and any clinical feature in controls and cases from each single sample

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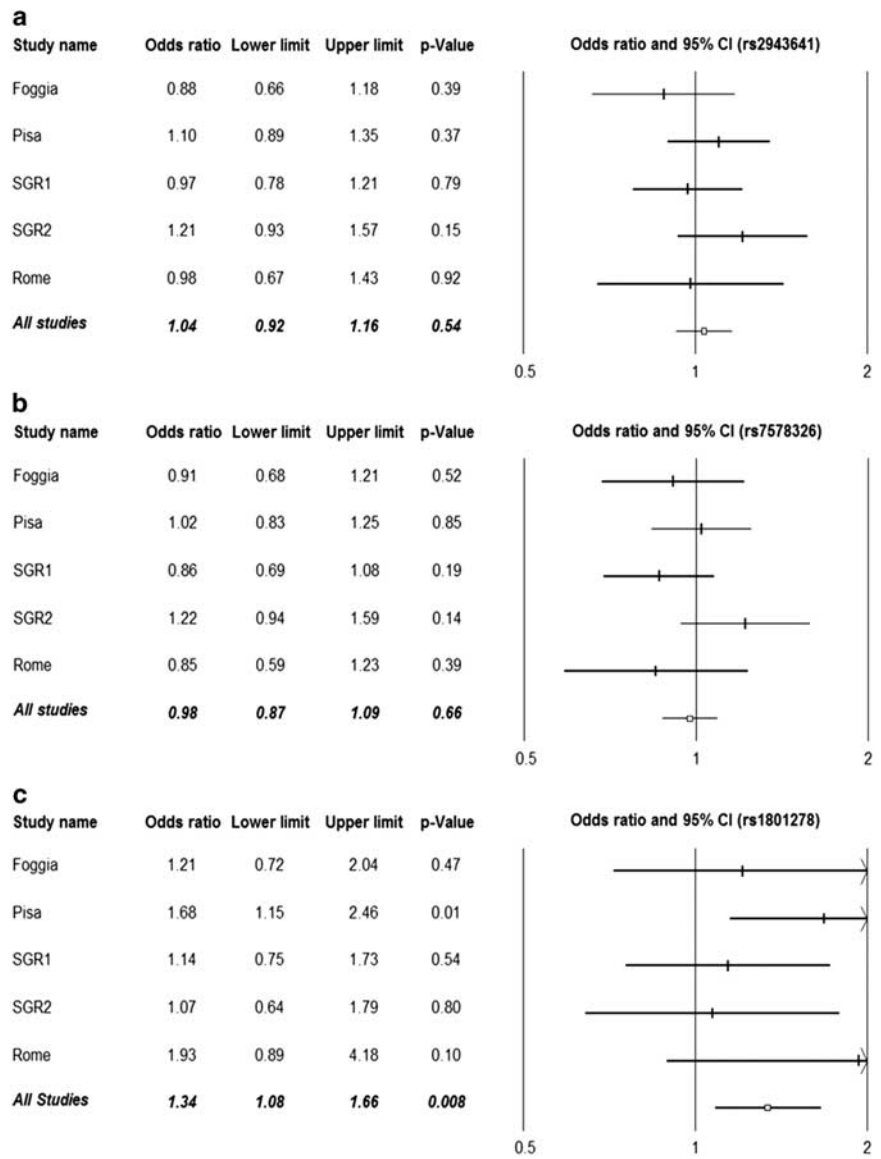

Figure 1. Meta-analysis of five case–control studies. The cumulative effect of the five studies on the association between rs2943641 (**a**), rs7578326 (**b**) and rs1801278 (**c**) polymorphisms and the failure to oral antidiabetes drugs (OADs) was tested by a fixed-effects model. Odds ratios (ORs) and 95% confidence intervals (Cis) for additive genetic model are shown. Sizes of OR symbols are proportional to the study sample size. SGR, San Giovanni Rotondo.

(data not shown) as well as in the pooled one (Supplementary Table 1).

Association between SNP rs7578326 and failure to OADs

Genotype frequencies at rs7578326, available in 1394 controls and 1180 cases, were in HWE in all samples (all P-values being >0.05).

Genotype distribution across controls and cases as well as the additive OR (95% CI) for failure to OADs in each single sample are shown in Figure 1b. As no SNP-by-sample heterogeneity on failure to OADs was observed ($P_{het}=0.31$), data from all samples were pooled and analyzed. No association was observed between rs7578326 and failure to OADs in each single sample and in the pooled sample as well (Figure 1b).

No association was observed between rs7578326 and any clinical feature in each single sample (data not shown) as well as in the pooled one (Supplementary Table 2).

Association between SNP rs1801278 and failure to OADs

Genotype frequencies at rs1801278 (that is, *IRS1* G972R) now evaluated in a total of 1396 controls and 1266 cases were in HWE in all samples (all *P*-values being >0.05).

Genotype distribution across controls and cases as well as the additive OR (95% CI) for failure to OADs in each single sample are shown in Figure 1c. As no SNP-by-sample heterogeneity on failure to OADs was observed ($P_{het}=0.45$), data from all samples were pooled and analyzed (Figure 1c). Individuals carrying the minor allele at rs1801278 (that is, carrying the *IRS1* R972 variant) tended to be more frequent among cases in all samples, and hence in the pooled sample they had 34% significantly higher risk of being cases ($P=0.008$).

No association was observed between rs1801278 and any clinical feature in each single sample (data not shown) as well as in the pooled one (Supplementary Table 3).

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When our present data were meta-analyzed with those previously obtained in an independent study,[28] an additive OR equal to 1.41 (1.15–1.72; $P = 0.001$) was obtained.

The association between rs1801278 (that is, *IRS1* G972R) and failure to OADs remained significant after adjusting for rs2943641 or for rs7578326 ($P = 0.028$ and $P = 0.037$, respectively), whereas an ~50% reduction on a log scale was observed after adjusting for disease duration (OR becoming 1.17, $P = 0.19$).

In order to exclude that our findings were influenced by the status ascertainment we used, a sensitivity analysis was carried out in the pooled sample by excluding from controls all individuals who were treated with only diet ($n = 292$), and from cases all individuals with HbA1c $< 8\%$ on insulin treatment ($n = 505$). In the remaining individuals the results obtained for all three SNPs were not different than that observed in the whole sample (Supplementary Table 4).

DISCUSSION

In a pooled analysis, comprising a total of 2662 diabetic individuals from central southern Italy, we report for the first time that rs2943641 and rs7578326, which are Tag SNPs of all other SNPs in the *IRS1* locus so far associated with T2D and cardiometabolic traits,[14–22] are not associated with failure to OADs. Conversely, our present data confirm and reinforce previous evidence for the association between SNP rs1801278 (*IRS1* G972R) and failure to OADs.[8] When present data are meta-analyzed with those previously reported by one of us,[28] an overall 40% increased risk of OAD failure is observed for carriers of the *IRS1* R972 variant, with a statistical significance that, though not formally excluding a false positive finding, becomes robustly credible. The credibility of our present finding on rs1801278 is also strengthened by all study samples showing a homogeneous trend of association toward the same direction (Figure 1c).

Data on all three SNPs investigated in this study add new insights into the role of the *IRS1* locus on the risk of failure to OADs. In fact, although rs2943641 and rs7578326 are established markers of T2D,[16,19] the role of rs1801278 (*IRS1* G972R) is at issue,[29] thus making unlikely that the association between this latter SNP and failure to OADs is mainly mediated by direct effects on glucose homeostasis. Rather, it becomes reasonable that the observed association with reduced responsiveness to OADs is secondary to yet unknown effects of the loss-of-function *IRS1* R972 variant on specific mechanism(s) of action of OADs. As a matter of fact, the *IRS1* R972 variant has been reported to affect *in vivo* both insulin sensitivity[12,13] and secretion[30,31] that are specifically targeted by metformin and sulfonylureas,[3] by far the most utilized OAD in our study. It can therefore be hypothesized that in a condition in which insulin sensitivity and/or secretion are genetically impaired, as in carriers of the *IRS1* R972 variant, metformin and sulfonylureas are less active, thus increasing the chance of a clinically manifest OAD failure.

Conversely, as the *IRS1* R972 is involved in anticipating disease onset,[29] it cannot be excluded that carriers of this variant are at higher risk of ODA failure because of being affected by diabetes of longer duration and, consequently, higher severity. That this can be, at least partly, the case is suggested by the observation that when diabetes duration is used as a covariate, the OR of OAD failure in *IRS1* R972 decreases from 1.34 to 1.17, representing an almost 50% reduction on a log scale. This may be considered a general phenomenon regarding failures to any treatment to T2D, as well as any progressive disease in which severity increases over time. In the context of T2D, failing to antihyperglycemic therapies rather than being caused by a drug-specific defective molecular mechanisms may be because of progressive deterioration of insulin secretion and sensitivity,[3] that are likely to affect treatment efficacies, regardless of their mechanisms of action.

We do acknowledge that cross-sectional study design and ascertainment strategy, based on the addition of insulin therapy for defining failure to OADs, can be viewed as a limitation of our study. Having admitted so, it is of note that a sensitivity analysis was carried out for addressing this subject, with no much change observed on the association with OAD failure for any of the three SNPs of interest. Hence, although we cannot exclude that in some patients adding insulin may have been secondary to metformin-related gastrointestinal symptoms and/or sulfonylureas-dependent recurrent hypoglycemic episodes, rather than OAD failure, it is unlikely that such possible incorrectness in status ascertainment has biased our results.

We also acknowledge that most of our patients were on a combination of metformin and sulfonylureas, thus making impossible obtaining information on the role of *IRS1* variability on the efficacy of each oral agent, as singly considered.

The strength of our study is the ethnic and geographical homogeneity across samples (all being from central southern Italy); it would be of interest to understand whether similar results can be obtained in samples characterized by different environmental and/or genetic background.

In conclusion, among the three SNPs at the *IRS1* locus that we investigated in the present study, only rs1801278 (*IRS1* G972R) is robustly associated with OAD failure in terms of both statistical significance and clinical effect size, with homozygous R972R individuals having an 80% higher risk of needing insulin addition on top of, or instead of, previous OAD as compared with their homozygous G972G counterparts.

Though the biology underlying this association is elusive and need to be addressed more deeply, such a high clinical impact points to rs1801278 (*IRS1* G972R) as a potential biomarker for pursuing the goal of stratified medicine in the field of antihyperglycemic treatment in T2D.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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