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The *IRS1* G972R polymorphism and glomerular filtration rate in patients with type 2 diabetes of European ancestry

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ORIGINAL ARTICLE

ABSTRACT

Background. In Mexican Americans, the *IRS1* G972R polymorphism (rs1801278) has been associated to such a marked reduction in glomerular filtration rate (GFR) (i.e. $\beta = -8.3$ mL/min/1.73 m^2) to be considered a major determinant of kidney function.
Methods. This was a cross-sectional study to investigate whether a similarly strong effect can also be observed among individuals of European ancestry. We investigated a total of 3973 White patients with type 2 diabetes. Standardized serum creatinine was measured by the modified kinetic Jaffè reaction and estimated GFR (eGFR) calculated by the modification diet renal disease (MDRD) formula; rs1801278 was genotyped by TaqMan assay.
Results. No significant association was observed, with R972 carriers showing only a modestly, not significant, lower eGFR level as compared with other subjects ($\beta = -1.82$ mL/min/1.73 m^2, P = 0.086).
Conclusions. Our data indicate that *IRS1* G972R is not a strong determinant of GFR in diabetic patients of European ancestry as in Mexican Americans. Since we had 100% power to detect the previously reported association, the risk our finding is a false negative one is minimal.

Kidney dysfunction predisposes to end-stage renal disease both in the general population and in diabetic patients. Several pieces of evidence indicate that low glomerular filtration rate (GFR) is heritable, thus suggesting it is under the influence of genetic determinants. A number of genetic variations known to affect whole body insulin sensitivity have been associated with reduced GFR in diabetic patients. In contrast to what was reported in Mexican Americans, *IRS1* G972R polymorphism has no a strong effect on eGFR) in patients with type 2 diabetes of European ancestry.

INTRODUCTION

Kidney dysfunction predisposes to end-stage renal disease and cardiovascular risk both in the general population and in diabetic patients [1]. Several pieces of evidence indicate that low glomerular filtration rate (GFR) is heritable, thus suggesting it is under the influence of genetic determinants [2].

Insulin resistance is pathogenic for renal damage both in the general population [3] and in patients with type 2 diabetes [4]. Since insulin resistance is also under genetic control, the two traits might share some common genetic determinants [5]. In fact, several genetic variations known to affect whole body insulin sensitivity have been associated with reduced GFR [6–9] in diabetic patients. Very recently, a comprehensive analysis of *IRS1*, which encodes for a central mediator of post-receptor insulin signaling, has suggested that variability at this locus plays an important role in kidney dysfunction in Mexican Americans. Among the several polymorphisms examined, the non-synonymous G972R (rs1801278) showed the strongest signal, with carriers of the R972 variant being characterized by a large reduction of GFR (i.e. 8.3 mL/min/1.73 m^2 lower than that of homozygous individuals for the G972 allele) [10]. Of note, this association accounted for 26% of the linkage signal with GFR that the same group had previously reported on 2q37 [11], the chromosomal region harboring *IRS1*. Thus, based on these recent findings, the *IRS1* G972R polymorphism appears to be a major modulator of GFR in Mexican Americans. The aim of this our study was to investigate whether such a strong effect can also be observed among diabetic patients of European ancestry. To this end, GFR and the G972R genotype were determined in five different samples of White patients with type 2 diabetes including a total of 3973 individuals.

MATERIALS AND METHODS

Five independent samples of patients with type 2 diabetes (defined according to ADA 2003 criteria), including a total of 3973 individuals of European ancestry, were studied. Selection criteria and clinical features of these populations have already been published [12]. Briefly, two cohorts of patients have been recruited at Scientific Institute Casa Sollievo della Sofferenza, San Giovanni Rotondo in different periods: the first consisted of 985 patients consecutively recruited between 21 November 2000 and 28 September 2005; the second comprised 641 patients recruited between 2 September 2008 and 5 October 2010. The third cohort consisted of 622 patients, consecutively recruited at Endocrine Unit of University of Foggia. The fourth sample comprised 942 patients consecutively recruited at the University Hospital of Pisa. Finally, the fifth sample included 783 patients living in the Boston area who were recruited at the Joslin Diabetes Center in Boston (MA) for a study of the genetics of CAD. In all sets, standardized serum creatinine was measured by using the modified kinetic Jaffè reaction. Estimated GFR was then calculated by using both the MDRD and CKD-EPI equations [13, 14].

Genotyping

All study participants were typed for SNP rs1801278 by the Genetic Core of the Mendel Institute (Italy) or by the Joslin DERC Genetics Core (USA) by means of TaqMan assay implemented on an HT7900 and an HT7700platform (Applied Biosystems, Foster City, California) respectively. 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.

Statistical analysis and power calculation

Clinical patients' characteristics are reported as mean and standard deviation for continuous variables and frequencies and percentages for categorical ones. Deviation from HWE of the rs1801278 was investigated by exact χ^2 test.

The effect of the genetic variant on eGFR was evaluated by means of multivariate linear model, and results were reported as regression coefficients (β).

Pooled data analyses were performed in an individual patient data meta-analysis fashion (i.e. adjusting for 'study sample') after excluding the presence of a significant SNP-by-sample interaction, according to a fixed-effects model.

The size of the four samples pooled and analyzed together had 100% power to detect with an alpha error of 0.05 a difference of eGFR across different genotypes similar in magnitude to that reported by Thameem *et al.* (i.e. 8.3 mL/min/1.73 m^2) [10].

All analyses were performed using SPSS, version 13.0 (SPSS Inc., Chicago, IL), and SAS, release 9.1 (SAS Institute, Cary, NC).

RESULTS

Clinical features of each study sample are listed in Table 1. As shown in Table 2, mean eGFR across genotype groups was not different in any of the five cohorts. Since no heterogeneity was observed (P = 0.337), data were pooled and meta-analyzed, adjusting for 'study sample'. A trend toward reduced eGFR in R972 carriers, which however did not reach a formal statistical significance, was observed ($\beta = -1.82$ mL/min/1.73 m^2, P = 0.086). Similar data were obtained if eGFR was obtained by the CKD-EPI equation ($\beta = -1.60$ mL/min/1.73 m^2, P = 0.073).

DISCUSSION

The established role of insulin resistance on kidney dysfunction [5] makes reasonable testing the role of genetic variants known to affect insulin signaling on GFR levels. Our data indicate that the *IRS1* G972R polymorphism (rs1801278), in contrast to what has been described in Mexican Americans from the San Antonio Family Diabetes/Gallbladder Study (SAFDGS) [10], is not a strong determinant of eGFR in diabetic patients of European ancestry. Since we had 100% power to detect, with an alpha error equal to 0.05, an association similar to that reported in SAFDGS [10], the risk of a false negative finding is minimal. Different ethnicity of study populations might well explain the

Table 1. Clinical features of diabetic patients from the five study cohorts

Study cohorts	SGR 1	SGR 2	Foggia	Pisa	Boston
Male/female	495/490	371/270	301/321	558/384	514/269
Age (years)	62.0 ± 9.7	62.2 ± 9.2	63.0 ± 11.8	59.2 ± 7.6	64.5 ± 6.8
Duration of diabetes (years)	10.8 ± 9.0	10.9 ± 9.1	13.4 ± 10.0	10.5 ± 9.1	12.6 ± 7.7
BMI (Kg/m^2)	31.1 ± 5.8	31.1 ± 6.3	30.2 ± 6.3	29.5 ± 5.5	32.2 ± 5.6
HbA1c (%)	8.7 ± 2.0	8.1 ± 1.8	9.0 ± 2.2	7.7 ± 2.7	7.4 ± 1.3
eGFR (mL/min/1.73 m^2)	75.5 ± 21.3	94.0 ± 27.0	86.1 ± 32	83.6 ± 19.1	70.2 ± 21.6

Data are expressed as males/females or mean ± SD.
SGR1, first cohort from San Giovanni Rotondo; SGR2, second cohort from San Giovanni Rotondo; BMI, body mass index; HbA1c, glycated hemoglobin; eGFR, estimated glomerular filtration rate as calculated according to MDRD equation.

Table 2. eGFR values in diabetic patients from the five study cohorts

Study cohort	eGFR (mL/min/1.73 m^2) according to *IRS1* G92R genotype			β value (P value)
	GG	GR	RR	
SGR 1 (*N*)	842	137	7	−1.41 (0.430)
	75.7 ± 21.0	74.3 ± 22.9	72.4 ± 16.9	
SGR 2 (*N*)	565	74	2	−0.085 (0.979)
	94.1 ± 26.8	93.0 ± 28.9	111.5 ± 26.2	
Foggia (*N*)	539	78	5	−6.71 (0.058)
	87.0 ± 33.0	81.3 ± 30.6	65.7 ± 20.9	
Pisa (*N*)	797	144	1	−1.18 (0.489)
	83.7 ± 19.2	82.7 ± 19.9	68.6	
Boston (*N*)	694	87	2	−0.02 (0.993)
	70.2 ± 21.7	69.9 ± 20.7	76.8 ± 5.6	
All samples (*N*)	3436	520	17	−1.82 (0.086)[a]
	81.2 ± 25.3	79.6 ± 24.8	75.3 ± 21.5	

Data are expressed as number of patients (*N*) and mean ± SD. eGFR, estimated glomerular filtration rate as calculated according to MDRD equation. β values are expressed as mL/min/1.73 m^2.
SGR1, first cohort from San Giovanni Rotondo; SGR2, second cohort from San Giovanni Rotondo; GG, patients homozygous for the G allele; GR, heterozygous patients; RR, patients homozygous for the R allele.
[a]Adjusted for 'study sample'.

difference between previous [10] and our present results. This is also suggested by the inability to replicate in 825 Caucasian the association the same authors observed in Mexican Americans [10]. Difference across different populations may be due to either environmental or genetic factors. As far as the latter is concerned, linkage disequilibrium (LD) structure across *IRS1* locus, as derived from HapMap data (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap3r2_b36/), seems quite similar in Mexican Americans and Europeans, thus making unlikely that genetic differences underlie the different association observed between the two populations. Of note, in individuals of European ancestry, several other SNPs at the *IRS1* locus, which are not in LD with G972R, have been recently associated with metabolic and cardiovascular abnormalities, possibly related to kidney dysfunction [15–22], thus leaving open the possibility that some of them do play an independent lead role in modulating GFR among Europeans.

In addition, while our samples exclusively comprised diabetic patients, only 28% study subjects were diabetics in SAFDGS [10]. It is, therefore, possible that diabetes acts as a negative modifier of the *IRS1* G972R effect on GFR, thus making difficult to assess it in diabetic individuals.

At this regard, it is worth nothing that our sample of 3973 patients had 80% power at alpha level of 0.05 to detect a β value

of −3.13 mL/min/1.73 m^2, thus leaving open the possibility that a smaller effect of *IRS1* G972R does exist. In addition, whether such a possible minor role acts in combination with other functional genetic variations of insulin signaling known to singly affect GFR [6–9], as it happens for insulin resistance and cardiovascular disease [23] as well as for insulin secretion and abnormal glucose homeostasis [24], is a possibility that remains to be addressed.

In conclusion, in contrast to what reported in Mexican Americans, *IRS1* G972R polymorphism has no a strong effect on eGFR in patients with type 2 diabetes of European ancestry. Further studies are certainly needed to deeper address the exact role of *IRS1* R972 variant on kidney function in diabetic patients.

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