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Joint effect of insulin signaling genes on all-cause mortality

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ABSTRACT

Objective: We have previously reported the combined effect of SNPs perturbing insulin signaling (*ENPP1* K121Q, rs1044498; *IRS1* G972R, rs1801278; *TRIB3* Q84R, rs2295490) on insulin resistance (IR), type 2 diabetes (T2D) and cardiovascular events. We here investigated whether such a combined effect affects also all-cause mortality in a sample of 1851 Whites of European ancestry. **Methods**: We investigated a first sample of 721 patients, 232 deaths, 3389 person-years (py). Replication was assessed in two samples of patients with T2D: the Gargano Mortality Study (GMS) of 714 patients, 127 deaths, 5426 py and the Joslin Kidney Study (JKS) comprising 416 patients, 214 deaths, 5325 py. **Results**: In the first sample, individuals carrying 1 or ≥2 risk alleles had 33% ($p = 0.06$) and 51% ($p = 0.02$) increased risk of mortality, as compared with individuals with no risk alleles. A similar, though not significant, trend was obtained in the two replication samples only for subject carrying ≥ 2 risk alleles. In a pooled analysis, individuals carrying ≥2 risk alleles had higher mortality rate as compared to those carrying 0 risk alleles (HR = 1.34, 95%CI = 1.08–1.67; $p = 0.008$), and as compared to those carrying only one risk allele (HR = 1.41, 95% CI = 1.13–1.75; $p = 0.002$). This association was independent from several possible confounders including sex, age, BMI, hypertension and diabetes status. **Conclusion**: Our data suggest that variants affecting insulin signaling exert a joint effect on all-cause mortality and is consistent with a role of abnormal insulin signaling on mortality risk.

Type 2 diabetes (T2D) and cardiovascular (CV) disease are major determinants of all-cause mortality [1] and are both characterized by insulin resistance (IR) [2,3], which is itself a predictor of all-cause mortality [4,5].

IR is, at least in part, genetically determined [6]. Thus, it is conceivable that genetic factors affecting IR may also affect all-cause mortality. Some clues on the genetic of IR have been provided by recent genome-wide association studies [7,8]. In addition, non-synonymous, functional variants of genes affecting the insulin signaling pathway (rs1044498 – *ENPP1* K121Q; rs1801278 – *IRS1* G972R; and rs2295490 – *TRIB3* Q84R; the only ones which have been thoroughly characterized in transfected cells as well as in human cells naturally carrying them [9–19]), have also been reported to exert a combined effect on IR, T2D and CV disease [20,21].

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Based upon this background, we investigated the combined effect of these insulin signaling single nucleotide polymorphism (SNPs) on all-cause mortality in a total of 1851 white individuals of European ancestry.

1. Materials and methods

1.1. Study design

Based on our previous observation of a combined SNPs effect on CV events in three cohorts analyzed together [21], we used these same pooled studies as a first sample to test the hypothesis of an association with all-cause mortality.

Subsequently, we tried to increase the robustness of our finding by investigating two additional replication cohorts.

1.1.1. First combined sample

This sample comprises the following cohorts:

1.1.1.1. Gargano Heart Study (GHS)-prospective design. Three-hundred-fifty-four Whites with T2D (ADA 2003 criteria) and coronary artery disease who were consecutively recruited at "Casa Sollievo della Sofferenza" Institute in San Giovanni Rotondo (Gargano, Center East Coast of Italy) from 2001 to 2008 [21,22]. All patients had either a stenosis >50% in at least one coronary major vessel at coronary angiography or a previous myocardial infarction (MI). The only exclusion criterion was the presence of poor life expectancy for non diabetes-related diseases.

1.1.1.2. Tor Vergata Atherosclerosis Study (TVAS). One-hundred-two Whites were consecutively recruited from 2005 to 2007 at "Tor Vergata" University Hospital (Rome); they all had been diagnosed with an acute MI. Exclusion criteria were the presence of malignancies and a previous medical record of diabetes, although 22 (15.7%) study participants turned out to have subclinical diabetes after an OGTT [21].

1.1.1.3. Cardiovascular Risk Extended Evaluation in Dialysis (CREED) database. Two-hundred-sixty-five Whites with end stage renal disease (ESRD) were recruited at the Reggio Calabria Hospital. Exclusion criteria were dialysis for less than 6 months, left ventricular ejection fraction <35%, history of circulatory congestion and hospitalization for inter-current illness including major infections. Out of these, 43 (16.2%) had diabetes [23].

1.1.2. Replication samples

1.1.2.1. Gargano Mortality Study (GMS). Seven-hundred-fourteen Whites with T2D (ADA 2003 criteria) were consecutively recruited from November 1st 2000 to September 30th 2005 at "Casa Sollievo della Sofferenza" Institute, for a study having all-cause mortality as the end-point [22,24]. The only exclusion criterion was the presence of poor life expectancy due to non diabetes-related disorders.

1.1.2.2. Joslin Kidney Study in type 2 diabetes (JKS). This cohort consists of a random sample ($n = 516$) of T2D patients from the Joslin Clinic enriched with individuals with proteinuria, who were recruited between 1993 and 1996 at the Joslin Diabetes Center, Boston, MA as previously described [25].

All subjects had diabetes diagnosed after age 25 according to WHO criteria and were treated with diet or oral agents for at least two years after the diagnosis. The present study was limited to 416 self-reported Whites for whom DNA samples were still available in 2013. Their survival status was updated as of December 31, 2011 by matching with the National Death Index.

Subjects from all studies underwent clinical examination and standardized interview at the time of recruitment, as previously reported [21–25]. Smoking habits and history of hypertension were recorded at time of examination. Hypertension was defined as systolic blood pressure >130 mmHg or diastolic blood pressure >85 mmHg or presence of antihypertensive therapy. For all studies the end-point was all-cause mortality. Confirmation of the event was obtained from death certificates or, in the case of GMS, by direct contact with patients and/or their relatives or by queries to the registry offices of the cities of residence.

Study protocol and the informed consent procedure were approved by each Institutional Ethic Committee. All participants gave written informed consent.

1.2. Genotyping

Genotyping was performed by TaqMan allele discrimination as previously described [20]. Genotyping quality was assessed by including in each 96 wells plate positive controls with known genotypes (i.e. homozygous for the major allele, heterozygous or homozygous for the minor allele, previously evaluated for each SNP of interest by direct Sanger sequencing, each in duplicates). The agreement rate was >99% for each SNP assay. Genotype distribution was in Hardy–Weinberg equilibrium (HWE) in all study samples (p value >0.1). Minor allele frequency of ENPP1, TRIB3 and IRS1 are shown in Supplemental Table 1.

1.3. Statistical methods

Patients' baseline characteristics were reported as mean ± standard deviation (SD) and percentages for continuous and categorical variables, respectively.

In all prospective studies, time-to-death analyses were performed using multivariable Cox proportional hazards regression models, and risks were reported as hazards ratios (HR) along with their 95% confidence intervals (95% CI). The overall survival was defined as the time between enrollment and death. For subjects who did not experience the end point, survival time was censored at the time of the last available follow-up attempt. Incidence rates for overall mortality were expressed as the number of events per 100 person-years (py%). Since no evidence of a linear association was observed between the number of risk alleles carried by each individual and the rate of all-cause mortality, a Cox-based tree regression analysis (RECPAM) was performed. A deviance test confirmed that a non-linear, dichotomized model of risk alleles was to be preferred as compared to the linear (additive) one ($p < 0.01$).

Pooled data analyses were performed in an individual patient data meta-analysis fashion [26] (i.e. adjusting for "study sample") after having observed no genotype-by-sample interaction.

In the whole sample ($n = 1851$), we had 80% power, at alpha = 0.05, to detect an HR = 1.35 or 1.49, for either 1 or ≥2 risk alleles with respect to 0 risk allele, assuming an incidence rate in the 0 risk allele group of 4% py.

A p-value <0.05 was considered as significant. All analyses were performed using SAS Release 9.1.3 (SAS Institute, Cary, NC, USA).

2. Results

2.1. First sample

Clinical features of study participants from GHS-prospective design, TVAS and CREED cohorts are summarized in Table 1 (first three panels).

The association between each SNP, considered individually, and all-cause mortality is shown in Supplemental Table 2. Although