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Infrequent *TRIB3* coding variants and coronary artery disease in type 2 diabetes

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ABSTRACT

Objective: Genes that modulate insulin sensitivity may also be involved in shaping the risk of coronary artery disease (CAD). The relatively common *TRIB3* Q84R polymorphism (rs2295490) has been associated with abnormal insulin signaling, endothelial dysfunction, insulin resistance, and pro-atherogenic phenotypes. The aim of our study was to investigate the association between low-frequency *TRIB3* coding variants and CAD in patients with type 2 diabetes (T2D).
Methods: Three case–control studies for CAD from Italy and US were analyzed, for a total of 1565 individuals, all with type 2 diabetes. Infrequent variants were identified by re-sequencing *TRIB3* exons in 140 "extreme cases" and 140 "super-controls" and then genotyped in all study subjects.
Results: *TRIB3* infrequent variants (n = 8), considered according to a collapsing rare variants framework, were significantly associated with CAD in diabetic patients from Italy (n = 700, OR = 0.43, 95% CI 0.20–0.91; p = 0.027), but not from the US (n = 865, OR = 1.22, 95% CI 0.69–2.18; p = 0.49). In the Italian sets, the association was especially strong among individuals who also carried the common R84 variant.
Conclusion: Although preliminary, our finding suggests a role of *TRIB3* low-frequency variants on CAD among Italian patients with T2D. Further studies are needed to address the role of *TRIB3* infrequent variants in other populations of both European and non-European ancestries.

1. Introduction

Coronary artery disease (CAD) is a leading cause of death worldwide, especially in patients with type 2 diabetes (T2D) [1]. CAD, as many other complex diseases, is under the combined control of both genetic and environmental factors. While the latter are well known [2], the former are only partially understood as indicated by the fact that all the frequent variants discovered to date by genome-wide associations studies (GWAS) [3,4] account for only a small proportion of the CAD heritability. An additional proportion of the CAD-predisposing genetic background may be explained by low-frequency/rare variants [5–8]. However, to date, no data have been made available in support of this hypothesis among patients with T2D – a condition characterized by high cardiovascular risk.

Insulin resistance is a well-established pathogenic factor for atherosclerosis and related cardiovascular disorders such as CAD

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[9,10]. Since insulin resistance [11] and CAD [3] are both in part under genetic control, they might share some common genetic background, that is, genes that modulate insulin sensitivity may also modulate CAD risk. Indeed, we have recently reported that some common variants that affect insulin signaling and are associated with insulin resistance, are also associated with major cardiovascular events [12–14].

Among these variants is a relatively common amino acid substitution (Q84R; rs2295490) in *TRIB3* – an inhibitor of insulin-stimulated Akt phosphorylation and downstream signaling [15]. This polymorphism, increasing TRIB3 inhibitory activity on insulin signaling [15–18], has been associated with endothelial dysfunction *in vitro* [17,19] and with several *in vivo* metabolic alterations including insulin resistance [16] and other pro-atherogenic phenotypes [16,19,20].

For these reasons, *TRIB3* is a prime candidate in the search for low-frequency coding variants predisposing to CAD among subjects with T2D.

2. Methods

2.1. Study participants

Individuals from three independent case–control studies of CAD among patients with T2D, namely the Gargano Heart Study-cross-sectional design (GHS, n = 481), the Catanzaro Study (CS, n = 219) and the Joslin Heart Study (JHS, n = 865) were investigated. Briefly, patients from the first two studies, GHS and CS, were recruited in Italy, at the Institute "Casa Sollievo della Sofferenza" in San Giovanni Rotondo (FG) and at Magna-Graecia University in Catanzaro, respectively. JHS participants were recruited at the Joslin Diabetes Center and Beth Israel Deaconess Medical Center in Boston. Recruitment procedures for these three studies have been previously described [4]. Briefly, CAD-cases were patients with T2D who had a stenosis greater than 50% in at least one major coronary artery or a main branch thereof that was documented by cardiac catheterization or had a previous MI. CAD-control participants had no clinical evidence of CAD and had a normal ECG response to an exercise treadmill test; control participants from the JHS were recruited if they were older than 55 years and have had diabetes for more 5 years.

In all three studies, participants were non-Hispanic Whites and were diagnosed with T2D according to the ADA 2003 criteria. Their clinical features are described in Table 1.

The study protocol and informed consent procedure were approved by the local human subject committees. All participants gave written informed consent.

2.2. Study design

2.2.1. Re-sequencing of the TRIB3 coding region

The entire *TRIB3* coding region was re-sequenced in 280 individuals from GHS by the Sanger method as previously described [16]. To increase the probability of detecting infrequent variants with biological effect [21] (i.e. that affect the risk of CAD), we selected 140 "extreme" cases that had a MI (rather than simply a coronary stenosis) and 140 super-controls who were free of CAD despite being older than 60 years.

2.2.2. Genotyping of TRIB3 infrequent coding variants

Genotyping of rs200422001, rs138380491, rs41281850, rs35051116, rs149447454, rs144632965, rs140801463, rs374473490, was carried out at the Joslin Genetics Core in a total of 1565 individuals from GHS, JHS and CS by means of custom TaqMan assays implemented on an ABI PRISM 7700 HT Sequence Detection System (Life, Foster City, CA). Genotyping of rs2295490 was carried out at the Mendel Institute in 1514 individuals, by means of custom TaqMan assay implemented on a HT 7900 platform (Life, Foster City, CA). Genotyping quality was tested by including six blinded duplicated samples in each 384-well assay. The average agreement rate was greater than 99%.

2.3. Statistical analysis

Our discovery sample of 280 patients had 95 and 99% probability to identify *TRIB3* variants with minor allele frequency (MAF) equal to 0.5 and 0.8%, respectively.

The association between each individual SNP, as well as between all SNPs considered together (i.e. by a burden test which collapses rare variants in a genetic region into a single burden variable) and CAD was evaluated by Fisher exact test and by logistic regression analysis, respectively. Results were reported as odds ratios (OR) along with their 95% confidence interval (CI). The burden test was chosen since it is recognized as the most powerful statistical tool when the majority of variants can be considered as pathogenic and effects can be assumed to be in the same direction

Table 1
Clinical features of study subjects from the three different samples.

	GHS		CS		JHS	
	Control	Case	Control	Case	Control	Case
N	264	217	138	81	448	417
Male/Female	116/148	145/72	62/76	55/26	266/182	296/121
Age at examination (yrs)	64.8 ± 8.1	59.8 ± 8.5	63.4 ± 8.9	60.6 ± 11.3	65 ± 7	64 ± 6
Age at diabetes (yrs)	50.6 ± 11	48.6 ± 9.6	50.3 ± 11.9	60.6 ± 12.3	52 ± 10	52 ± 8
BMI (kg/m^2)	30.1 ± 4.7	31.2 ± 5.6	30.2 ± 4.6	31.1 ± 5.9	32.2 ± 6.4	32.3 ± 5.9
Diabetes duration (yrs)	14.3 ± 9.3	11.3 ± 8.1	13.1 ± 9.8	9.8 ± 8.8	13 ± 9	12 ± 7
HbA1C (%)	8.5 ± 1.9	8.6 ± 1.9	7.7 ± 3.9	8.5 ± 4.2	7.3 ± 1.2	7.5 ± 1.4
Glucose-lowering therapy						
Diet only (%)	13	10	35.5	24.7	52.8	43.8
Oral agents (%)	47	46	42.2	40.6	16.6	22.3
Insulin ± oral agents (%)	40	44	22.3	34.7	30.6	33.9
Antihypertensive therapy (%)	48.5	51.5	68.4	85.1	70.8	82.2
Lipid-lowering therapy (%)	38.2	61.8	27.4	52.5	38.4	67.5
Ever smoked						
Current smokers (%)	38.7	61.3	16.9	15.8	6.3	8.2
Former smokers (%)	50.8	49.2	25.6	42.6	32.1	59.3

GHS: Gargano Heart Study; CS: Catanzaro Study; JHS: Joslin Heart Study. BMI: body mass index; HbA1c: glycated haemoglobin; yrs: years.
Continuous variables are reported as means ± SD, categorical variables as percentages.