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The Type 2 Diabetes and Insulin-Resistance Locus Near *IRS1* Is A Determinant of HDL Cholesterol and Triglycerides Levels Among Diabetic Subjects

Rajani Sharma¹, Sabrina Prudente², Francesco Andreozzi³, Christine Powers¹, Gaia Mannino³, Simonetta Bacci⁴, Ernest V. Gervino^{5,6}, Thomas H. Hauser^{5,6}, Elena Succurro³, Luana Mercuri², Elizabeth H. Goheen¹, Hetal Shah^{1,6}, Vincenzo Trischitta^{2,7,8}, Giorgio Sesti³, and Alessandro Doria^{1,6,9}

¹Research Division, Joslin Diabetes Center, San Giovanni Rotondo, Italy

²IRCCS Casa Sollievo della Sofferenza, Mendel Laboratory, San Giovanni Rotondo, Italy

³Department of Experimental and Clinical Medicine, University Magna Græcia, Catanzaro, Italy

⁴Unit of Endocrinology, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy

⁵Cardiovascular Division, Beth Israel Deaconess Medical Center, Boston, MA

⁶Department of Medicine, Harvard Medical School, Boston, MA

⁷Research Unit of Diabetes and Endocrine Diseases, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy

⁸Department of Experimental Medicine, "Sapienza" University, Rome, Italy

⁹Department of Epidemiology, Harvard School of Public Health, Boston, MA

Abstract

OBJECTIVE—SNP rs2943641 near the insulin receptor substrate 1 (*IRS1*) gene has been found to be associated with type 2 diabetes (T2D) and insulin-resistance in genome-wide association studies. We investigated whether this SNP is associated with cardiovascular risk factors and coronary artery disease (CAD) among diabetic individuals.

METHODS—SNP rs2943641 was typed in 2,133 White T2D subjects and tested for association with BMI, serum HDL cholesterol and triglycerides, hypertension history, and CAD risk.

RESULTS—HDL cholesterol decreased by 1 mg/dl ($p=0.0045$) and serum triglycerides increased by 6 mg/dl ($p=0.018$) for each copy of the insulin-resistance allele. Despite these effects, no association was found with increased CAD risk (OR=1.00, 95% CI 0.88–1.13).

CONCLUSIONS—The insulin-resistance and T2D locus near the *IRS1* gene is a determinant of lower HDL cholesterol among T2D subjects. However, this effect is small and does not translate into a detectable increase in CAD risk in this population.

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Address for correspondence: Alessandro Doria, MD, PhD, MPH, Section on Genetics & Epidemiology, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215, TEL (617) 732-2406, FAX (617) 732-2667.

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Keywords

coronary artery disease; polymorphisms; insulin-resistance; type 2 diabetes

Introduction

Genetic factors influence the risk of coronary artery disease (CAD) both in the general and the diabetic populations (1;2). Finding the genes that are responsible for this effect may provide tools to identify high-risk individuals and devise new preventive therapies. Insulin-resistance and its associated lipid abnormalities such as low HDL cholesterol and increased triglycerides are among the factors that have been implicated in the pathogenesis of CAD (3). Since also insulin-resistance is under genetic control, genes contributing to its etiology may also be involved in shaping cardiovascular risk (4;5).

A recent genome-wide association study (GWAS) has identified a SNP on chromosome 2q36 (rs2943641) that was associated with type 2 diabetes at a genome-wide level of statistical significance among subjects of European descent (6). The same SNP was also associated with insulin-resistance, although not at a genome-wide level (6). This polymorphism is placed in a non-coding region located about 500 kb from the gene for insulin receptor substrate 1 (*IRS1*) - one of the primary phosphorylation targets of the insulin receptor (7). Interestingly, SNPs in strong linkage disequilibrium with rs2943641 ($r^2=0.8-0.95$) were found to be associated with HDL levels and CAD in large GWAS of the general population (8;9), although the association with CAD could not be replicated in other studies (10).

In the present study, we investigated whether SNP rs2943641 is associated with cardiovascular risk factors related to insulin resistance and with an increased risk of CAD among individuals with type 2 diabetes.

Methods

Study subjects

Three series of CAD-positive cases and CAD-negative controls, all with type 2 diabetes, were included in the study. One series was recruited in Boston at the Joslin Diabetes Center and Beth Israel Deaconess Medical Center as part of the Joslin Heart Study (JHS); the other two were recruited in Italy, at the Institute 'Casa Sollievo della Sofferenza' in San Giovanni Rotondo as part of the Gargano Heart Study (GHS) and the Magna-Graecia University in Catanzaro (CZ). The recruitment criteria were previously described for the JHS and GHS series (11;12). Cases and controls from CZ were recruited according to the same criteria as in the GHS series. All subjects gave their informed consent according to protocols approved by the local research ethic committees.

Biochemical measurements

Serum HDL cholesterol and triglycerides (TG) were measured by enzymatic-colorimetric methods using a Vitros 5.1 Analyzer (JHS), a Roche Modular P Chemistry Analyzer (GHS), or a Roche Cobas® 4000 analyzer (CZ).

Genotyping

All study subjects were typed for SNP rs2943641 by the Joslin DERC Genetics Core by means of TaqMan assays (Applied Biosystems, Foster City, CA). Genotyping quality was

tested by including 12 HapMap samples in each 384-well assay. The agreement rate with the HapMap database genotypes was >99%.

Data analysis

Phenotypic differences among carriers of different rs2943641 genotypes were evaluated according to an additive model with 1 d.f. as in previous studies (6;9). The association with quantitative variables was evaluated by linear regression analysis adjusting for age, gender, and case-control status; the association with CAD was evaluated by logistic regression analysis. Summary estimates of the SNP effect across studies were obtained by adding a “study” indicator to the models. Heterogeneity was assessed by adding appropriate interaction terms. A one-sided p value of 0.01 was considered significant based on five different traits being tested for association with rs2943641 in one pre-specified direction (i.e., allele C being associated with lower HDL, higher TG, higher BMI, higher odds of hypertension, and higher odds of CAD, consistent with the previously reported association between allele C and insulin-resistance (6)). The study had 80% power ($\alpha=0.01$) to detect a 2.9% decrease in HDL cholesterol, a 5.7% increase in TG, a 0.57 kg/m² increase in BMI, an OR of hypertension of 1.34, and an OR of CAD of 1.23 per copy of allele C (calculations performed by means of the PS software, available at <http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>).

Results

A total of 2,133 type 2 diabetic subjects were typed for SNP rs2943641 at 2q36. Salient characteristics of these subjects are shown in Supplemental Table 1. Genotyping was successful for 2,037 (95.4%) of them, including 923 CAD-positive cases and 1,114 CAD-negative controls. Genotype distributions were in Hardy-Weinberg equilibrium ($p>0.001$) in all case and control series. Both among cases and controls, the frequencies of the rs2943641 risk allele (“C”) were higher in the admixed US population of the JHS than in the two studies from Italy (GHS and CZ), suggesting the possibility of ethnic differences in the frequency of this SNP (see Supplemental Table 2). In agreement with this hypothesis, a principal component (PC) derived from a set of 192 SNPs informative for stratification along the European North-South cline (13) showed a significant association with rs2943641 in the JHS ($p=0.01$). Thus, all analyses involving the JHS were adjusted for this PC to avoid spurious results due to population structure.

We first examined the association between rs2943641 and cardiovascular risk factors related to insulin-resistance (Table 1). In GHS and CZ, HDL levels progressively decreased with increasing dosage of the insulin-resistance allele C. A similar association between allele C and decreased HDL levels was observed in JHS, though the trend was not as clear. When the three studies were considered together in a meta-analysis, the inverse relationship between number of C alleles and HDL levels was statistically significant ($p=0.0045$). HDL levels were estimated to decrease by 2.3% (about 1 mg/dl) for each copy of allele C that was carried. The effect was somewhat stronger among non-obese ($BMI<30$) than obese ($BMI\geq 30$) subjects, with a 4.4% decrease per allele in the former vs. 1.1% in the latter group, but the interaction did not reach statistical significance ($p=0.09$) (Supplemental Table 3). Similarly, no significant evidence of heterogeneity was observed among studies or between CAD cases and controls. Differences across rs2943641 genotypes were also observed for triglyceride levels ($p=0.018$), with an estimated increase of 3.8% (about 6 mg/dl) per copy of allele C. For both HDL and triglycerides, results were similar if the analysis was adjusted by the presence of therapy with statins (Supplemental Table 4). No association was found between rs2943641 and BMI or a history of hypertension.

No significant evidence of association between rs2943641 allele C and increased CAD risk was found in any of the three studies under the pre-specified log-additive model (Table 2). When the three case-control series were considered together in a meta-analysis, the summary OR was equal to 1.00 (95% CI 0.88–1.13, $p=0.51$), with no significant evidence of heterogeneity among studies (Table 3) or between BMI strata (Supplemental Table 4). Although not statistically significant, a tendency toward association with the allele C was observed in the meta-analysis according to a dominant model of inheritance explored as a post-hoc analysis (OR=1.15, 95% CI=0.89–1.49, $p=0.14$).

Discussion

Our data confirm the association between 2q36 locus and HDL levels reported by Teslovich et al. (9) and extend this observation to type 2 diabetic subjects. Since this locus is also associated with type 2 diabetes (6), one can envision a scenario according to which non-diabetic carriers of the rs2943641 risk allele are more likely to become diabetic, and once they have developed diabetes, they are more likely to have an atherogenic lipid profile than other diabetic subjects. Whether the genetic effect that we found among type 2 diabetes subjects is restricted to lipid traits or also concerns other abnormalities related to insulin-resistance remains to be clarified. Similar to previous reports (6), we did not detect associations with other cardiovascular risk factors related to insulin-resistance such as overweight or hypertension. However, these data should be interpreted with caution since these traits can be influenced by the diabetic milieu and/or diabetes treatment.

Despite the effect on HDL cholesterol, rs2943641 was not significantly associated with increased CAD risk. These negative findings should be considered in the context of the expected magnitude of the genetic effect and the power of our study. In our three studies combined, the odds of CAD increased by 2.4% for each 1 mg/dl decrease in HDL cholesterol levels, similar to the risk estimates obtained in prospective studies (14). Since each copy of rs2943641 allele C was associated with a 1 mg/dl decrease in HDL cholesterol levels, the log-additive OR of CAD that one should expect for this SNP through its effect on HDL is in the order of 1.025. While our study had 80% power to detect ORs as low as 1.23, it had less than 10% power to detect ORs of such small magnitude. The small effect size might be similarly responsible for the discordant findings of association with CAD in the general population (8;10).

Available evidence points to *IRS1*, which is placed 500 kb from rs2943641 in telomeric direction, as the gene mediating this genetic effect. In the report by Rung et al., the risk allele was associated with a ~30% decrease in *IRS1* protein levels and decreased insulin induction of *IRS1*-associated phosphatidylinositol-3-OH kinase activity in skeletal muscle, suggesting an effect on the initial step of the insulin signaling cascade (15). The recent GWAS report of a nominally significant association ($p=0.0046$) between a SNP in an intron of the *IRS1* gene (rs4675095) and insulin-resistance further highlights the potentially important role played by *IRS1*-dependent abnormalities of insulin signaling in the etiology of insulin-resistance (16). Of note, this other SNP is not in LD with rs2943641, suggesting the existence of multiple sources of genetic variability in *IRS1* function contributing to insulin-resistance.

In conclusion, the T2D and insulin-resistance locus near the *IRS1* gene is a determinant of lower HDL and possibly of higher TG levels among T2D individuals. While this genetic effect is small and does not significantly increase the risk of CAD in this population, it is conceivable that the activity of the cellular pathways influenced by this SNP can be modulated by drugs to a much larger extent than what is observed as the consequence of natural variation. Thus, dissection of the molecular mechanisms underlying this genetic

effect may be instrumental to identify critical targets for the development of new interventions aimed at improving the lipid profile of diabetic subjects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Cardiovascular risk factors related to the insulin-resistance syndrome according to rs2943641 genotypes in the three series of type 2 diabetic subjects.

Table 1

Study	Characteristic	rs2943641 [†]			P _{add}
		T/T	T/C	C/C	
JHS	n (M/F)	57/31	254/140	254/136	
	BMI (kg/m ²)	32.4 ± 0.6	32.0 ± 0.3	32.5 ± 0.3	0.25
	HDL cholesterol (mg/dl) [‡]	42.4 ± 1.5	43.7 ± 0.7	41.4 ± 0.71	0.088
	Triglycerides (mg/dl) [‡]	189 ± 15	173 ± 7	192 ± 7	0.025
	Hypertension (%) [¶]	84.1	76.9	79.7	0.55
GHS	n (M/F)	61/60	190/147	164/130	
	BMI (kg/m ²)	30.7 ± 0.5	31.2 ± 0.3	30.8 ± 0.3	0.57
	HDL cholesterol (mg/dl) [‡]	47.0 ± 1.2	45.0 ± 0.7	44.3 ± 0.8	0.019
	Triglycerides (mg/dl) [‡]	149 ± 9	151 ± 5	159 ± 6	0.15
	Hypertension (%) [¶]	75.6	76.7	74.4	0.66
CZ	n (M/F)	38/30	85/70	105/85	
	BMI (kg/m ²)	31.5 ± 0.6	0.9 ± 0.4	30.8 ± 0.4	0.80
	HDL cholesterol (mg/dl) [‡]	48.2 ± 1.7	47.5 ± 1.1	46.6 ± 1.0	0.13
	Triglycerides (mg/dl) [‡]	153 ± 12	156 ± 8	162 ± 7	0.29
	Hypertension (%) [¶]	95.6	92.9	95.3	0.41
All	n (M/F)	156/121	529/358	523/352	
	BMI (kg/m ²)	31.5 ± 0.4	31.4 ± 0.2	31.5 ± 0.2	0.47
	HDL cholesterol (mg/dl) [‡]	45.9 ± 0.9	45.3 ± 0.6	43.9 ± 0.6	0.004
	Triglycerides (mg/dl) [‡]	166 ± 8	162 ± 5	174 ± 5	0.016
	Hypertension (%) [¶]	83.3	79.6	81.4	0.61

Data for quantitative variables are least square means ± SE estimated by a linear regression model including rs2943641, age, gender, case-control status, study center (in the summary analysis), and population structure PC (in the JHS and the summary analysis) as predictors.

[†] Genotypes were missing for 3 subjects from JHS, 33 subjects from SHR, and 60 subjects from CZ.

[‡] HDL cholesterol and triglycerides were analyzed after log-transformation.

Table 2

Association between rs2943641 and CAD in the three studies.

Study		rs2943641			p
		T/T (%)	T/C (%)	C/C (%)	
JHS	CAD- (n=441)	0.113	0.429	0.458	0.47
	CAD+ (n=431)	0.088	0.476	0.436	
	Log-additive OR (95% CI)		1.01 (0.82–1.24)		
GHS	CAD- (n=410)	0.171	0.434	0.395	0.41
	CAD+ (n=342)	0.149	0.465	0.386	
	Log-additive OR (95% CI)		1.03 (0.84–1.26)		
CZ	CAD- (n=263)	0.160	0.373	0.468	0.68
	CAD+ (n=150)	0.173	0.380	0.447	
	Log-additive OR (95% CI)		0.94 (0.71–1.23)		
All	Log-additive OR (95% CI)		1.00 (0.88–1.13)		0.51