The $\text{ENPP1}$ Q121 Variant Predicts Major Cardiovascular Events in High-Risk Individuals

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<th>Citation</th>
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The ENPP1 Q121 Variant Predicts Major Cardiovascular Events in High-Risk Individuals

Evidence for Interaction With Obesity in Diabetic Patients

Simonetta Bacci, Stefano Rizza, Sabrina Prudente, Belinda Spoto, Christine Powers, Antonio Facciorusso, Antonio Pacilli, Davide Lauro, Alessandra Testa, Yuan-Yuan Zhang, Giuseppe Di Stolfo, Francesca Mallamaci, Giovanni Tripepi, Rui Xu, Davide Mangiacotti, Filippo Aucella, Renato Lauro, Ernest V. Gervino, Thomas H. Hauser, Massimiliano Copetti, Salvatore De Cosmo, Fabio Pellegrini, Carmine Zoccali, Massimo Federici, Alessandro Doria, and Vincenzo Trischitta

OBJECTIVE—Insulin resistance (IR) and cardiovascular disease may share a common genetic background. We investigated the role of IR-associated ENPP1 K121Q polymorphism (rs1044498) on cardiovascular disease in high-risk individuals.

RESULTS—Incidence of cardiovascular events per 100 person-years was 4.2 in GHS, 10.8 in TVAS, and 11.7 in CREED. Hazard ratios (HRs) for KQ+QQ versus individuals carrying the K121/K121 genotype (KK) individuals were 1.47 (95% CI 0.80–2.70) in GHS, 2.31 (95% CI 1.22–4.34) in TVAS, and 1.36 (95% CI 0.88–2.10) in CREED, and 1.56 (95% CI 1.15–2.12) in the three cohorts combined. In the 395 diabetic patients, the Q121 variant predicted cardiovascular events among obese but not among nonobese individuals (HR 5.94 vs. 0.62, P = 0.003 for interaction). A similar synergism was observed in cross-sectional studies of 339 type 2 diabetic patients (n = 169 from Italy, n = 170 from the U.S.).

CONCLUSIONS—The ENPP1 K121Q polymorphism is an independent predictor of major cardiovascular events in high-risk individuals. In type 2 diabetes, this effect is exacerbated by obesity. Future larger studies are needed to confirm our finding.

Diabetes 60:1000–1007, 2011

Morbidity and mortality due to cardiovascular disease (CVD) are highly prevalent (1), mostly because of the epidemics of obesity and type 2 diabetes (2–4). Environmental and genetic factors both contribute to CVD (5). Insulin resistance and related abnormalities are among the factors that have been implicated in the etiology of CVD (11–17). Because insulin resistance is also under genetic control, the two conditions may share a common genetic background (18–20), with genes that contribute to impaired insulin sensitivity being prime candidates for a predisposing effect on CVD. The ectoenzyme nucleotide pyrophosphate phosphodiesterase (ENPP1) inhibits insulin receptor signaling (21). A nonsynonymous polymorphism (K121Q, rs1044498) of the ENPP1 gene has been described (22), with the Q121 variant determining a gain of function resulting in an increased ability to inhibit insulin receptor signaling (22–24). This variant has been associated with insulin resistance in most (22,25–27) but not all (28) large studies. In agreement with the hypothesis of a common genetic “soil” between insulin resistance and CVD, the Q121 variant has been also associated with atherosclerosis-related phenotypes in European (24,29,30) but not in Brazilian (31) or in Chinese (32) samples. Most of these associations have been mainly observed among heavier individuals (25–27,30,33), thereby suggesting a gene-by-adiposity interaction.

The aim of our study was to investigate the role of the ENPP1 K121 variant and its interaction with obesity (i.e., BMI ≥30 kg/m²) in accelerating major cardiovascular events in very high-risk individuals.

RESEARCH DESIGN AND METHODS

Study participants
Prospective studies. Sample 1—the Gargano Heart Study, prospective analysis. The study included 340 whites from Italy with type 2 diabetes (according to American Diabetes Association 2000 criteria) and coronary
artery disease (CAD), as indicated by previous myocardial infarction (MI) or >50% stenosis of at least one major vessel at coronary angiography, or both. These individuals were cases of the cross-sectional case-control Gargano Heart Study (GHS) (30) and were consecutively recruited at the Scientific Institute "Casa Sollievo della Sofferenza" (Foster City, CA) on the HT7900 platform (Applied Biosystems). The failure rate was <1%. Genotyping quality was assessed by including positive controls for continuous or categoric variables, and as frequencies and percentages for categorical variables. Comparisons between genotype groups were performed by Pearson x^2 test. Because of the low number of QQ individuals, only the dominant genetic model was tested by comparing individuals carrying the K121/Q121 or the Q121/Q121 genotype (Q121) (i.e., QQ heterozygotes + QQ homozygotes) to K121 homozygotes (KK). In prospective studies, a time-to-event analysis was conducted by means of Cox proportional hazards regression models using the Breslow approach in the case of ties and reported as hazard ratios (HRs) along with their 95% CI. The time to event was defined as the time between enrollment date and the date of the first cardiovascular event. For censored subjects, the time variable was defined as the time between the enrollment date and the date of the last available clinical data. The assumption of proportionality of the hazards was tested by using scaled Schoenfeld residuals. In cross-sectional studies, the association between ENPP1 Q121 variant and age at MI was analyzed by multiple linear regression analysis, and results are given as β regression coefficients.

Pooled data analyses were performed in an individual patient data meta-analysis fashion (38) (i.e., adjusting for "study sample") after excluding genotype-by-sample interactions. Genotype-by-obesity interaction was tested by adding a cross-product term to the regression model. The discriminatory power of prediction models was assessed by estimating the survival c-index (39) and by measuring the integrated discrimination improvement (IDI) (40).

RESULTS

Characteristics of cohort members at baseline. We studied three cohorts of subjects who were at very high risk of major cardiovascular events: the GHS—individuals with type 2 diabetes and previously diagnosed CAD; the Tor Vergata Atherosclerosis Study (TVAS)—individuals from the general population who had experienced an MI; and the Cardiovascular Risk Extended Evaluation in Dialysis (CREED)—individuals with ESRD who required dialysis. Clinical characteristics of study subjects at study entry are summarized in Table 1. No significant differences in baseline characteristics across genotype groups were observed in any of the three studies.

ENPP1 K121Q polymorphism as a predictor of major cardiovascular events. The average mean (SD) follow-up was 37.1 (19.4) months (range 1–91) in the GHS, 30.6 (11.3) months (range 1–37) in the TVAS, and 36.3 (22.0) months (range, 1–69) in the CREED. During follow-up, 43 major cardiovascular events occurred in the GHS, 39 in the TVAS, and 94 in the CREED, resulting in respective incidence rates of 4.2, 10.8, and 11.7 per 100 person-years (Table 2). In all studies, incidence rates per 100 person-years were numerically higher in Q121 carriers than in KK homozygotes: 5.4 vs. 3.6 in the GHS, 19.2 vs. 8.1 in the TVAS, and 14.1 vs. 10.8 in the CREED (Table 2). The difference was significant in the TVAS (P = 0.025) and in a pooled analysis of the three studies (P = 0.005). No difference in the magnitude of the genetic effect was observed among studies (P = 0.32 for interaction).

In a time-to-event analysis, the HR of cardiovascular events for Q121 carriers versus KK homozygotes was 1.47 (95% CI 0.90–2.30, P = 0.21) in the GHS, 2.31 (95% CI 1.22–4.34, P = 0.01) in the TVAS, and 1.36 (95% CI 0.88–2.10, P = 0.16) in the CREED (Fig. 1A, B, and C, respectively; P = 0.32 for gene-by-sample interaction). In a pooled analysis (i.e., individual patient data meta-analysis) of the three studies, which included 737 subjects with 176 events, the HR for Q121 carriers versus KK homozygotes was 1.56 (95% CI 1.15–2.12, P = 0.004; Fig. 1D). Age at study entry (HR 1.04 [95% CI 1.03–1.06], P < 0.0001), diabetes (2.23 [95% CI 1.50–3.31], P < 0.0001), and smoking status (1.71 [95% CI 1.24–2.36], P = 0.001) were additional predictors of incident events. BMI (HR 1.03 [95% CI 0.99–1.06], P = 0.08), hypertension (1.50 [95% CI 0.99–2.27], P = 0.054), and sex
**Interaction between Q121 variant and obesity in predicting major cardiovascular events.** Given the previous evidence for an ENPP1-by-obesity interaction in the modulation of traits related to insulin resistance (25–27,29,30,39–41), we investigated this hypothesis in our study. In the GHS, which entirely consisted of patients with type 2 diabetes, we indeed observed a significant interaction between the Q121 variant and obesity. An association between the variant and risk of events was present among the 159 subjects who had a BMI ≥30 kg/m² (HR 3.56 [95% CI 1.21–10.5], P = 0.02), but not among the 171 individuals who had a BMI <30 kg/m² (0.91 [95% CI 0.40–2.06], P = 0.82; P = 0.039 for interaction).

No evidence of gene-by-obesity interaction was instead observed in the TVAS (P = 0.53) and the CREED (P = 0.41). Because approximately 85% of these two cohorts consisted of nondiabetic individuals, we hypothesized that the genotype-by-obesity interaction might be specific to diabetes. Indeed, a pattern consistent with such an effect was also observed in these two studies when the analysis was restricted to individuals with diabetes, even though the small sample size prevented statistical significance (data not shown).

Thus, we further investigated the Q121-by-obesity interaction in a pooled analysis of the three studies after stratification by diabetes status. In the diabetic stratum (n = 395), the Q121 variant was associated with an increased risk of incident events among the 177 obese (Fig. 2A) but not among the 218 nonobese (Fig. 2B) individuals, with adjusted HRs of 5.94 (95% CI 1.88–18.78, P = 0.002) vs. 0.62 (95% CI 0.32–1.24, P = 0.18). The interaction between the Q121 variant and obesity was significant (P = 0.003). By contrast, no evidence of interaction was observed in the nondiabetic stratum (n = 344, P = 0.26), with adjusted HRs of 0.82 (95% CI 0.22–3.11, P = 0.77) in the 39 obese individuals and 1.85 (95% CI 1.18–2.90, P = 0.008) in the 305 nonobese subjects.

Among obese diabetic individuals, the addition of the K121Q genotype to the multivariable model produced a slight improvement from 0.802 to 0.831 in risk discrimination when this was assessed by the survival c-index (P = 0.003). A much larger effect, approaching statistical significance, was observed when the improvement was assessed by IDI with a 4.56% improvement (95% CI −0.27 to 9.42, P = 0.09).

**TABLE 2**

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<tr>
<td>Person-years</td>
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<tr>
<td>Nonfatal stroke</td>
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<tr>
<td>CV death</td>
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<tr>
<td>Total events</td>
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<td>25</td>
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<tr>
<td>Incidence rate</td>
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**TABLE 3**

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<th>Nonfatal stroke</th>
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<td>11</td>
<td>43</td>
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*Per 100 person-years. CV, cardiovascular.
Interaction between Q121 variant and obesity on age at MI in cross-sectional studies. To seek replication of the gene-by-obesity interaction observed in patients with diabetes, we analyzed the association between the Q121 variant and age at MI in two cross-sectional samples of individuals with type 2 diabetes who had had a previous MI. One sample was from the Gargano area in Italy, the other was from Boston. Salient clinical features of the

FIG. 1. Kaplan-Meier survival curves are shown for major cardiovascular events in GHS (A), TVAS (B), and CREED (C). D: Estimates generated by Cox regression in the pooled analysis are shown.

FIG. 2. Survival curves for major cardiovascular events in obese (A) and nonobese (B) patients with type 2 diabetes. Curves are estimates generated by Cox regression in the pooled analysis of the three prospective studies.
study subjects are summarized in Table 3. Because no significant genotype-by-sample interaction was observed in the association with age at MI ($P = 0.11$), pooled analyses were performed by adjusting for “study sample.” To make the analysis comparable to that of prospective studies, sex, smoking status, hypertension, and BMI, but not age (due to its collinearity with age at MI—and diabetes—because all study participants were diabetic) were included as covariates. Among obese subjects, 64 Q121 carriers had had the MI almost 3 years earlier than the 124 KK homozygotes, at 54.5 (9.6) vs. 57.2 (8.9) years of age (due to its collinearity with age at MI—diabetes). Our results indicate that the Q121 variant is independent of that of age, sex, BMI, diabetes, and cigarette smoking. Our findings are in agreement with a previous cross-sectional study of 445 MI survivors from Central Europe (29). By contrast, case-control genome-wide association studies reported that a single nucleotide polymorphism (SNP, rs7767502), which is in perfect linkage disequilibrium with the ENPP1 K121Q polymorphism, was not associated with CAD (6–10). Several differences between our study and the genome-wide association studies, such as the prospective versus cross-sectional designs, the different end points under investigation, and the different baseline cardiovascular risk, with only the patients enrolled in our study being very high-risk as per selection criteria, might be responsible for this apparent discordance.

A further important result of our study is that the effect of the Q121 variant was modulated by obesity in diabetic patients among whom the risk of incident events was five times higher in Q121 than in KK genotype carriers. Although not the aim of our study, one can infer that obese individuals (Fig. 2A) as a whole tend to have a lower risk of future cardiovascular events than nonobese patients (Fig. 2B; adjusted HR 0.68 [95% CI 0.41–1.24], $P = 0.13$). This paradoxical protective effect of obesity resembles that observed in patients with CAD (41), ESRD (42), heart failure (43), and older age (44), all conditions heavily over-represented in our samples. In this context, the Q121 variant seems to eliminate the paradoxical protective effect of obesity.

An important finding was that the Q121 variant-by-obesity interaction observed in the prospective study was replicated in a cross-sectional study on age at MI in diabetic patients. Information on the K121Q genotypes tended to improve risk prediction in these patients when the improvement was measured by the IDI, the approach that is currently favored to evaluate predictive ability increase conferred by a new marker when added to a well-performing model (39). Thus, pending further validation in larger studies, one can hypothesize clinical implementation of the Q121 variant as a marker of early cardiovascular events among obese diabetic patients. Given the increasing incidence worldwide of both obesity and diabetes (2–4) and the poor ability to stratify cardiovascular risk among diabetic patients, a large sector of society would be likely to benefit in the future from the availability of such a test.

The synergistic effect of the genetic marker and obesity in the modulation of cardiovascular risk resembles results repeatedly reported in the risk modulation of insulin resistance and related traits (25–27,30,33,45–49). Placed in a broader perspective, this is an excellent example of genetic heterogeneity (i.e., different genetic effects being at play in different population subgroups) and clearly illustrates how accounting for such heterogeneity may be critical to dissect the genetic architecture of multifactorial diseases.

Understanding the mechanisms through which the Q121 variant is associated with CVD is beyond the scope of this study. However, one can speculate that the Q121 variant exacerbates cardiovascular risk by inducing systemic insulin resistance (22,25–27) and proatherogenic phenotypes.
It may also act by way of a direct detrimental effect on insulin-dependent endothelial function, as suggested by the observation that human endothelial cells carrying the Q121 variant show impaired insulin receptor signaling and, most importantly, reduced release of nitric oxide (24), a potent vasodilator whose deficiency is an established early step in the pathway development of atherosclerosis (50).

One can hypothesize that the interaction between the Q121 variant and obesity is sustained by the different sites of action on the insulin-signaling pathway. Although ENPP1 acts at the insulin receptor level (21,23), obesity acts by different mechanisms, mostly at a postreceptor level (51). It is, therefore, possible that postreceptor insulin-signaling abnormalities are necessary for the Q121 variant to be fully effective in inducing insulin resistance and, eventually, related clinical outcomes.

The three cohorts of very high-risk individuals that we studied were quite different from each other: one comprised only patients with type 2 diabetes and CAD, another included patients with a previous MI who did not have frank type 2 diabetes, and the third included only patients with ESRDs. Despite such apparent phenotypic heterogeneity, the effect of the Q121 variant was not heterogeneous across the three studies. Not only did this allow us to analyze the three cohorts together, increasing statistical power, but it also suggests that our findings may be generalizable to all high-risk patients, irrespective of their background clinical characteristics. Whether the predictive role of the Q121 variant extends to situations characterized by a more moderate cardiovascular risk remains to be determined.

We acknowledge that, mainly because of the relatively small size of our samples, the significance level of our findings is still compatible with a false-positive result. However, this seems unlikely given that the association between Q121 was not heterogeneous across the three cohorts and, importantly, was further confirmed in cross-sectional studies as far as the interaction with obesity in diabetic patients is concerned. We also acknowledge that due to the relatively small sample size of the studies that we analyzed, we cannot exclude that the gene-by-obesity interaction that we observed among diabetic patients also occurs among nondiabetic individuals, as is the case for the modulation of insulin resistance (25–27). Therefore, our findings need further replication in larger samples before they can be considered as established. Finally, because this study was entirely performed in individuals of European ancestry, we do not know whether our findings can be extended to populations of different race.

In conclusion, pending confirmation in further larger studies, the Q121 variant has the potential to become a clinical tool for identifying those very high-risk patients who are especially prone to major cardiovascular events and need, therefore, to be targeted with specific and even more aggressive preventive strategies.

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No potential conflicts of interest relevant to this article were reported.

S.B. designed the study, acquired, analyzed, and interpreted the data; and wrote the manuscript. S.R. and S.P. acquired, analyzed, and interpreted the data and reviewed the manuscript. D.S., C.P., A.F., A.P., D.L., A.T., Y.-Y.Z., and G.D.S. acquired data and reviewed the manuscript. F.M. reviewed the manuscript. G.T., R.X., D.M., F.A., R.L., E.V.G., and T.H.H. acquired data and reviewed the manuscript. M.C. acquired and analyzed the data and reviewed and edited the manuscript. S.D.C. acquired and interpreted the data and reviewed the manuscript. F.P.
acquired and analyzed the data and reviewed and edited the manuscript. C.Z. and M.F. acquired data and reviewed and edited the manuscript. A.D. and V.T. designed the study, analyzed and interpreted the data, and wrote the manuscript.

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