ENPP1 Q121 Variant, Increased Pulse Pressure and Reduced Insulin Signaling, and Nitric Oxide Synthase Activity in Endothelial Cells

Simonetta Bacci, Rosa Di Paola, Claudia Menzaghi, Patrizia Di Fulvio, Sara Di Silvestre, Fabio Pellegrini, Roberto Baratta, Antonella Marucci, Sandra Mastroianno, Grazia Fini, Gloria Formoso, Agostino Consoli, Francesco Pellegrini, Lucia Frittitta, Assunta Pandolfi, Vincenzo Trischitta

Objective—Insulin resistance induces increased pulse pressure (PP), endothelial dysfunction (ED), and reduced bioavailability of endothelium-derived nitric oxide (NO). The genetic background of these 3 cardiovascular risk factors might be partly common. The ENPP1 K121Q polymorphism is associated with insulin resistance and cardiovascular risk.

Methods and Results—We investigated whether the K121Q polymorphism is associated with increased PP in white Caucasians and with ED in vitro. In 985 individuals, (390 unrelated and 595 from 248 families), the K121Q polymorphism was associated with PP ($P=8.0 \times 10^{-4}$). In the families, the Q121 variant accounted for 0.08 of PP heritability ($P=9.4 \times 10^{-4}$). This association was formally replicated in a second sample of 475 individuals ($P=2.6 \times 10^{-2}$) but not in 2 smaller samples of 289 and 236 individuals ($P=0.49$ and 0.21, respectively). In the individual patients’ data meta-analysis, comprising 1985 individuals, PP was associated with the Q121 variant ($P=1.2 \times 10^{-3}$). Human endothelial cells carrying the KQ genotype showed, as compared to KK cells, reduced insulin-mediated insulin receptor autophosphorylation ($P=0.03$), Ser473-Akt phosphorylation ($P=0.03$), and NO synthase activity ($P=0.003$).

Conclusions—Our data suggest that the ENPP1 Q121 variant is associated with increased PP in vivo and reduced insulin signaling and ED in vitro, thus indicating a possible pathogenic mechanism for the increased cardiovascular risk observed in ENPP1 Q121 carriers. (Arterioscler Thromb Vasc Biol. 2009;29:00-00.)

Key Words: ●●●

Increased pulse pressure (PP), mostly a consequence of arterial stiffness, has been associated with cardiovascular events, including myocardial infarction (MI). Increased PP is associated with endothelial dysfunction (ED) and related reduced bioavailability of endothelium-derived nitric oxide (NO). Both increased PP and ED are, at least partly, genetically determined. Insulin resistance is pathogenic for both abnormalities and is under genetic control too; thus, it is possible that these conditions share some common genetic background.

ENPP1 (also known as plasma cell antigen 1 [PC-1]) is a class II membrane glycoprotein known to adversely influence insulin sensitivity by binding to and inhibiting insulin receptor (IR) function. A missense polymorphism, K121Q, has been described in the ENPP1 gene. As compared to the K121 variant, a stronger inhibitor of the IR insulin receptor substrate-1 (IRS-1)/phosphatidylinositol 3-kinase (PI3-K) activity pathway and, in most, although not all studies, has been associated with insulin resistance and type 2 diabetes. Most importantly, in this specific context, the ENPP1 Q121 variant modulates susceptibility to atherosclerosis and premature MI. To get insights about the pathogenic mechanisms underlying the deleterious cardiovascular effect of the ENPP1 Q121 variant, we investigated in humans whether it was associated with increased PP. In endothelial cells, insulin stimulates Akt phosphorylation at Ser473 and subsequently increases NO release, a potent vasodilator and a strong in vivo regulator of arterial stiffness and PP. Thus, we also tested whether the ENPP1 Q121 variant exerts a direct effect on some crucial steps of insulin signaling and on NO synthase activity in cultured human umbilical vein endothelial cells (HUVECs).
In Vivo Studies

First Stage Study
Nine hundred eighty-five white Caucasians from Gargano and surrounding area (Southern-East Italy) were investigated. Of them, 390 were unrelated subjects and 595 were from 248 pedigrees, comprising 75 nuclear families, 153 sibships, and 20 extended sibships.

Replication Attempt
As for replication, 3 additional samples of white Caucasians were studied. Four hundred seventy-five obese (ie, BMI ≥30 Kg/m²) and 289 nonobese individuals (ie, BMI <30 Kg/m²) were recruited from the outpatient Obesity Clinic at the Endocrine Unit and the hospital staff of Garibaldi Hospital in Catania (Sicily, Southern Italy), respectively. Finally, 236 newly diagnosed and never-treated hypertensive patients (systolic blood pressure >90 mm Hg) were recruited at the Catanzaro University Hospital (Calabria, Southern Italy). As per selection criteria, all subjects from these 4 samples had fasting plasma glucose <126 mg/dL, were free from overt cardiovascular disease (ie, by means of self-report), and were not treated with medications known to interfere with either glucose or lipid metabolism and with blood pressure.

The study, approved by local ethical committees, was performed according to the Helsinki Declaration. All study individuals gave written informed consent. In all individuals clinical examination, according to the Helsinki Declaration. All study individuals gave

In Vitro Studies

Cell Cultures
Umbilical cords were obtained from healthy mothers delivering at the Pescara Town Hospital consecutively asked to participate in the study. Those who were willing to participate signed a written consent form. Primary HUVECs were obtained and cultured as previously described. Because of our previous data showing that HUVECs carrying either the IRS-1 R972 or the TRIkB R84 variant (or the IRS-1 G972R or the TRIkB Q84R polymorphism, respectively) had reduced insulin-stimulated Akt activation and NO synthase activity, we have used HUVECs lines in this study (at least 2 different strains of KK- and of KQ-HUVECs) were chosen among those carrying wild-type IRS-1 G972G972 and TRIkB Q84Q84 genotypes and used for each experiment.

Insulin-Stimulated IR Phosphorylation
HUVECs were starved for 12 hours in serum-deprived medium and incubated with or without insulin (100 nmol/L). Fifty micrograms of total cell lysates were immunoprecipitated with anti-IR β-subunit antibody (C19, Santa Cruz Biotechnology), separated by the same SDS-PAGE and transferred to nitrocellulose membrane (Amersham Pharmacia Biotech). Blots were probed with anti-PY antibody (4G10, Millipore), stripped, and reprobed with anti-IR-β-subunit antibody (C19, Santa Cruz Biotechnology). Immunocomplexes were detected with the ECL Western Blotting System (Amersham Pharmacia Biotech) and quantified with a computerized densitometric system. Densities of IR-β-subunit phosphorylation were divided by the amount of immunoprecipitated IR-β-subunit and the ratio indicated as arbitrary units.

Insulin Stimulation of Akt Phosphorylation and NO Synthase Activity
HUVECs were starved for 2 hours in serum-deprived medium and incubated with or without insulin (100 nmol/L). Because in our hands phosho-Akt levels in HUVECs are comparable after 2, 5, and 10 minutes insulin stimulation (data not shown), the longest incubation (ie, 10 minutes) was chosen. Equal amounts of total cell lysate proteins were resolved by SDS-PAGE, transferred to nitrocellulose membrane, immunoblotted with anti-Akt, anti-Ser473-Akt, and β-actin primary antibodies, and incubated with peroxidase-conjugated secondary antibodies. Proteins were detected using enhanced chemiluminescence and quantified with a computerized densitometric system. Densities of Akt phosphorylation were divided by those of total Akt content and the ratio indicated as arbitrary units.

Genotyping
Genomic DNA from both blood samples and umbilical cord arteries and genotyping were obtained as previously described. Genotypic data were analyzed using the Hardy-Weinberg equilibrium (HWE). Association between PP, SBP, or DBP values according to smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available).

Statistical Methods
General features of study subjects are reported in Table 1 as means ± SE. A χ² test was used to assess whether genotype distribution was in Hardy-Weinberg equilibrium (HWE). Association between PP, SBP, or DBP values and K121Q polymorphism in 3 samples (obese and nonobese groups from Sicily, and hypertensive patients from Calabria) was assessed with a multivariate linear regression model. As for the Gargano sample, to account for within-family correlation in PP, SBP, and DBP values, a multivariate linear mixed model using a linear mixed model29–31 was used to account for potential between-sample heterogeneity (tested as a genotype-by-sample interaction).30 All 4 samples were adjusted for age, sex, smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available). PP (as well SBP and DBP) values according to smoking habit (except for the Calabria sample, where the latter variable was not available).

Table 1. Features of the 1985 Study Subjects

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gargano</th>
<th>Obese* Sicily</th>
<th>Nonobese Sicily</th>
<th>Calabria</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>985</td>
<td>475</td>
<td>289</td>
<td>236</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>377/608</td>
<td>127/348</td>
<td>138/151</td>
<td>131/105</td>
</tr>
<tr>
<td>Age, y</td>
<td>39.4±0.4</td>
<td>37.2±0.54</td>
<td>36.7±0.73</td>
<td>44.7±0.35</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>26.0</td>
<td>40.4</td>
<td>34.3</td>
<td>NA</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.9±0.1</td>
<td>42.6±0.35</td>
<td>24.5±0.17</td>
<td>26.1±0.19</td>
</tr>
<tr>
<td>HOMA_IR</td>
<td>1.8±0.04</td>
<td>4.5±0.11</td>
<td>1.7±0.06</td>
<td>2.1±0.08</td>
</tr>
<tr>
<td>PG, mg/dL</td>
<td>90.3±0.3</td>
<td>97.9±0.55</td>
<td>86.7±0.56</td>
<td>89.8±0.43</td>
</tr>
<tr>
<td>HDL, μmol/L</td>
<td>120.0±0.4</td>
<td>7.8±0.26</td>
<td>9.6±0.36</td>
<td>NA</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>53.3±0.4</td>
<td>45.0±0.38</td>
<td>45.8±0.73</td>
<td>NA</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>95.7±1.9</td>
<td>123±3.00</td>
<td>99.8±3.94</td>
<td>NA</td>
</tr>
</tbody>
</table>

Data are reported as means ± SE. BMI indicates body mass index; HOMA_IR, homeostasis model assessment of insulin-resistance; PG, plasma glucose; IRI, immunoreactive insulin; NA, not available. *Obesity is defined as BMI ≥30 kg/m².
Table 2. Pulse Pressure (PP), Systolic Blood Pressure (SBP), and Diastolic Blood Pressure (DBP) of the 1985 Subjects According to Sample Location and ENPP1 K121Q Genotype

<table>
<thead>
<tr>
<th></th>
<th>K121K</th>
<th>K121Q</th>
<th>Q121Q</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>716</td>
<td>248</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>38.1±0.4</td>
<td>40.8±0.6</td>
<td>39.8±2.0</td>
<td>8.0×10⁻⁴</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>114.8±0.5</td>
<td>117.0±0.8</td>
<td>117.1±2.8</td>
<td>1.1×10⁻²</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>76.7±0.4</td>
<td>76.2±0.6</td>
<td>78.3±1.9</td>
<td>0.84</td>
</tr>
<tr>
<td>Obese Sicily</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>326</td>
<td>137</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>43.8±0.4</td>
<td>44.6±0.7</td>
<td>49.3±2.3</td>
<td>2.6×10⁻²</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>123.9±0.6</td>
<td>124.7±1.0</td>
<td>130.5±3.4</td>
<td>0.14</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>80.1±0.5</td>
<td>80.1±0.7</td>
<td>81.2±2.4</td>
<td>0.95</td>
</tr>
<tr>
<td>Nonobese Sicily</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>202</td>
<td>78</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>41.7±0.6</td>
<td>40.1±0.9</td>
<td>44.2±2.7</td>
<td>0.49</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>115.8±0.8</td>
<td>115.0±1.2</td>
<td>117.8±3.6</td>
<td>0.78</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>74.1±0.6</td>
<td>74.8±1.0</td>
<td>73.5±2.9</td>
<td>0.74</td>
</tr>
<tr>
<td>Calabria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>167</td>
<td>64</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>57.8±1.0</td>
<td>58.0±1.7</td>
<td>72.9±5.9</td>
<td>0.21</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>150.4±1.5</td>
<td>149.5±2.4</td>
<td>169.3±8.5</td>
<td>0.45</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>92.5±0.8</td>
<td>91.4±1.3</td>
<td>96.4±4.8</td>
<td>0.91</td>
</tr>
<tr>
<td>IPD metaanalysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>1411</td>
<td>527</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>45.1±0.3</td>
<td>46.3±0.4</td>
<td>49.5±1.4</td>
<td>1.2×10⁻³</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>125.7±0.4</td>
<td>126.7±0.6</td>
<td>131.0±1.9</td>
<td>1.7×10⁻²</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>80.6±0.3</td>
<td>80.3±0.4</td>
<td>82.1±1.4</td>
<td>1</td>
</tr>
</tbody>
</table>

Data are reported as adjusted means±SE. *P values refer to the additive model after adjustment for age, sex, and smoking habit (except for the Calabria sample, where smoking was not available); assessed with linear (mixed) models. IPD indicates individual patients’ data.

Student t test. All statistical analyses were performed using SPSS Statistical Package (Version 13) and SAS Release 9.1 (SAS Institute). Probability values <0.05 were considered statistically significant.

Results

In Vivo Studies

Clinical features of all study subjects are shown in Table 1. Genotype distribution was in Hardy–Weinberg Equilibrium in each sample.

First Stage Study

In the first stage study (ie, the sample from Gargano) of 985 non-diabetic individuals, 716 carried the K121/K121 (ie, KK), 248 the K121/Q121 (ie, KQ), and 21 the Q121/Q121 (ie, QQ) genotype. In the multivariate linear mixed model the ENPP1 K121Q polymorphism was significantly associated (P=8.0×10⁻⁴) with PP and to a lower extent SBP but not with DBP (Table 2). Of note, the association between Q121 variant and PP was still significant when adjusting also for HOMAIR (P=6.0×10⁻⁴). In the 289 nonobese individuals, 202 carried the KK, 64 the KQ, and 9 the QQ genotype. No significant association was observed between PP values and the ENPP1 genotypes (Table 2).

Replication Attempt

As for replication, 3 additional samples of white Caucasians (ie, 2 from Sicily and 1 from Calabria) were studied.

Samples FROM SICILY

Of the 475 obese unrelated individuals, 326 carried the KK, 137 the KQ, and 12 the QQ genotype. In the multivariate linear model the K121Q polymorphism was significantly (P=2.6×10⁻²) associated with PP (Table 2). No significant associations were observed with SBP and DBP (Table 2). The association between Q121 variant and PP remained significant after adjusting also for HOMAIR (P=2.1×10⁻²). Because a small proportion (22.3%) of untreated hypertensive patients was observed in this sample, data were analyzed after adjusting also for hypertensive status and showed that the association between the Q121 variant and PP remained significant (P=4.0×10⁻²).

Of the 289 nonobese unrelated individuals, 202 carried the KK, 78 the KQ, and 9 the QQ genotype. No significant association was observed between PP values and the ENPP1 genotypes (Table 2).

Combined Analysis

Individual Patients’ Data Meta-Analysis

The pooled dataset of the 4 samples was adjusted for age, gender, smoking habit, and sample location. The genotype-by-sample interaction did not show between-samples heterogeneity in the K121Q polymorphism effect on PP (P=0.16). Therefore, a fixed effects genotype individual patients’ meta-analysis was performed, where the use of a linear mixed model was still necessary to account for the within-family correlation of 1 of the samples (ie, the Gargano sample). The K121Q polymorphism was strongly associated with PP (P=1.2×10⁻³, β=1.53) and SBP (P=1.7×10⁻², β=1.50) but not with DBP (P=1.0; Table 2). The association with PP did not change after adjusting also for HOMAIR (P=1.4×10⁻³). No significant gene × obesity interaction was observed in the K121Q polymorphism effect on PP (P=0.93). Neither in this pooled analysis (P=0.67) nor in any sample singly considered (data not shown) was a genotype-by-gender interaction observed, thus making unlikely the possibility of a gender-specific effect of the K121Q polymorphism on PP.

In Vitro Studies

Impact of ENPP1 K121Q Variant on Insulin-Stimulated IR Phosphorylation

Insulin-stimulated IR β-subunit phosphorylation was lower in KQ- (ie, 2.5-, 4.0-, and 1.1-fold stimulation over basal unstimu-
lated phosphorylation level) than KK-HUVECs (14.1-, 17.2-, and 7.0-fold stimulation). A representative experiment is shown in Figure 1A. Mean data are shown in Figure 1B.

Impact of ENPP1 K121Q Variant on Insulin-Stimulated Akt Phosphorylation
Insulin-induced Akt phosphorylation at Ser473 was markedly impaired in KQ- (ie, virtually no stimulation over basal unstimulated phosphorylation level) as compared to KK-HUVECs (ie, 1.49-, 1.50-, and 1.48-fold stimulation over basal unstimulated levels). Mean data are shown in Figure 2. In contrast, no significant differences were observed in Akt and β-actin protein expression (Figure 2).

Impact of ENPP1 K121Q Variant on Insulin-Stimulated NO Synthase Activity
To assess the impact of ENPP1 K121Q variant on NO synthase activity, insulin-stimulated conversion of L-[3H]arginine into L-[3H]citrulline was measured. Basal NO synthase activity was not different between KK- and KQ-HUVECs (Figure 3). In contrast, after 100 nmol/L insulin stimulation, virtually no changes in NO synthase activity was observed in KQ-HUVECs, whereas a clear increase (ie, 1.88-, 1.77-, and 1.83-fold stimulation) was observed in KK-HUVECs. Mean data are shown in Figure 3. Preincubation with 1 mmol/L L-NAME (ie, an NO synthase inhibitor), abolished insulin-stimulated NO synthase activity in KK-HUVECs and had no effect on KQ-HUVECs (Figure 3).

Discussion
Our present study, performed in 4 samples of white Caucasians from Southern Italy comprising a total of 1985 individuals, suggests that the ENPP1 Q121 variant is independently associated with increased PP, a useful clinical marker of arterial stiffness. When looking at the individual samples analyzed, statistically significant association was observed in the first stage study and in 1 of the 3 samples analyzed in the replication attempt, thus providing evidence of formal replication of the observed association. As an additional novelty of the present study, data from families indicated that the Q121 variant plays a role in PP heritability. Because the first sample mostly comprised healthy individuals of young-middle average age, the effect of the Q121 variant on PP might represent a very early alteration in the multi-step proatherogenic process. At variance with what was observed in the modulation of other phenotypes including insulin sensitivity, type 2 diabetes, and atherosclerosis, no significant gene-by-obesity interaction was observed in modulating PP.

The Q121 variant was associated with SBP in 1 sample; however, no replication was observed in the other samples. In the pooled analysis, the Q121 variant showed a weaker association with SBP, as compared to that with PP. Similarly, data from families indicated that the Q121 variant explained a much smaller proportion of SBP heritability as compared to that of PP. Some associations of the Q121 variant with incidence of hypertension and increased SBP have been reported in previous studies; unfortunately no measurement of PP were available in these studies, thus making it impossible to compare previous data with our present findings. It is of note that in the Diabetes Genetics Initiative (www.broad.mit.edu/diabe-
phorylation, Akt phosphorylation, and NO synthase activity. Although we cannot exclude that other yet unknown genetic variants able to affect insulin-induced NO synthase activity have played a role in the results we obtained, if by chance they have been carried by KQ- but not KK-HUVECs, these data are in line with the notion that, as compared to the K121, the Q121 variant is a stronger inhibitor of upstream insulin signaling at the level of IR/IRS-1/PI3-Kinase activity pathway.14,15 Because reduced NO bioavailability at the endothelium may increase arterial stiffness and PP,6,7 it may be hypothesized that the effect of ENPP1 Q121 on PP is mediated, at least partly, by a direct detrimental effect on insulin-dependent endothelial function. A direct effect on the arterial wall is also suggested by the observation that, in both samples, the association between the Q121 variant and high PP values was independent from HO-MAβ, a surrogate of “systemic” insulin resistance. A deleterious role on insulin signaling and NO synthase activity has been reported also in HUVECs naturally carrying other genetic determinants of human insulin resistance.24,25 Thus suggesting that this is a generalized phenomenon related to “insulin resistance genes.”

It is quite well established that PP and arterial stiffness are strong predictors of premature cardiovascular events,2 and of early coronary disease.3 Thus, our present data suggest a possible pathogenic pathway contributing to the reported increased risk of early MI carried by Q121 carriers.16,17 False-positive results are not uncommon in association studies. In our case, the risk of population stratification was minimized by reporting the association in 2 genetically homogenous samples (both being recruited in restricted regions of Southern Italy) and by including families in the sample from Gargano. The lack of nominal association observed in the 2 additional samples is likely to be a consequence of low statistical power because of small sample size. As a matter of fact the power to detect an effect similar to that observed in the first sample from Gargano was ≤50% both in the sample comprising nonobese individuals from Sicily and in that from Calabria. In the case of the sample from Calabria, also the intrinsic nature of the study (comprising only hypertensive patients in whom the range of PP distribution is reduced because limited to the high end of the PP values spectrum) might have reduced the chance to detect significant difference across genotype groups.

Also, the low probability value reached in the pooled analysis very much reduces the probability of a false-positive result. Thus, although the need of further replications in larger cohorts remains an essential next step to achieve even more robust, possibly genome-wide, P level of significance, the risk of a false-positive finding seems to be modest.

In conclusion, our data suggest that the ENPP1 Q121 variant is associated with PP in white Caucasians from Southern Italy and with reduced insulin signaling and insulin-mediated NO synthase activity in human endothelium.

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Disclosures

References


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