Published in final edited form as:
Am J Kidney Dis. 2009 February ; 53(2): 273–280. doi:10.1053/j.ajkd.2008.07.040.

The Q121 Variant of *ENPP1* Gene is Associated with Decreased Kidney Function Among Patients With Type 2 Diabetes

Salvatore De Cosmo, MD[1], **Antonio Minenna, MD**[1], **Yuan-Yuan Zhang, PhD**[2], **Robert Thompson, MD**[2], **Giuseppe Miscio, PhD**[3], **Monica Vedovato, MD**[4], **Anna Rauseo, MD**[1], **Alois Saller, MD**[5], **Sandra Mastroianno, MD**[1], **Fabio Pellegrini, MSc**[3,6], **Roberto Trevisan, MD**[7], **Paola Fioretto, MD**[5], **Alessandro Doria, MD**[2], and **Vincenzo Trischitta, MD**[1,3,8,9]

[1] Unit of Endocrinology, Scientific Institute “Casa Sollievo della Sofferenza”, San Giovanni Rotondo, Italy

[2] Joslin Diabetes Center, Boston, MA (USA)

[3] Research Unit of Diabetes and Endocrine Diseases, Scientific Institute “Casa Sollievo della Sofferenza”, San Giovanni Rotondo, Italy

[4] Chair of Metabolic Diseases, University of Padova, Padova, Italy

[5] Department of Medical and Surgical Sciences, University of Padova, Padova, Italy

[6] Department of Clinical Pharmacology and Epidemiology, Consorzio Mario Negri Sud, S. Maria Imbaro, Italy

[7] Unit of Diabetes, Ospedali Riuniti of Bergamo, Bergamo, Italy

[8] CSS-Mendel Institute, Rome, Italy

[9] Department of Clinical Sciences, “Sapienza” University, Rome, Italy

Abstract

Background—Insulin resistance plays a role in diabetic kidney complications. The *ectonucleotide pyrophosphatase/phosphodiesterase* (*ENPP1)* (rs1044498) K121Q polymorphism has been associated with insulin resistance and related vascular complications among patients with type 2 diabetes (T2D), in many although not all studies. This study investigated whether the *ENPP1* Q121 variant modulates the risk of reduced GFR in T2D.

Study design—Cross-sectional study.

Setting & Participants—Two Diabetes Units from Italy (Gargano e Padua) and one from the US (Boston) recruited a total of 1392 patients with T2D.

Predictors—The *ENPP1* Q121 variant.

Measurements—Estimated glomerular filtration rate (eGFR) from serum creatinine, urinary albumin excretion, blood pressure, HbA1c, Triglycerides, Total cholesterol, HDL-Cholesterol

Outcomes—Reduced GFR levels (i.e. eGFR <60 ml/min/1.73m^2).

Results—In the Gargano and Boston populations, according to dominant model of inheritance, Q121 carriers (i.e. individual with either KQ or QQ genotype) had an increased risk of reduced GFR:

Address for correspondence: Dr. Salvatore De Cosmo (sdecosm@tin.it), Endocrinologia, Scientific Institute “Casa Sollievo della Sofferenza” V.le Cappuccini 1, 71013 San Giovanni Rotondo (FG), ITALY, and Dr. Vincenzo Trischitta (vincenzo.trischitta@uniroma1.it), Istituto CSS-Mendel, V.le R. Margherita 261, 00198 Roma, Italy.

ORs=1.69 (95%CI 1.1–2.6) and 1.50 (95%CI 1.0–2.2), respectively. In the Padua set the association was in the same direction but didn't reach a formal statistical significance (OR=1.77, 95%CI 0.7–4.5). When the three studies were pooled, Q121 carriers showed and increased risk of reduced GFR (OR=1.58, 95%CI 1.2–2.1, p=0.002). Also pooled mean differences of absolute GFR values were different across genotype groups with Q121 carriers showing lower GFR, as compared to KK individuals (p=0.04).

Limitations—p values not approaching a genome-wide level of significance.

Conclusions—Our data suggest that patients with T2D carrying the *ENPP1* Q121 variant are at increased risk of reduced GFR.

Keywords

gene polymorphism; insulin resistance; vascular diabetic complications; PC-1

Background

Chronic kidney disease (CKD) is a worldwide public health problem because of its devastating adverse outcomes such as end stage renal disease (ESRD) and increased cardiovascular morbidity and mortality (1–3). Several pieces of evidence indicate that, among patients with type 2 diabetes (T2D), glomerular filtration rate (GFR) is heritable, suggesting that, in addition to environmental factors, genetic determinants exert significant influences on this trait (4–5).

Insulin resistance and related metabolic abnormalities have been suggested to play a role in renal damage in the general population and in diabetic patients as well (6–7). Since insulin resistance is also under genetic control (8), the two traits might share common genetic determinants. Membrane glycoprotein ENNP1 (also known as PC-1) inhibits insulin receptor signalling and, when overexpressed, causes insulin resistance (9). It has been recently reported that the *ENPP1* K121Q polymorphism (i.e. rs1044498 A>C, with a minor allele frequency varying from 13% in HapMap CEU to 93% in HapMap YRI), with Q121 being a gain of function variant characterized by a stronger ability to inhibit insulin receptor signalling (10), is associated with insulin resistance (11–16), T2D (17) and coronary artery disease (18–19). Among patients with type 1 diabetes, several (20–21) although not all (22) studies have indicated that the Q121 variant is associated with reduced renal function. In a small pilot study we previously suggested a similar association also in T2D (23). Encouraged by these preliminary results, in the present study, we sought to investigate, in three independent samples, comprising altogether 1392 individuals, whether the Q121 variant confers an increased risk of reduced GFR in T2D.

Research design and Methods

Study patients

We studied three independent samples of patients with T2D, World Health Organization (WHO) criteria, two from Italy and one from Boston. Each sample was recruited according to the Helsinki Declaration and study protocol was approved by the local Ethical Committees. All participants provided written informed consent. Selection criteria and clinical features of these three populations have already been published (7,23–24). Briefly, the first sample from Italy consisted of 684 Whites with T2D who were consecutively recruited in the Gargano area at the Scientific Institute "Casa Sollievo della Sofferenza" in San Giovanni Rotondo (Central Italy). The inclusion criteria were diabetes diagnosed after age 30 years, absence of ketones at diagnosis, and absence of clinically evident autoimmune disease. These criteria were chosen to minimize the risk of including late-onset type 1 diabetic patients. Exclusion criteria were non diabetic kidney disease, cancer, life-threatening diseases.

Elevated urinary albumin excretion (UAE) was diagnosed when albumin/creatinine ratio (ACR) was ≥ 2.5 mg/mmol in men and 3.5 mg/mmol in women; 208 (30%) patients had elevated UAE. In this sample we also considered the presence of cardiovascular risk factors strictly related to the metabolic syndrome (MS) such as arterial hypertension, dyslipidemia, and abdominal obesity as previously reported (25). An individual MS-related score (MS-r score) was assigned ranging from 0 (diabetes only) to 3 according to the presence of abdominal obesity and/or dyslipidemia and/or arterial hypertension.

The second sample included 583 White with T2D, living in the Boston area, who were recruited at the Joslin Diabetes Center, in Boston (MA, USA) for a study on the genetics of coronary artery disease (CAD), a condition which was present in 244 (42%) patients (24). This information was missing in the other two samples. In the Boston sample the CAD-positive cases were recruited among patients with T2D who underwent cardiac catheterization at the Beth Israel Deaconess Medical Center. CAD-negative controls were Joslin patients who were aged 55 or older, had diabetes for 5 years or more, and had a negative cardiovascular history and a normal exercise treadmill test.

The third sample, comprising 125 Whites with T2D and increased UAE rate living in Northeast Italy, was recruited at the University of Padua (23). Inclusion criteria were as follows: ≤70 years of age, serum creatinine ≤2.4 mg/dl, persistent microalbuminuria or macroalbuminuria and absence of non diabetic renal disease.

In the latter two samples, measurements of GFR and UAE rate have been simultaneously performed, while no UAE measurement are available in the Boston sample.

In all three sets standardised serum creatinine was measured by the modified kinetic Jaffè reaction. Estimated GFR (eGFR) was calculated with the 4-variable Modification Diet in Renal Diseases (MDRD) Study equation (26) IDMS-traceable. Reduced GFR defined as eGFR <60 ml/min/1.73 m^2. This value of GFR identifies patients with CKD stage 3–5 (27). Patients with eGFR < and ≥ 60 ml/min/1.73 m^2 were considered as cases and controls, respectively, in the case-control study for reduced GFR. In the Gargano sample, 57/208 (27%) individuals with albuminuria and 60/476 (13%) with normoalbuminuria had reduced GFR. As said before, all individuals had albuminuria in the sample from Padua, whereas no data on UAE were available from the Boston sample.

Genotyping

High–molecular weight DNA for genotyping was extracted from peripheral blood (5–10 ml) by a proteinase K–phenol/chloroform standard method, resuspended in 10 mmol/l Tris- HCl, pH 8.0, 1 mmol/l EDTA, and stored at 4°C. PCR technique, specific primers, and experimental conditions used for genotyping with the *Ava*II restriction enzyme have been previously described (11). The genotyping failure was <2%. Genotyping quality was checked by directly sequencing 10% of randomly selected samples. The agreement rate of re-sequenced samples was >99%. The *ENPP1* K121Q genotype distribution was in Hardy-Weinberg equilibrium within each sample (Gargano sample: χ^2=3.51, p=0.06; Boston sample: χ^2= 0.02, p=0.88; Padua χ^2= 0.05, p=0.82).

Statistical analysis

Data are reported as mean ± SD or median (range). Mean differences were compared by unpaired Student's *t* or 1-way ANOVA F-tests, as appropriate. Differences between categorical variables were tested by Pearson's χ^2. The effect of the K121Q variant on the risk of reduced GFR was evaluated by a multivariate logistic regression and expressed as Odds Ratios (ORs) and 95% OR confidence intervals (CIs). The effect of the K121Q variant on absolute GFR

values was assessed by a multivariate linear regression. Both the models were adjusted for the following confounders: sex, age, duration of diabetes, HbA1c, hypertension and BMI. Furthermore, a random-effects individual data meta-analysis was performed with generalized hierarchical linear models to estimate both pooled GFR mean differences and pooled reduced GFR risk between genotypes (28). The same set of confounders was considered. Between-studies heterogeneity was assessed testing K121Q variant by study interaction. Considering realistic a fifty percent increased risk of reduced GFR in patients carrying the Q121Q variant, the size of sample studied allows us to detect such effect, with a power of 80% and a $p < 0.05$ which was considered as significant. All the analyses were performed using SPSS package Version 13.0 (SPSS Inc., Chicago, IL, USA) and SAS Release 9.1 (SAS Institute, Cary, NC).

Results

Clinical features in each sample are shown in table 1, left panel. Right panel of table 1 indicates that these features were not different across the three genotypes groups in any sample. EGFR data are presented in table 2. The lowest mean GFR value in the Boston sample is very likely justified by the fact that this set was enriched with individuals having clinical coronary artery disease, a condition that is known to be associated with reduced GFR (29). Indeed, in this sample GFR values were significantly lower in individuals with coronary artery disease than in those without it (65±22 vs 74±19 ml/min/1.73m^2, respectively; $p<0.0001$). When GFR values were analyzed across genotype groups they tended to be lower in Q121 carriers in all 3 samples, (Table 2). Since no heterogeneity in the genetic effect was observed across the study populations (p for heterogeneity=0.784) data from the three samples were then pooled and meta-analysed. According to a dominant model of inheritance, Q121 carriers had significantly (p=0.04) lower e-GFR as compared to KK individuals (Table 2).

The risk of reduced GFR is shown in Table 3. Similarly to what discussed about absolute GFR levels, the different proportion of reduced GFR across the three samples is likely to be due to the intrinsic nature of the Boston sample which is enriched by individuals with overt coronary artery disease. In the Gargano set, according to a dominant model of inheritance, carriers of the Q121 variant had increased risk of reduced GFR (table 3, Panel A). This association remained significant (p=0.04 for all models) also after adjusting for potential confounders which were not available in the other 2 samples, including either antihypertensive (p=0.04), or renin-angiotensin system blockers (p=0.01), or anti-diabetic therapy (p=0.04) or smoking habit (p=0.04). The association between the Q121 variant and kidney function was still observed when the MS-r score was added in the multivariate model (p=0.04). In addition, no interaction between Q121 variant and MS-r score was present (p=0.2) in modulating the risk of reduced GFR.

A significant association between the Q121 variant and reduced GFR was also observed in the second set from the Boston area (adjusted OR=1.50, 95% CI 1.0–2.2 (table 3, panel B). This association remained significant (p=0.037) also after adjusting for the presence of coronary artery disease which was highly prevalent in this set because of the study design (see above). The Q121 variant tended to be more prevalent also in the third sample from Padua, though not reaching a nominal statistical significance with the present sample size. (table 3, panel C). Since no heterogeneity in the genetic effect was observed across the study populations (p=0.602), data from the three samples were pooled and meta-analysed. The risk of having reduced GFR in patients carrying the Q121 variant was significantly increased independently of several covariates (adjusted OR=1.58; 95% CI 1.2–2.1) (table 3, Panel D). This association was significant among obese (BMI ≥30 Kg/m^2) (n=762) but not among non obese (n=630) patients (OR=1.96; 95% CI 1.4– 2.8 and 1.21; 95% CI 0.8–1.9, respectively), thus indicating a clear interaction between obesity and *ENPP1* K121Q polymorphism (p for interaction=0.03) in modulating the risk of reduced GFR.

In the 2 samples having also data on UAE (i.e. Gargano and Padua samples) no association was observed between this variable and the Q121 variant (Table 1).

Discussion

Our data indicate that the Q121 variant of the *ENPP1* gene is independently associated with reduced GFR in patients with T2D. When reduced GFR values were taken into account, Q121 carriers showed an average reduction of approximately 3 ml/min/1.73m^2 and a 60% increase in the risk for reduced GFR. Since study patients had a mean disease duration of 11 years, they are likely to have lost 22–27.5 ml/min since diabetes diagnosis (30–32). Thus, the 3 ml/min/1.73m^2 difference observed across different genotypes represents approximately 15% of the probable GFR loss occurred since disease diagnosis. Such an effect is clinically relevant when considering that the overall impact of well known modifiable risk factors such as blood pressure, glycemic control, albumin excretion rate and smoking habit on GFR loss is 24% (32) and is entirely consistent with the role of a “minor” gene in the context of a complex multi-factorial trait. Similarly, also when considering the risk of reduced GFR, the 60 % increased risk given by the Q121 variant is not weaker than that attributable to major changes in systolic blood pressure (20% increased risk for 10 mmHg difference) or HbA1c (5% increased risk for 1% difference) (33).

Despite the association with GFR values and reduced GFR, no association was found between Q121 variant and albuminuria. Similarly, two previous studies in patients with type 1 diabetes reported a significant association between Q121 variant and reduced GFR, but not increased albuminuria (20–21). This might be due to the antihypertensive therapy ongoing on virtually all diabetic patients which is known to be more effective in improving proteinuria, than in preventing GFR loss and which may have overridden the deleterious effect of *ENPP1* Q121 on the former parameter. Alternatively, AER and GFR might be regulated by different genes, as recently discussed (34). Thus, AER may be expression of microangiopathy while GFR is more likely to be affected by macroangiopathy; the Q121 variant is probably associated with macroangiopathy, more than with micro, as suggested by the reported association with pro-atherogenic phenotypes (17,18). As a matter of fact, a possible mechanism accounting for the reduction of renal function in patients carrying the Q121 variant is the predisposing effect of this variant to insulin-resistance (11–16) and atherosclerosis (18–19), which are both independent risk factors for renal dysfunction in patients with T2D (7,35). We would like also to speculate that a reduced insulin sensitivity is likely to underlie the association between the Q121 variant and reduced GFR despite the fact that the association between the Q121 variant and reduced GFR was still observed when in the multivariate model the MS-r score was added thus making uncertain the role of insulin resistance in mediating it. This might be due to the metabolic background of the samples studied which, because of diabetes itself, hypertension and reduced GFR present in several patients, show an “acquired insulin resistance phenotype” that may have overridden and masked the deleterious role of genetic insulin resistance. Alternatively, it may be postulated a direct deleterious role of the Q121 variant on endothelial function and/or arterial wall which has been recently proposed (36)

Although BMI was not associated with the Q121 variant, it significantly interacted with the variant in increasing the risk of reduced GFR acting, therefore, as a “modifier” of the gene effect. The observed gene/BMI interaction resembles that reported in the modulation of T2D (37–38), atherosclerosis (19) and hyperinsulinemia (39), thus suggesting that this is a generalized phenomenon underlying the effect of the Q121 variant on several phenotypes related to insulin resistance.

We acknowledge that the observed association, although significant, was certainly less than optimal, thus leaving open the possibility of a false positive result. However, such a possibility

is unlikely given the similar trend of association observed across the 3 samples analyzed which suggests that this is a true generalized phenomenon.

A potential limitation of our study is the different recruitment criteria used in the three different cohorts, one being a general diabetic population (i.e. the one from Gargano), one being enriched by coronary artery disease (i.e. the one from Boston) and one being enriched by micro or macroalbuminuria (i.e. the one from Padua). This clinical heterogeneity might, eventually, have resulted in genetic heterogeneity (i.e. different gene effect in different samples). Such a possibility has been formally excluded by testing for gene-samples interaction which turned out not to be operating in modulating GFR values and reduced GFR risk. Lack of heterogeneity allowed us to pool and analyse together data from the three populations, thus increasing statistical power.

An additional limitation of this study may be the underestimation of true GFR by the MDRD Study equation; despite this is true across the entire GFR range, this imprecision is reported to be more important for GFR above 60 ml/min/1.73m^2 (40–41). Since, on average, KK patients have a higher eGFR than Q121 carriers, the underestimation of true GFR is likely to have enriched the group of cases with KK people, thus reducing the strength of the association between the Q121 variant and reduced GFR (i.e. biasing our results towards the null hypothesis) further minimizing the risk that the significant association we observed is a spurious one.

In addition, we acknowledge that, although we have adjusted our final result for place of recruitment, a possible difference in practice patterns in diabetic control, use of antidiabetic medications modulating insulin resistance, dietary difference or different extents of survival biases in the two countries, might have influenced our finding. Furthermore, since potential confounders such as antihypertensive, renin-angiotensin system blockers, anti-diabetic therapy and smoking habit were available only for the Gargano sample, we can't claim the complete independence of the gene effect from these latter variables across all samples studies.

It is worth to note that haplotypes comprising the K121Q polymorphism have been reported to be associated with insulin resistance states in some but not all studies (37,42–46) as well as in type 2 diabetes enriched by end stage renal disease (47). Thus, we can't exclude that a comprehensive analysis of the entire *ENPP1* gene might provide further information about the association with kidney function in patients with T2D.

In conclusion, our data indicate that, among patients with T2D, those carrying the *ENPP1* Q121 variant have an increased risk of kidney dysfunction and reduced GFR. If this association is confirmed in other populations, so that it can be considered a general phenomenon, it might serve the important function of helping the identification of diabetic individuals who are prone to renal dysfunction and should be targeted with specific preventive and therapeutic strategies early in the course of diabetes.

Acknowledgments

This work was supported by a research grant from Italian Minister of Health (RF 2003). NIH grants HL73168 and DK36836 (Genetics Core of the Diabetes & Endocrinology Research Center at the Joslin Diabetes Center), William Randolph Hearst Fellowship (to Y-Y Z.).

References

1. He, J.; Whelton, PK. Epidemiology of end-stage renal disease. In: Brenner, BM.; Kurokawa, K., editors. International Handbook of Hypertension. Haslemere, United Kingdom: Euromed Communications; 1999.

2. Muntner P, He J, Hamm L, Loria C, Whelton PK. Renal insufficiency and subsequent death resulting from cardiovascular disease in the United States. J Am Soc Nephrol 2002;13:745–53. [PubMed: 11856780]

3. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis 1998;32 (Suppl 3):S112–9. [PubMed: 9820470]

4. Langefeld CD, Beck SR, Bowden DW, Rich SS, Wagenknecht LE, Barry I, Freedman BI. Heritability of GFR and albumin in Caucasians with type 2 diabetes mellitus. Am J Kidney Dis 2004;43:796–800. [PubMed: 15112169]

5. Placha G, Poznik GD, Dunn J, Smiles A, Krolewski B, Glew T, Puppala S, Hneider J, Rogus JJ, Rich SS, Duggirala R, Warram JH, Krolewski AS. A genome-wide linkage scan for genes controlling variation in renal function estimated by serum cystatin C levels in extended families with type 2 diabetes. Diabetes 2006;55:3358–65. [PubMed: 17130480]

6. Chen J, Muntner P, Hamm LL, Jones DW, Batuman V, Fonseca V, Whelton PK, He J, et al. The Metabolic Syndrome and Chronic Kidney Disease in U.S. Adults. Ann Intern Med 2004;140:167–174. [PubMed: 14757614]

7. De Cosmo S, Trevisan R, Minenna A, Vedovato M, Viti R, Santini S, Dodesini A, Fioretto, Trischitta V. Insulin Resistance and the Cluster of Abnormalities Related to the Metabolic Syndrome Are Associated With Reduced Glomerular Filtration Rate in Patients With Type 2. Diabetes Care 2006;29:432–434. [PubMed: 16443904]

8. Almind K, Doria A, Kahn CR. Putting the genes for type II diabetes on the map. Nat Med 2001;7:277–9. [PubMed: 11231616]

9. Maddux BA, Sbraccia P, Kumakura S, Sasson S, Youngren J, Fisher A, Spencedr S, Grupe A, Henzel W, Stewart TA, Reaven GM, Goldfine ID. Membrane glycoprotein PC-1 and insulin resistance in non-insulin-dependent diabetes mellitus. Nature 1995;373:448–451. [PubMed: 7830796]

10. Costanzo BV, Trischitta V, Di Paola R, Spampinato D, Pizzuti A, Vigneri R, Frittitta L. The Q allele variant (GLN121) of membrane glycoprotein PC-1 interacts with the insulin receptor and inhibits insulin signaling more effectively than the common K allele variant (LYS121). Diabetes 2001;50:831–6. [PubMed: 11289049]

11. Pizzuti A, Frittitta L, Argiolas A, Baratta R, Goldfine ID, Bozzali M, Ercolino T, Scarlato G, Iacoviello L, Vigneri R, Tassi V, Trischitta V. A polymorphism (K121Q) of the human glycoprotein PC-1 gene coding region is strongly associated with insulin resistance. Diabetes 1999;48:1881–1884. [PubMed: 10480624]

12. Frittitta L, Baratta R, Spampinato D, Di Paola R, Pizzuti A, Vigneri R, Trischitta V. The Q121 PC-1 variant and obesity have additive and independent effects in causing insulin resistance. J Clin Endocrinol Metab 2001;86:5888–91. [PubMed: 11739459]

13. Kubaszek A, Pihlajamaki J, Karhapaa P, Vauhkonen I, Laakso M. The K121Q polymorphism of the PC-1 gene is associated with insulin resistance but not with dyslipidemia. Diabetes Care 2003;26:464–7. [PubMed: 12547881]

14. Abate N, Carulli L, Cabo-Chan A, Chandalia M, Snell PG, Grundy SM. Genetic polymorphism PC-1 K121Q and ethnic susceptibility to insulin resistance. J Clin Endocrinol Metab 2003;88:5927–5934. [PubMed: 14671192]

15. Gonzalez-Sanchez JL, Martinez-Larrad MT, Fernandez-Perez C, Kubaszek A, Laakso M, Serrano-Rios M. K121Q PC-1 gene polymorphism is not associated with insulin resistance in a Spanish population. Obes Res 2003;11:603–605. [PubMed: 12740448]

16. Gu HF, Almgren P, Lindholm E, Frittitta L, Pizzuti A, Trischitta V, Groop LC. Association between the human glycoprotein PC-1 gene and elevated glucose and insulin levels in a paired-sibling analysis. Diabetes 2000;49:1601–1603. [PubMed: 10969847]

17. McAteer JB, Prudente S, Bacci S, Lyon HN, Hirschhorn JN, Trischitta V, Florez JC. for the ENPP1 Consortium. The ENPP1 K121Q polymorphism is associated with type 2 diabetes in European populations: evidence from an updated meta-analysis in 42,042 subjects. Diabetes 2008;57:1125–30. [PubMed: 18071025]

18. Endler G, Mannhalter C, Sunder-Plassmann H, Schillinger M, Klimesch A, Exner M, Kapiotis S, Meier S, Kunz F, Raiger E, Huber K, Wagner O, Sunder-Plassmann R. The K121Q polymorphism

in the plasma cell membrane glycoprotein gene predisposes to early myocardial infarction. J Mol Med 2002;80:791–795. [PubMed: 12483464]

19. Bacci S, Ludovico O, Prudente S, Zhang YY, Di Paola R, Mangiacotti D, Rauseo A, Nolan D, Duffy J, Fini G, Salvemini L, Amico C, Vigna C, Pellegrini F, Menzaghi C, Doria A, Trischitta V. The K121Q polymorphism of the ENPP1/PC-1 gene is associated with insulin resistance/atherogenic phenotypes, including earlier onset of type 2 diabetes and myocardial infarction. Diabetes 2005;54:3021–5. [PubMed: 16186408]
20. De Cosmo S, Argiolas A, Miscio G, Thomas S, Piras GP, Trevisan R, Cavallo Perin P, Bacci S, Zucaro L, Margaglione M, Frittitta L, Pizzuti A, Tassi V, Viberti GC, Trischitta VA. PC-1 aminoacid variant (K121Q) is associated with faster progression of renal disease in patients with type 1 diabetes and albuminuria. Diabetes 2000;49:521–524. [PubMed: 10868979]
21. Canani LH, Ng DP, Smiles A, Rogus JJ, Warram JH, Krolewski AS. Polymorphism in ecto-nucleotide pyrophosphatase/phosphodiesterase 1 gene (ENPP1/PC-1) and early development of advanced diabetic nephropathy in type 1 diabetes. Diabetes 2002;51:1188–93. [PubMed: 11916943]
22. Jacobsen P, Grarup N, Tarnow L, Pedersen O, Parving HH. PC-1 amino acid variant (K121Q) has no impact on progression of diabetic nephropathy in type 1 diabetic patients. Nephrol Dial Transplant 2002;17:1408–1412. [PubMed: 12147787]
23. De Cosmo S, Trevisan R, Della Vestra M, Vedovato M, Argiolas A, Solini A, Saller A, Damone F, Tiengo A, Trischitta V, Fioreto P. PC-1 Amino Acid Variant Q121 Is Associated With a Lower Glomerular Filtration Rate in Type 2 Diabetic Patients With Abnormal Albumin Excretion Rates. Diabetes Care 2003;26:2898–902. [PubMed: 14514598]
24. Boonyasrisawat W, Eberle D, Bacci S, Zhang YY, Nolan D, Gervino EV, Johnstone MT, Trischitta V, Shoelson SE, Doria A. Tag polymorphisms at the A20 (TNFAIP3) locus are associated with lower gene expression and increased risk of coronary artery disease in type 2 diabetes. Diabetes 2007;56:499–505. [PubMed: 17259397]
25. De Cosmo S, Minenna A, Ludovico O, Mastroianno S, Di Giorgio A, Pirro L, Trischitta V. Increased urinary albumin excretion, insulin resistance, and related cardiovascular risk factors in patients with type 2 diabetes. Diabetes Care 2005;28:910–915. [PubMed: 15793194]
26. Levey AS, Greene T, Kusek JW, Beck GJ. A simplified equation to predict glomerular filtration rate from serum creatinine. J Am Soc Nephrol 2000;11:155A.
27. National Kidney Foundation. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. Am J Kidney Dis 2007;49(suppl 2):S1–S180.
28. Higgins JP, Whitehead A, Turner RM, Omar RZ, Thompson SG. Meta-analysis of continuous outcome data from individual patients. Stat Med 2001;20:2219–41. [PubMed: 11468761]
29. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med 2004;351:1296–305. [PubMed: 15385656]
30. Murussi M, Gross JL, Silveiro SP. Glomerular filtration rate changes in normoalbuminuric and microalbuminuric Type 2 diabetic patients and normal individuals A 10-year follow-up. J Diabetes Complications 2006;20:210–5. [PubMed: 16798471]
31. Gaede P, Tarnow L, Vedel P, Parving HH, Pedersen O. Remission to normoalbuminuria during multifactorial treatment preserves kidney function in patients with type 2 diabetes and microalbuminuria. Nephrol Dial Transplant 2004;19:2784–8. [PubMed: 15328385]
32. Rossing K, Christensen PK, Hovind P, Tarnowe L, Rossing P, Parving H-H. Progression of nephropathy in type 2 diabetic patients. Kidney Intern 2004;66:1596–1605.
33. Retnakaran R, Cull CA, Thorne KI, Adler AI, Holman RR. for the UKPDS Study Group. Risk Factors for Renal Dysfunction in Type 2 Diabetes U.K. Prospective Diabetes Study 74. Diabetes 2006;55:1832–1839. [PubMed: 16731850]
34. Placha G, Canani LH, Warram JH, Krolewski AS. Evidence for different susceptibility genes for proteinuria and ESRD in type 2 diabetes. Adv Chronic Kidney Dis 2005;12:155–69. [PubMed: 15822051]
35. Fioretto P, Mauer M, Brocco E, Velussi M, Frigato F, Muollo B, Sambataro M, Abaterusso C, Baggio B, Crepaldi G, Nosadini R. Patterns of renal injury in NIDDM patients with microalbuminuria. Diabetologia 1996;39:1569–1576. [PubMed: 8960844]

36. Bacci S, Menzaghi, Di Paola R, Di fluvio P, Di Silvestre S, Mastroianno S, Formoso G, Consoli A, Pandolfi A, Trischitta V. The ENPP1 Q121 Variant plays a role in the susceptibility to increased pulse pressure. Diabetes 2008;57 (suppl 1):A205.
37. Meyre D, Bouatia-Naji N, Tounian A, Samson C, Lecoeur C, Vatin V, Ghoussaini M, Wachter C, Hercberg S, Charpentier G, Patsch W, Pattou F, Charles MA, Tounian P, Clement K, Jouret B, Weill J, Maddux BA, Goldfine ID, Walley A, Boutin P, Dina C, Froguel P. Variants of ENPP1 are associated with childhood and adult obesity and increase the risk of glucose intolerance and type 2 diabetes. Nat Genet 2005;37:863–7. [PubMed: 16025115]
38. Bochenski J, Placha G, Wanic K, Malecki M, Sieradzki J, Warram JH, Krolewski AS. New polymorphism of ENPP1 (PC-1) is associated with increased risk of type 2 diabetes among obese individuals. Diabetes 2006;55:2626–30. [PubMed: 16936213]
39. Abate N, Chandalia M, Satija P, Adams-Huet B, Grundy SM, Sandeep S, Radha V, Deepa R, Mohan V. ENPP1/PC-1 K121Q polymorphism and genetic susceptibility to type 2 diabetes. Diabetes 2005;54:1207–13. [PubMed: 15793263]
40. Chudleigh RA, Dunseath G, Evans W, Harvey JN, Evans P, Ollerton R, Owens DR. How Reliable Is Estimation of Glomerular Filtration Rate at Diagnosis of Type 2 Diabetes? Diabetes Care 2007;30:300–305. [PubMed: 17259498]
41. Rigalleau V, Lasseur C, Raffaitin C, Beauvieux MC, Barthe N, Chauveau P, Combe C, Gin H. Normoalbuminuric renal insufficient diabetic patients: A lower risk group. Diabetes Care 2007;30:2034–2039. [PubMed: 17485574]
42. Böttcher Y, Körner A, Reinehr T, Enigk B, Kiess W, Stumvoll M, Kovacs P. ENPP1 variants and haplotypes predispose to early onset obesity and impaired glucose and insulin metabolism in German obese children. J Clin Endocrinol Metab 2006;91:4948–52. [PubMed: 16968801]
43. Bochenski J, Placha G, Wanic K, Malecki M, Sieradzki J, Warram JH, Krolewski AS. New polymorphism of ENPP1 (PC-1) is associated with increased risk of type 2 diabetes among obese individuals. Diabetes 2006;55:2626–30. [PubMed: 16936213]
44. Lyon HN, Florez JC, Bersaglieri T, Saxena R, Winckler W, Almgren P, Lindblad U, Tuomi T, Gaudet D, Zhu X, Cooper R, Ardlie KG, Daly MJ, Altshuler D, Groop L, Hirschhorn JN. Common variants in the ENPP1 gene are not reproducibly associated with diabetes or obesity. Diabetes 2006;55:3180–4. [PubMed: 17065359]
45. Weedon MN, Shields B, Hitman G, Walker M, McCarthy MI, Hattersley AT, Frayling TM. No evidence of association of ENPP1 variants with type 2 diabetes or obesity in a study of 8,089 U.K. Caucasians. Diabetes 2006;55:3175–9. [PubMed: 17065358]
46. Meyre D, Bouatia-Naji N, Vatin V, Veslot J, Samson C, Tichet J, Marre M, Balkau B, Froguel P. ENPP1 K121Q polymorphism and obesity, hyperglycaemia and type 2 diabetes in the prospective DESIR Study. Diabetologia 2007;50:2090–6. [PubMed: 17704904]
47. Keene KL, Mychaleckyj JC, Smith SG, Leak TS, Perlegas PS, Langefeld CD, Freedman BI, Rich SS, Bowden DW, Sale MM. Association of the distal region of the Ectonucleotide Pyrophosphatase/Phosphodiesterase 1 (ENPP1) gene with type 2 diabetes in an African American population enriched for nephropathy. Diabetes 2008:db07-0886.

Table 1
Clinical features of diabetic patients in whole samples and across *ENNP1* genotype groups

KK: patients carrying the K121/K121 genotype; KQ: patients carrying the K121/Q121 genotype; QQ: patients carrying the Q121/Q121 genotype

	Whole sample	KK	KQ	QQ
GARGANO sample				
Male/Female	360/324	253/228	91/86	16/10
Age (yrs)	61.3 ± 9.6	61.1± 9.7	61.8 ± 9.0	61.9± 10.1
Duration of diabetes (yrs)	10.7 ± 8.9	10.6 ± 9.1	11.1 ± 8.5	10.8 ± 9.6
BMI (Kg/m^2)	30.9± 5.7	31.0 ± 5.6	31.0 ± 5.8	30.0 ± 4.6
HbA1c (%)	8.7 ± 2.0	8.6 ±2.0	8.8 ± 2.0	8.4 ± 1.8
Hypertension (%)	84	83	86	89
Normo-/Micro-/Macro-albuminuria (%)	70/25/5	71/25/4	71/25/4	63/37/0
BOSTON sample				
Male/Female	361/222	256/152	99/61	6/9
Age (yrs)	64.7 ± 6.9	64.7 ± 7.0	64.7 ± 6.8	64.4 ± 5.5
Duration of diabetes (yrs)	12.9 ± 7.6	12.8 ± 7.4	13.2 ± 8.1	13.1 ± 7.3
BMI (Kg/m^2)	32.2 ± 6.1	32.0 ± 6.5	32.6 ± 5.4	31.1 5.1
HbA1c (%)	7.4 ± 1.3	7.4 ± 1.3	7.4 ± 1.3	7.3 ± 0.9
Hypertension (%)	81	79	83	79
Normo-/Micro-/Macro-albuminuria (%)	N.A.	N.A.	N.A.	N.A.
PADUA sample				
Male/Female	100/25	73/14	24/11	3/0
Age (yrs)	56.7 ± 8.3	55.8 ± 8.5	58.9 ± 7.6	56.3 ± 6.0
Duration of diabetes (yrs)	11.4 ± 7.3	11.1 ± 6.9	11.9 ± 8.4	11.7 ± 5.7
BMI (Kg/m^2)	28.4 ± 4.1	28.3 ± 4.3	28.4 ± 3.7	30.3 ± 3.0
HbA1c (%)	8.5 ± 1.7	8.6 ± 1.8	8.4 ± 1.7	8.4 ± 0.6
Hypertension (%)	94	93	94	100
Normo-/Micro-/Macro-albuminuria (%)	0/37/73	0/33/67	0/49/51	0/0/100

BMI: body mass index

HbA1c: glycated haemoglobin

UAE: urinary albumin excretion

Table 2

GFR values of diabetic patients in whole samples and across *ENNP1* genotype groups

	Whole sample	KK	KQ	QQ	*KQ+QQ*
Gargano sample	**(n=684)**	**(n=481)**	**(n=177)**	**(n=26)**	**(n=203)**
GFR (ml/min/1.73m^2)	74 (8–144)	75 ±18	72 ± 20	73 ± 23	*72 ± 20*^
Boston sample	**(n=583)**	**(n=408)**	**(n=160)**	**(n=15)**	**(n=175)**
GFR (ml/min/1.73m^2)	70 (7–145)	71 ± 20	69 ± 22	72 ± 23	*69 ± 22**
Padua sample	**(n=125)**	**(n=87)**	**(n=35)**	**(n=3)**	**(n=38)**
GFR (ml/min/1.73m^2)	78 (28–154)	79 ± 24	75 ± 25	75 ± 19	*75 ± 24*^^
All samples	**(n=1392)**	**(n=976)**	**(n=372)**	**(n=44)**	**(n=416)**
GFR (ml/min/1.73m^2)	73 (7–154)	74 ± 20	71 ± 21	73 ± 22	*71 ± 21***

Data are given as mean ± SD or mean and range in parenthesis

KK: patients carrying the K121/K121 genotype; KQ: patients carrying the K121/Q121 genotype; QQ: patients carrying the Q121/Q121 genotype

GFR: glomerular filtration rate (GFR conversion factor for SI unit is 0.01667)

^ p=0.05 KQ+QQ vs KK patients

* p=0.5 KQ+QQ vs KK patients

^^ p= 0.7 KQ+QQ vs KK patients

** p=0.04 KQ+QQ vs KK patients

All statistical analyses are adjusted for age, sex, duration of diabetes, HbA1c, hypertension, BMI, and **place of recruitment

Am J Kidney Dis. Author manuscript; available in PMC 2010 September 16.

Am J Kidney Dis. Author manuscript; available in PMC 2010 September 16.

Table 3

Patients without and with reduced GFR across *ENNP1* genotype groups

							Genetic model		
							Additive	**Recessive**	**Dominant**
Panel A – Gargano sample	**KK**		**KQ**		**QQ**		**OR**	**OR**	**OR**
	n	**%**	**n**	**%**	**n**	**%**	**(95%C.I.)**	**(95%C.I.)**	**(95%C.I.)**
Reduced GFR − n	408	72	137	24	22	4	1.33 (1.0–1.9) P=0.1	0.88 (0.30–2.6) P=0.8	1.55 (1.0–2.3) P=0.04
							1.43 (1.0–2.1)^ P=0.07	0.83 (0.2–2.8)^ P=0.8	1.69 (1.1–2.6)^ P=0.02
Reduced GFR + n	73	62	40	34	4	4			
Panel B – Boston sample	**KK**		**KQ**		**QQ**		**OR**	**OR**	**OR**
	n	**%**	**n**	**%**	**n**	**%**	**(95%C.I.)**	**(95%C.I.)**	**(95%C.I.)**
Reduced GFR − n	291	72	99	25	11	3	1.32 (0.9–1.8) P=0.1	0.80 (0-3-2.5) P=0.8	1.47 (1.0–2.1) P=0.04
							1.39 (1.0–2.0)^ P=0.08	1.04 (0.3–3.9)^ P=0.9	1.50 (1.0–2.2)^ P=0.05
Reduced GFR + n	117	64	61	34	4	2			
Panel C – Padua sample	**KK**		**KQ**		**QQ**		**OR**	**OR**	**OR**
	n	**%**	**n**	**%**	**n**	**%**	**(95%C.I.)**	**(95%C.I.)**	**(95%C.I.)**
Reduced GFR − n	70	73	24	25	2	2	1.72 (0.8–3.7) P=0.2	1.68 (0.2–19.2) P=0.7	1.90 (0.80–4.5) P=0.1
							1.66 (0.7–3.8)^ P=0.2	3.4 (0.3–45)^ P=0.4	1.77 (0.7–4.5)^ P=0.2
Reduced GFR + n	17	59	11	38	1	3			
Panel D – All samples	**KK**		**KQ**		**QQ**		**OR**	**OR**	**OR**
	n	**%**	**n**	**%**	**n**	**%**	**(95%C.I.)**	**(95%C.I.)**	**(95%C.I.)**
Reduced GFR − n	769	72	260	25	35	3	1.34 (1.1–1.7) P=0.01	0.83 (0.4–1.7) P=0.622	1.52 (1.2–2.0) P=0.002
							1.41 (1.1–1.8)^^ P=0.006	0.98 (0.4–2.2)^^ P=0.9	1.58 (1.2–2.1)^^ P=0.002
Reduced GFR + n	207	63	112	34	9	3			

KK: patients carrying the K121/K121 genotype; KQ: patients carrying the K121/Q121 genotype; QQ: patients carrying the Q121/Q121 genotype;

GFR: glomerular filtration rate (GFR conversion factor for SI unit is 0.01667)

Reduced GFR − = patients with GFR ≥ 60 ml/min/1.73m^2

Reduced GFR + = patients with GFR < 60 ml/min/1.73m^2

OR (95% C.I.): odds ratio (95% confidence interval);

^ adjusted for age, sex, duration of diabetes, HbA1c, hypertension, BMI;

^^ adjusted also for place