The K121Q Polymorphism of the PC-1 Gene Is Associated With Insulin Resistance but not With Dyslipidemia

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OBJECTIVE — To investigate the relationship of the K121Q polymorphism of the plasma cell glycoprotein 1 (PC-1) gene with insulin resistance, insulin secretion, and lipids and lipoproteins.

RESEARCH DESIGN AND METHODS — Altogether, 110 normoglycemic subjects (group I) underwent a hyperinsulinemic-euglycemic clamp for evaluation of insulin sensitivity. The first-phase insulin secretion was determined by the intravenous glucose tolerance test (IVGTT) in a separate sample of 295 normoglycemic subjects (group II).

RESULTS — The 121Q allele (genotypes K121Q and Q121Q) compared with the K121K genotype was related to higher fasting insulin levels (group I: 69.6 ± 45.6 vs. 51.9 ± 28.4 pmol/l [mean ± SD], $P = 0.050$; group II: 66.6 ± 38.8 vs. 53.8 ± 26.6 pmol/l, $P = 0.009$). In group I, subjects carrying the 121Q allele compared with subjects with the K121K genotype had lower rates of whole-body glucose uptake (51.17 ± 12.07 vs. 60.12 ± 14.86 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P = 0.012$) and nonoxidative glucose disposal (33.71 ± 10.51 vs. 41.51 ± 13.36 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P = 0.015$) during the clamp. In group II, there was no significant difference between the 121Q allele carriers and subjects with the K121K genotype in total first-phase insulin secretion during the first 10 min of the IVGTT (2,973 ± 2,224 vs. 2,520 ± 1,492 $pmol \cdot l^{-1} \cdot min^{-1}$, $P = 0.415$). No association of the K121Q polymorphism with serum lipids and lipoproteins was found.

CONCLUSIONS — In healthy normoglycemic Finnish subjects, the K121Q polymorphism of the PC-1 gene is associated with insulin resistance but not with impaired insulin secretion or dyslipidemia.

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Type 2 diabetes is an inherited disorder characterized by defects in insulin secretion and insulin action. However, the genetic basis of type 2 diabetes is known only in rare cases (1). Plasma cell glycoprotein 1 (PC-1, ENPP1) is a promising candidate gene for type 2 diabetes. It may inhibit the insulin receptor (IR) by interacting directly with the α subunit of the IR (2). PC-1 binds to the connecting domain of the IR, which moves the two β subunits together, transactivating them (3,4). Thus, PC-1 inhibits autophosphorylation of the IR (2) and impairs insulin signaling downstream of the IR (5). Accordingly, human studies show an association of increased adipose tissue (6) and skeletal muscle (7) PC-1 content and decreased plasma levels of the soluble form of PC-1 (8) with insulin resistance. The 121Q variant (Gln121) in exon 4 of the PC-1 gene has been shown to interact with the IR, and it has a greater inhibitory action on the IR than the 121K allele variant (Lys121) (9). Indeed, the K121Q genotype has been shown to be associated with insulin resistance in Caucasian Sicilians (10) and with higher glucose and insulin levels in Finnish and Swedish populations (11). The K121Q variant was not associated with type 2 diabetes in Danish Caucasians (12) or in Oji Cree (13). The association of the K121Q polymorphism with insulin sensitivity was previously demonstrated in only one study applying the euglycemic clamp technique (10), a gold standard for the measurement of insulin sensitivity. However, in that study, indirect calorimetry, allowing the evaluation of oxidative and nonoxidative glucose disposal, was not combined with the clamp. Furthermore, the association of the K121Q polymorphism with insulin secretion or with dyslipidemias has not been previously studied. Therefore, we investigated whether the K121Q polymorphism of the PC-1 gene is related to insulin sensitivity, insulin secretion, or lipid and lipoprotein levels in normoglycemic Finns.

RESEARCH DESIGN AND METHODS

Subjects

The first study group (group I) consisted of 110 unrelated healthy normoglycemic subjects from our two previous population studies (14,15) whose DNA was available (82 men and 28 women, age 52 ± 8 years, BMI 26.4 ± 4.1 kg/m^2). A hyperinsulinemic-euglycemic clamp and indirect calorimetry were performed on these subjects to determine insulin sensitivity). Subjects were randomly selected among healthy Finns, and the protocol of both population studies was identical. In addition, in a separate sample of 295 healthy normoglycemic subjects (group II) (150 men and 145 women, age 44 ± 12 years, BMI 25.6 ± 3.7 kg/m^2), an intravenous glucose tolerance test (IVGTT) was performed to determine their first-phase insulin secretion (16). All study subjects (groups I and II, $n = 405$) had normal glucose tolerance according to the

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Abbreviations: AUC, area under the curve; IR, insulin receptor; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Characteristics of subjects studied according to the K121Q polymorphism of the PC-1 gene in subjects belonging to group I (euglycemic clamp) and group II (IVGTT)*

	Group I			Group II		
	K121K	K121Q and Q121Q	*P*	K121K	K121Q and Q121Q	*P*
n	88	22	—	240	55	—
Age	50 ± 8	54 ± 6	0.044	44 ± 12	44 ± 12	NS
BMI (kg/m^2)	25.8 ± 3.6	27.4 ± 3.3	NS	25.6 ± 3.7	25.6 ± 3.7	NS
Systolic blood pressure (mmHg)	132 ± 14	136 ± 12	NS	130 ± 16	129 ± 15	NS
Diastolic blood pressure (mmHg)	84 ± 7	85 ± 7	NS	85 ± 10	85 ± 10	NS
Fasting glucose (mmol/l)	5.4 ± 0.5	5.9 ± 0.5	0.002	5.2 ± 0.6	5.2 ± 0.6	NS
Fasting insulin (pmol/l)	51.9 ± 28.4	69.6 ± 45.6	0.050	53.8 ± 26.6	66.6 ± 38.8	0.009
AUC glucose (mmol · l^{-1} · min^{-1})	731 ± 140	811 ± 176	0.034	717 ± 157	710 ± 159	NS
AUC insulin (pmol · l^{-1} · min^{-1})	29,418 ± 21,660	44,238 ± 43,651	0.087	31,712 ± 21,014	35,735 ± 24,348	NS
Total cholesterol (mmol/l)	5.77 ± 1.15	5.80 ± 1.04	NS	6.07 ± 1.05	5.93 ± 1.16	NS
HDL cholesterol (mmol/l)	1.34 ± 0.33	1.29 ± 0.26	NS	1.45 ± 0.33	1.42 ± 0.32	NS
Triglycerides (mmol/l)	1.35 ± 1.06	1.49 ± 0.61	NS	1.46 ± 0.80	1.51 ± 1.02	NS

Data are means ± SD. AUC indicates the area under the curve in the oral glucose tolerance test.

World Health Organization criteria (17), and they did not have any chronic diseases or continuous drug treatment that could affect carbohydrate metabolism.

Research design

The study protocol was approved by the Ethics Committee of the University of Kuopio. All subjects underwent an oral glucose tolerance test (OGTT) (75 g glucose), and concentrations of plasma glucose and insulin in the fasting state and at 60 and 120 min of OGTT were measured using standard methods. Subjects from group I participated in the euglycemic-hyperinsulinemic clamp (insulin infusion rate 480 pmol · m^{-2} body surface area · min^{-1}, blood glucose level was clamped at 5.0 mmol/l with an intravenous infusion of 20% glucose solution) and indirect calorimetry (measurement of O_2 consumption and CO_2 production, during the fasting state and last 30 min of the clamp) to evaluate energy expenditure, the degree of insulin sensitivity, and oxidative and nonoxidative glucose disposal as previously described (15). The rate of glucose oxidation was calculated according to the formula by Ferrannini and colleagues (18). Subjects from group II underwent an IVGTT (an intravenous injection of a glucose bolus of 0.3 g glucose/kg) after a 12-h overnight fast. The first-phase insulin secretion was estimated by calculating the area under the curve (AUC) for insulin response during the first 10 min of the IVGTT (samples taken at 4, 6, 8, and 10 min after the glucose bolus). Serum lipids and lipoproteins were measured using standard methods (19).

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by the proteinase K-phenol-chloroform extraction method. Exon 4 of the PC-1 gene was amplified by polymerase chain reaction with forward primer 5′-CTGTGTTCACTTTG GACATGTTG-3′ and reverse primer 5′-GACGTTGGAAGATACCAGGTTG-3′ (10). The reaction was performed in a total volume of 20 μl containing 50 ng genomic DNA, primers (0.5 μmol/μl), 0.375 units DNA polymerase (DynaZyme, Finnzymes, Espoo, Finland), and 100 μmol/l dNTP. PCR conditions were as follows: denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 62°C for 40 s, and extension at 72°C for 40 s with a final extension at 72°C for 4 min. The K121Q polymorphism was screened by the *Eco*47I restriction enzyme, followed by PAGE of the digested PCR products.

Statistical analysis

Data were analyzed with the SPSS/Win program (version 10.0; SPSS, Chicago). Data are given as means ± SD. The Student's *t* test for independent samples and ANOVA with covariates were used to compare the effect of the polymorphism on continuous variables. Triglyceride and insulin values were log-transformed before statistical analyses to achieve a normal distribution.

RESULTS — The frequency of the 121Q allele was 10.5% in group I and 9.8% in group II and did not differ from previous reports (10–13). The frequencies of genotypes in both groups (group I: K121K 80.0%, K121Q 19.1%, Q121Q 0.9%; group II: K121K 81.4%, K121Q 17.6%, Q121Q 1.0%) were in Hardy-Weinberg equilibrium. The subjects with the Q121Q genotype were combined with the K121Q genotype in all statistical analyses because of the small number of these subjects (4 among 405 subjects). BMI, systolic and diastolic blood pressure, and lipids and lipoproteins did not differ between subjects with the K121K genotype and the subjects with the 121Q allele in both groups, but subjects carrying the Q allele in group II were older ($P = 0.044$) (Table 1).

In group I, age- and sex-adjusted fasting plasma glucose levels (5.9 ± 0.5 vs. 5.4 ± 0.5 mmol/l, $P = 0.002$), glucose AUC (811 ± 176 vs. 731 ± 140 mmol · l^{-1} · min^{-1}, $P = 0.034$), and fasting insulin levels (69.6 ± 45.6 vs. 51.9 ± 28.4 pmol/l, $P = 0.050$) were significantly higher in subjects with the 121Q allele than in the subjects with the K121K genotype. In group II, fasting insulin levels were significantly higher in subjects with the 121Q allele than in subjects with the K121K genotype (66.6 ± 38.8 vs. 53.8 ± 26.6 pmol/l, $P = 0.009$), but no differences in fasting glucose levels and glucose

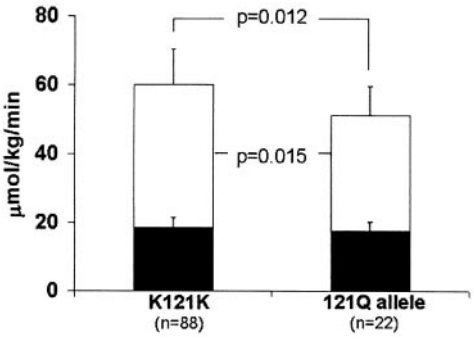

Figure 1—*Insulin sensitivity measured as the rates of whole-body glucose uptake, glucose oxidation (■), and nonoxidative glucose disposal (□) during the hyperinsulinemic-euglycemic clamp in 110 healthy normoglycemic subjects according to the K121Q polymorphism of the PC-1 gene.*

and insulin AUC in the OGTT were observed. When groups I and II were pooled (n = 405), age- and sex-adjusted fasting insulin levels (67.4 ± 40.6 vs. 53.3 ± 27.0 pmol/l, P = 0.001) and insulin AUC (38,164 ± 31,027 vs. 31,099 ± 21,180 pmol · l^{-1} · min^{-1}, P = 0.024) were higher in subjects with the 121Q allele than in subjects with the K121K genotype, but no difference between the groups was observed with respect to fasting and 2-h glucose levels or glucose AUC. Further adjustment for BMI did not change the results (P value for fasting insulin 0.001, and for insulin AUC 0.025).

In group I, the age- and sex-adjusted rates of whole-body glucose uptake were lower in subjects with the 121Q allele than in subjects with the K121K genotype (51.17 ± 12.07 vs. 60.12 ± 14.86 µmol · kg^{-1} · min^{-1}, P = 0.012, Fig. 1). In these subjects, the PC-1 polymorphism affected the rates of nonoxidative glucose disposal (33.71 ± 10.51 vs. 41.51 ± 13.36 µmol · kg^{-1} · min^{-1}, P = 0.015) but did not significantly affect the rates of glucose oxidation (17.47 ± 3.89 vs. 18.58 ± 3.19 µmol · kg^{-1} · min^{-1}, P = 0.148). The results remained essentially similar if the rates of whole-body glucose uptake were expressed as µmol · m^{-2} · min^{-1} (the 121Q allele versus the K121K genotype 2,127 ± 435 vs. 2,410 ± 519 µmol · m^{-2} · min^{-1}, P value after adjustment for age and sex 0.014, and after adjustment for age, sex, and BMI 0.045). Free fatty acid levels in the fasting state and during the clamp, the rates of lipid oxidation, the respiratory quotient, and the energy expenditure were similar in subjects with the K121K genotype and in subjects with the 121Q allele (data not shown).

In group II, first-phase insulin secretion, measured as insulin concentrations at 4 min (422.4 ± 323.6 vs. 355.9 ± 220.5 pmol/l, P = 0.360) and total insulin AUC during the first 10 min of the IVGTT (2,973 ± 2,224 vs. 2,520 ± 1,492 pmol · l^{-1} · min^{-1}, P = 0.415, Fig. 2) and insulin AUC above basal fasting insulin (2,408 ± 2007 vs. 2014 ± 1,381 pmol · l^{-1} · min^{-1}, P = 0.386), was not significantly different in subjects with the 121Q allele compared with the subjects with the K121K genotype. Similarly, total glucose AUC during the first 10 min of the IVGTT (103.9 ± 10.2 vs. 104.4 ± 10.6 mmol · l^{-1} · min^{-1}, P = 0.772) did not differ between the groups.

CONCLUSIONS — This study shows that subjects with the 121Q allele of the PC-1 gene had lower insulin sensitivity measured with the hyperinsulinemic-euglycemic clamp than subjects with the K121K genotype. Moreover, our findings imply that although PC-1 impairs insulin signaling at the receptor level, in subjects with the 121Q allele, nonoxidative glucose disposal may be more severely affected than glucose oxidation. However, the rates of glucose oxidation were also decreased, albeit not significantly, and therefore our results need confirmation in other studies. Finally, the lack of differences between the subjects with the 121Q allele and K121K genotype in energy expenditure, respiratory quotient, lipid oxidation, and free fatty acid levels suggests that substrate oxidation and energy metabolism were not significantly affected in subjects with the 121Q allele. Although there are several mechanisms via which the 121Q allele could induce insulin resistance, we cannot exclude the possibility that the effect of this PC-1 polymorphism on insulin sensitivity may be indirect, because the PC-1 gene can be in linkage disequilibrium with other functional polymorphisms.

The K121Q polymorphism of the PC-1 gene did not affect first-phase insulin secretion, as shown in the IVGTT (Fig. 2). In humans, no data on the expression of PC-1 in the pancreas have been reported, but in mice, no expression of PC-1 in the pancreatic tissue was observed (20). Although the role of the soluble form of PC-1 in the regulation of insulin secretion is unknown, it is unlikely that PC-1 affects insulin secretion directly.

In this study, the 121Q allele of the PC-1 gene was not associated with elevated serum triglycerides or low HDL cholesterol, which are typical components of the insulin resistance syndrome. The lack of the association between the K121Q polymorphism and lipids and lipoproteins could be related to different effects of PC-1 in skeletal muscle and adipose tissue. Indeed, PC-1 has been shown to regulate insulin signaling in skeletal muscle (7), but no association of PC-1 with IR tyrosine kinase activity (6,21) or glucose uptake (21) in adipocytes has been observed. Thus, our results suggest that the main site of action of PC-1 could be in skeletal muscle, which accounts for most of insulin-stimulated glucose disposal. However, this hypothesis needs further functional studies.

In conclusion, the present study indicates that the K121Q polymorphism of the PC-1 gene is associated with insulin

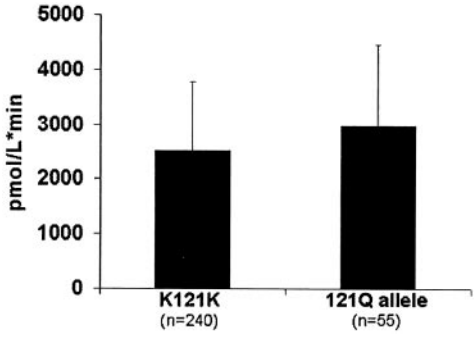

Figure 2—*Total insulin secretion measured as the AUC during the first 10 min of the IVGTT in a separate sample of 295 healthy normoglycemic subjects according to the K121Q polymorphism of the PC-1 gene.*

resistance in normoglycemic healthy Finns. No defect in insulin secretion was observed in subjects with the 121Q allele, and this allele did not affect the levels of serum lipids or lipoproteins in healthy subjects.

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