

A Polymorphism (K121Q) of the Human Glycoprotein PC-1 Gene Coding Region Is Strongly Associated With Insulin Resistance

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The genes responsible for insulin resistance are poorly defined. Plasma cell differentiation antigen (PC-1) glycoprotein inhibits insulin receptor signaling and is associated with insulin resistance. We describe here a novel polymorphism in exon 4 of the PC-1 gene (K121Q) and demonstrate that it is strongly associated with insulin resistance in 121 healthy nonobese (BMI <30 kg/m²) nondiabetic (by oral glucose tolerance test [OGTT]) Caucasians from Sicily. Compared with 80 KK subjects, Q allele carriers (*n* = 41, 39 KQ and 2 QQ) showed higher glucose and insulin levels during OGTT (*P* < 0.001 by two-way analysis of variance) and insulin resistance by euglycemic clamp (M value = 5.25 ± 1.38 [*n* = 24] vs. 6.30 ± 1.39 mg · kg⁻¹ · min⁻¹ [*n* = 49], *P* = 0.005). Q carriers had higher risk of being hyperinsulinemic and insulin resistant (odds ratio [CI]: 2.99 [1.28–7.0], *P* < 0.001). Insulin receptor autophosphorylation was reduced (*P* < 0.01) in cultured skin fibroblasts from KQ versus KK subjects. Skeletal muscle PC-1 content was not different in 11 KQ versus 32 KK subjects (33 ± 16.1 vs. 17.5 ± 15 ng/mg protein, *P* = 0.3). These results suggest a cause-effect relationship between the Q carrying genotype and the insulin resistance phenotype, and raise the possibility that PC-1 genotyping could identify individuals who are at risk of developing insulin resistance, a condition that predisposes to type 2 diabetes and coronary artery disease. *Diabetes* 48:1881–1884, 1999

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Additional information can be found in an on-line appendix at www.diabetes.org/diabetes/appendix.asp.

ANOVA, analysis of variance; OGTT, oral glucose tolerance test; PC-1, plasma cell differentiation antigen; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism.

Insulin resistance, a major characteristic of most patients with type 2 diabetes, also occurs in ~25% of healthy nonobese subjects (1–6). In both diabetic and nondiabetic subjects, insulin resistance is associated with an increased risk of dyslipidemia, hypertension, and coronary artery disease (1–6). Insulin resistance has a genetic predisposition, but the genes responsible are poorly known (4,5,7,8). Membrane glycoprotein plasma cell differentiation antigen (PC-1), when overexpressed, is an inhibitor of insulin receptor tyrosine kinase activity (9). PC-1 content is increased in muscle, fat, and fibroblasts of insulin-resistant subjects (10–13). Moreover, the amount of PC-1 overexpression is directly correlated with the amount of insulin resistance (10,13). Human PC-1 gene is located at chromosomal region 6q22–q23, where both STS D6S290 (linked to type 2 diabetes in Mexican Americans) (14), and the gene responsible for transient neonatal diabetes (15) have been mapped. We have now identified a polymorphic variant of PC-1 that is strongly associated with insulin resistance.

Four overlapping λ-wisconsin cosmid clones containing the PC-1 gene were isolated by screening a human chromosome 6 specific genomic library with human full-length PC-1 cDNA. Cosmids were digested with different four-base cutter restriction enzymes, blotted, and hybridized to 24-bp oligonucleotides based on the cDNA sequence (16). Positive fragments were cloned and sequenced. Intron-exon junctions were deduced comparing genomic and cDNA sequences. The full-length mRNA is transcribed from 25 exons (see on-line appendix at www.diabetes.org/diabetes/appendix.asp). All exons were analyzed in 40 unrelated and unselected individuals by single-strand conformation polymorphism (SSCP). Polymerase chain reaction (PCR) products showing different migration patterns at SSCP were cloned using a TA Cloning Kit (Invitrogen, Carlsbad, CA), and four clones for each sample were sequenced from both ends. Only exon 4 (nucleotide 447–571 of the cDNA) (16), which codes for an extracellular domain, showed a polymorphic variant. A frequent first-position A → C transversion at codon 121 (considering the second in-frame ATG as the start codon) was observed. This base change substitutes a glutamine for a lysine in a cysteine-rich region of PC-1 (K121Q; GenBank accession numbers AF067177 and AF067178. GenBank numbers of all other

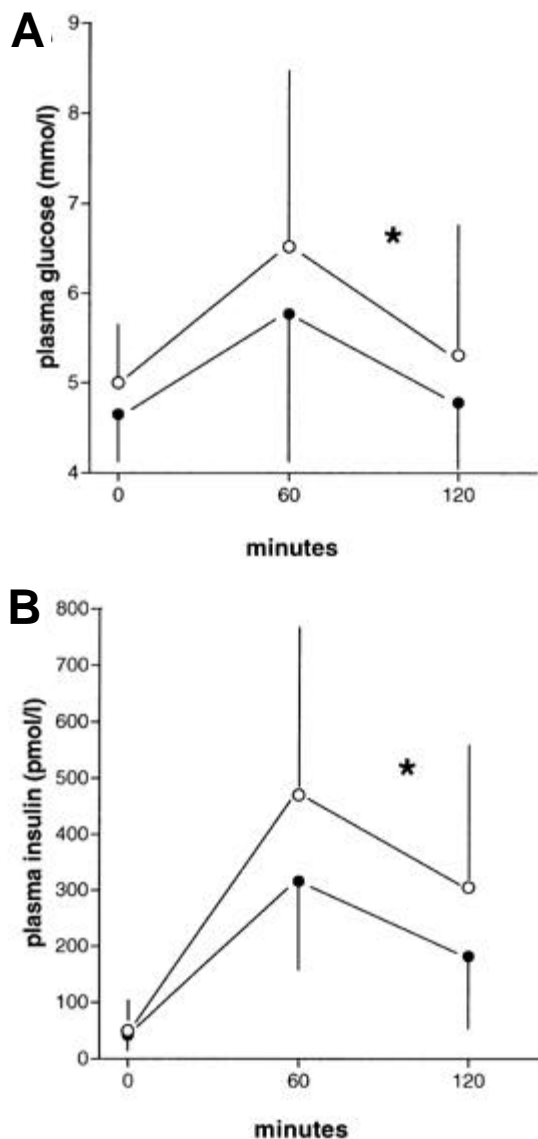


FIG. 1. Plasma glucose (A) and insulin (B) profiles during OGTT of KK subjects (●) and Q carriers (○). * $P < 0.001$ vs. KK subjects by two-way ANOVA.

exons are AF11280–AF110304) and creates an *Ava*II enzyme recognition site (Fig. 1). In 160 uncharacterized blood donors, the Q allele frequency was 17.5%, with genotype frequencies in agreement with the Hardy-Weinberg equilibrium.

We next investigated 121 nonobese (BMI < 30 kg/m²) normotensive (blood pressure $< 140/90$ mmHg) normal glucose tolerant (by oral glucose tolerance test [OGTT]) nonrelated white subjects who were residents of Sicily. Clinical charac-

teristics are given in Table 1. These individuals had a wide range of plasma insulin levels during the OGTT (Table 1), which in the presence of normal glucose tolerance indicates a wide range of insulin sensitivity (1,6,13). This wide range was confirmed by euglycemic-hyperinsulinemic clamp in a subgroup of 73 individuals; M values of insulin-stimulated glucose disposal ranged from 2.34 to 9.62 mg · kg⁻¹ · min⁻¹. We searched for an association between the K121Q polymorphism and insulin resistance.

Some 41 subjects were Q allele carriers, with a Q allele frequency of 17.8%. Q carriers showed higher glucose and insulin profiles during the OGTT ($P = 0.0002$ and $P < 0.0001$, respectively, two-way analysis of variance [ANOVA]) (Fig. 1). Insulin sensitivity was lower in Q carriers ($n = 24$) compared with that in KK subjects matched for age, sex, and BMI ($n = 49$) (M value = 5.25 ± 1.38 vs. 6.30 ± 1.39 mg · kg⁻¹ · min⁻¹, respectively, $P = 0.005$); similar insulin levels were observed at steady state: 485 ± 165 versus 460 ± 78 pmol/l. Blood pressure, serum HDL cholesterol, and triglyceride levels were not different between the two groups (data not shown). Total cholesterol was slightly higher in Q allele carriers (216 ± 42 vs. 199 ± 37 mg/dl, $P = 0.04$). There were two QQ allele subjects, both of whom were first-degree relatives of type 2 diabetic patients. One of them, a 25-year-old man, was studied by hyperinsulinemic clamp, and he had the lowest M value (2.34) in our series. All 25 exons from this QQ subject and from 2 KK individuals were screened by SSCP and sequencing. No further base change was detected.

Subjects were then subdivided into tertiles according to the plasma insulin levels at 120 min during the OGTT (tertiles 1 and 2: low insulin levels, tertile 3: high insulin levels), and the mean M values significantly decreased from tertile 1 to tertile 3 (7.04 ± 1.14 vs. 4.92 ± 1.21 mg · kg⁻¹ · min⁻¹, $P < 0.001$, one-way ANOVA). Q allele frequency was similar in subjects from tertiles 1 and 2 (11.2%, $n = 40$, and 12.2%, $n = 41$), but it was higher in tertile 3 insulin-resistant subjects (27.5%, $n = 40$) ($P = 0.009$, χ^2 in a 2×3 table). Based on these data, Q carriers had higher risk of being in tertile 3 (insulin resistant) than in tertile 1 (insulin sensitive) (odds ratio [CI]: 2.99 [1.28–7.0]).

Because type 2 diabetes is the most likely clinical outcome of insulin resistance (1–6), we also studied 132 white type 2 diabetic residents of Sicily (age 66.5 ± 8.0 years, 60 men, 72 women, BMI 28.9 ± 4.5 kg/m²) as a positive control group for insulin resistance. To minimize the possible inclusion of individuals affected by late-onset type 1 diabetes (5), these patients were selected on the basis of one first-degree relative with type 2 diabetes, age of diabetes onset > 45 years, BMI > 21 kg/m², and no need of insulin therapy. In these patients, Q allele frequency (20.8%) was intermediate between nondiabetic subjects of tertiles 2 and 3. There was no difference between

TABLE 1
Clinical and metabolic characteristics of the subjects studied

Genotype	n	Sex (M/F)	Age (years)	BMI (kg/m ²)	Plasma glucose (mmol/l)		Plasma insulin (pmol/l)	
					0	120	0	120
KK	80	45/35	36.6 ± 12.3	24.0 ± 3.0	4.6 ± 0.4	4.8 ± 1.0	42 ± 21	182 ± 161
KQ + QQ	39 + 2	26/15	38.2 ± 13.7	23.9 ± 3.2	$5.0 \pm 0.6^*$	5.3 ± 1.4	50 ± 34	$305 \pm 260^*$

Data are means \pm SD. Plasma glucose and insulin levels are from before (0) and 120 min after (120) OGTT. * $P < 0.01$ vs. KK subjects.

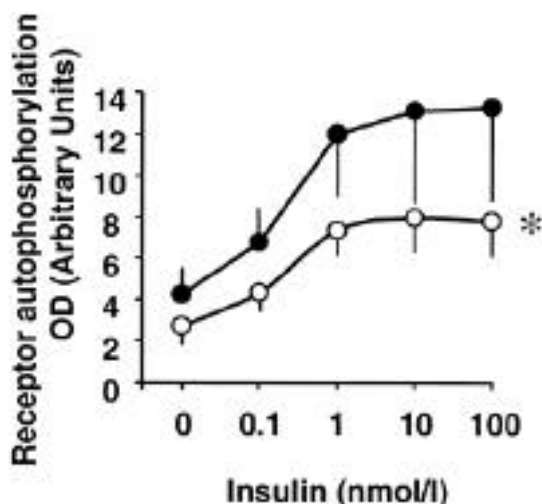


FIG. 2. Insulin receptor autophosphorylation in fibroblasts from five KQ (○) and five KK (●) subjects. After exposure to insulin, cells were solubilized, receptors were immunocaptured, and autophosphorylation was measured and normalized for protein content as described (13). * $P < 0.01$ vs. KK subjects. OD, optical densities.

obese (BMI >30 kg/m², $n = 92$, 21.1%) and nonobese ($n = 43$, 20.2%) diabetic patients.

Insulin receptor tyrosine kinase activity as measured by insulin receptor autophosphorylation was studied in cultured skin fibroblasts (13) from five QK and five matched KK subjects. Cells were selected on the basis of similar PC-1 protein content (13): 50.3 ± 8.7 and 60.8 ± 15.4 ng/0.1 mg protein. QK fibroblasts had reduced insulin receptor tyrosine kinase activity ($P < 0.01$, two-way ANOVA) (Fig. 2). No difference was observed in insulin receptor content and affinity as indicated by ¹²⁵I-labeled insulin binding studies (13).

PC-1 content (13) was not different in external oblique muscle (obtained at cholecystectomy), from 11 QK and 32 KK matched subjects (33 ± 16.1 vs. 27.5 ± 15 ng/mg protein, $P = 0.3$).

The data in this study demonstrate, therefore, that a PC-1 polymorphism is associated with insulin resistance. In our prior studies, we noted an increase in PC-1 content of muscle, fat, and fibroblasts of insulin-resistant subjects (9–13) that correlated with decreased insulin sensitivity (10,13). Because increased PC-1 content is also associated with decreased insulin receptor tyrosine kinase activity in cultured cells (13), it is likely that overexpression of PC-1 directly results in decreased insulin receptor function. In the present study, when QK fibroblasts were compared with KK fibroblasts with similar PC-1 content, the QK cells had reduced insulin receptor tyrosine kinase activity. Since these data were obtained after several passages in culture, the reduced insulin receptor tyrosine activity in QK allele cells is very likely genetic in origin and due to an enhanced effect of the Q allele on insulin receptor function. In the present study, we also found that PC-1 content was similar in muscle from QK and KK subjects, indicating that insulin resistance in Q carriers was not due to increased PC-1 expression. Taking these data together, we speculate that PC-1 may impair insulin receptor function and insulin sensitivity in different individuals by two different mechanisms. The first mechanism is overexpression of the common form of the PC-1 protein. The second mechanism is normal expression of the variant

PC-1 protein, which might be more potent in inhibiting insulin receptor tyrosine kinase activity. It is also possible that the PC-1 K121Q polymorphism might be in linkage disequilibrium with other mutations in the PC-1 gene and/or other genes that cause insulin resistance. In either case, our data suggest that PC-1 genotyping could identify individuals at risk for insulin resistance and its sequelae.

RESEARCH DESIGN AND METHODS

Subjects. There were 121 unrelated healthy white Sicilian subjects studied. By selection criteria, they were nonobese (BMI <30 kg/m²), normotensive (blood pressure $<140/90$ mmHg), and normal glucose tolerant (by OGTT). Subjects were on a weight-maintaining diet. Informed consent was obtained from all participants in the study, which was approved by the local ethics committee. Insulin-stimulated glucose disposal measurements by the euglycemic-hyperinsulinemic clamp (1) were performed with insulin infusion at a constant rate (1.0 mU · kg⁻¹ · min⁻¹) and a variable glucose infusion to maintain plasma glucose within 10% of baseline value.

Polymorphism screening. SSCP was performed as follows. Amplification reactions were performed for 35 cycles in 25 μ l containing 50 ng of genomic DNA, 50 pmol of each nucleotide primer, and 0.5 U of Taq polymerase (in 1.5 mmol/l MgCl₂, 10 mmol/l Tris-HCl, 50 mmol/l KCl, 0.1% Triton X-100, pH 8.8). PCR products were denatured for 5 min at 87°C in 90% formamide, 20 mmol/l EDTA, and 10 mmol/l NaOH. Samples were then chilled on ice, loaded on a native 8–12% (according to amplimers size) acrylamide (optimized from 19:1 to 29:1 acrylamide-bisacrylamide) gel (0.04 \times 20 \times 42 cm) (with or without 10% glycerol) in Tris borate EDTA buffer and electrophoresed at 10 W constant power for 13–16 h at room temperature. Gels were then treated by silver staining. PCR products showing different migration patterns at SSCP were cloned in a TA-cloning vector (Stratagene), and four clones for each sample were automatically sequenced from both ends using a Perkin Elmer ABI 373A automatic sequencer (Norwalk, CT). Exon 4 amplimers were obtained using oligonucleotides 4Fw (5'-ctgtgttcactctggacatgttg-3') and 4Rv (5'-gacgttggaagatacagggttg-3') as primers. PCR products were digested using *Ava*II restriction enzyme and run on 12% native polyacrylamide gel for 2 h at 500 V. Gels were then stained by silver nitrate. K alleles are displayed as single uncut bands of 238 bp, while Q alleles are shown as a doublet of 148 and 90 bp. All genotyping was carried out in duplicate for each individual, and the investigator was unaware of sample origin.

Skin fibroblast culture and insulin receptor autophosphorylation. Fibroblast cultures were established from 4-mm forearm skin-punch biopsies (13). ¹²⁵I-Insulin binding data were obtained by inhibition-competition studies (13). Receptor autophosphorylation was determined by exposing cells for 10 min to increasing insulin (0–100 nmol/l) concentrations. Cells were then solubilized, and receptor phosphorylation was determined as previously described (13).

Statistical analysis. Values are given as means \pm SD. Mean values were compared by Student's *t* test or Mann-Whitney *U* test for two groups and by one-way ANOVA for more than two groups. Glucose and insulin profiles during OGTT and insulin dose-response curves of insulin receptor autophosphorylation were compared by two-way ANOVA testing. Allele frequencies were compared by χ^2 test.

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