

Brief Genetics Report

Association Between the Human Glycoprotein PC-1 Gene and Elevated Glucose and Insulin Levels in a Paired-Sibling Analysis

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We studied whether there is an association between the single nucleotide polymorphism c.533A>C (K121Q) in the glycoprotein PC-1 gene and features of the metabolic syndrome in case-control and intrafamily association studies in 922 subjects from Finland and Sweden. No difference was observed in the Q allele frequency between control subjects and type 2 diabetic subjects (12.9 vs. 15.1%). The QK genotype was associated with higher fasting plasma glucose (FPG) concentrations than the KK genotype in type 2 diabetic patients ($P < 0.001$) and their relatives ($P < 0.05$). A permutation test of siblings discordant for the QK and KK genotypes also showed that the nondiabetic siblings with the QK genotype had higher FPG (6.1 ± 2.0 vs. 5.4 ± 0.6 mmol/l, $P < 0.001$) and fasting insulin (7.0 ± 3.6 vs. 4.8 ± 2.6 mU/l, $P < 0.05$) concentrations than the carriers of the KK genotype. In addition, diabetic siblings with the QK genotype had higher systolic blood pressure (147.0 ± 18.0 vs. 140.0 ± 18.7 mmHg, $P < 0.05$) and higher fasting (9.9 ± 3.0 vs. 8.8 ± 2.8 mmol/l, $P < 0.05$) and 2-h plasma glucose (17.3 ± 8.5 vs. 12.9 ± 4.2 mmol/l, $P < 0.05$) concentrations than the diabetic carriers of the KK genotype. The present study shows that, although the Q allele of the human glycoprotein PC-1 gene is associated with surrogate measures of insulin resistance, it may not be enough to increase the susceptibility to type 2 diabetes. *Diabetes* 49:1601-1603, 2000

Insulin resistance is seen in first-degree relatives of patients with type 2 diabetes and has been considered an inherited trait. Insulin resistance has also been considered a common denominator of several abnormalities, including glucose intolerance, hypertension, and dys-

lipidemia (1,2). Previous studies indicated that the membrane glycoprotein PC-1 inhibits insulin receptor tyrosine kinase (IR-TK) activity and subsequent cellular signaling and that its content is increased in skeletal muscle, fat, and fibroblasts from insulin-resistant subjects (3-9). Thus, the glycoprotein PC-1 gene, which is located on chromosome 6q22-q23 (10), may be a candidate gene for insulin resistance. Recently, a novel single nucleotide polymorphism c.533A>C (K121Q) (GenBank D12485) in the exon 4 of the glycoprotein PC-1 gene was described and found to be associated with insulin resistance in Caucasians from Sicily (11). In addition, IR-TK activity was reduced in cultured skin fibroblasts from individuals carrying the QK genotype (11). The present study was designed to examine if the Q allele of the glycoprotein PC-1 gene is associated with features of insulin resistance and type 2 diabetes using a case-control and an intrafamily design, including studies of sib-pairs discordant for the Q allele.

The frequency of the Q allele in all of the 922 subjects from Finland and Sweden was 13.8% and in Hardy-Weinberg equilibrium. Eight (2%) of the 392 type 2 diabetic patients, 2 (0.5%) of the 383 relatives of type 2 diabetic patients, and 1 of the 147 nondiabetic control subjects were homozygous for the Q allele. There was no significant difference in the Q allele frequency among type 2 diabetic patients (15.1%), their relatives (12.9%), and nondiabetic control subjects (12.9%). Among the type 2 diabetic patients, carriers of the QK genotype had higher fasting plasma glucose concentrations than homozygous carriers of the K allele ($P < 0.001$) (Table 1). A similar difference was seen in the relatives of type 2 diabetic patients ($P < 0.05$). However, no significant difference was found in fasting plasma glucose concentrations between the carriers of the KK and QK genotypes in the nondiabetic unrelated subjects.

We also performed a family association study in siblings discordant for the Q allele using a permutation test. In total, 49 type 2 diabetic sibling pairs (82 siblings from 41 families and 12 triplets from 4 families) and 30 nondiabetic sibling pairs (28 siblings from 14 families and 24 triplets from 8 families) were analyzed. The mean difference of the paired-siblings analysis between KK and QK sibs is summarized in Table 2. Nondiabetic siblings with the QK genotype had higher fasting plasma glucose ($P = 8 \times 10^{-5}$) and insulin ($P = 0.02$) concentrations than siblings with the KK genotype. Type 2 diabetic siblings with the QK genotype also had higher fasting glucose ($P = 0.02$) and 2-h glucose ($P = 0.03$) concentrations compared with diabetic

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FPG, fasting plasma glucose; IR-TK, insulin receptor tyrosine kinase; PCR, polymerase chain reaction.

TABLE 1
Characteristics of nondiabetic and diabetic subjects and their relatives with the KK, QK, and QQ genotypes of the glycoprotein PC-1 gene

| | Nondiabetic control subjects | | | Relatives of type 2 diabetic patients | | | Type 2 diabetic patients | | |
|---------------------------------|------------------------------|--------------|---------|---------------------------------------|--------------|-------------|--------------------------|--------------|--------------|
| | KK | QK | QQ | KK | QK | QQ | KK | QK | QQ |
| <i>n</i> (M/F) | 110 (50/60) | 36 (15/21) | 1 (0/1) | 308 (140/168) | 73 (35/38) | 2 (1/1) | 304 (148/156) | 80 (35/45) | 8 (5/3) |
| Age (years) | 56 ± 13 | 53 ± 15* | 46 | 45 ± 16 | 43 ± 14 | 67 ± 14 | 58 ± 14 | 59 ± 15 | 64 ± 12 |
| Age at onset (years) | — | — | — | — | — | — | 51 ± 13 | 53 ± 11 | 57 ± 9 |
| BMI (kg/m ²) | 25.3 ± 3.5 | 25.5 ± 3.4 | 23.5 | 26.7 ± 4.7 | 26.5 ± 4.9 | 25.1 ± 0.5 | 30.6 ± 5.5 | 31.0 ± 5.3 | 30.5 ± 5.8 |
| Waist-to-hip ratio | 0.87 ± 0.10 | 0.85 ± 0.09 | 0.74 | 0.89 ± 0.10 | 0.90 ± 0.11 | 0.98 ± 0.01 | 0.94 ± 0.08 | 0.93 ± 0.10 | 0.97 ± 0.11 |
| Systolic blood pressure (mmHg) | 134.0 ± 17.9 | 128.5 ± 14.1 | 133.0 | 129.2 ± 17.8 | 130.0 ± 15.4 | 145.0 ± 7.1 | 142.5 ± 17.8 | 148.9 ± 21.9 | 153.8 ± 29.5 |
| Diastolic blood pressure (mmHg) | 81.0 ± 9.4 | 78.4 ± 1.6 | 72.0 | 78.6 ± 10.8 | 78.6 ± 10.8 | 82.5 ± 10.6 | 82.2 ± 10.3 | 83.1 ± 12.1 | 85.4 ± 10.3 |
| FP glucose (mmol/l) | 5.3 ± 0.5 | 5.6 ± 0.6 | 5.4 | 5.6 ± 1.0 | 6.0 ± 1.1* | 5.8 ± 0.6 | 9.5 ± 3.7 | 10.8 ± 3.0† | 10.7 ± 3.9 |
| 2-h glucose (mmol/l) | 5.4 ± 2.0 | 5.9 ± 1.8 | 5.1 | 6.7 ± 2.8 | 6.7 ± 2.5 | 6.8 ± 2.9 | 9.7 ± 7.6 | 11.1 ± 10.4 | 6.5 ± 9.4 |
| FS insulin (mU/l) | 4.7 ± 3.0 | 5.7 ± 5.3 | 5.2 | 6.1 ± 4.5 | 7.5 ± 7.3 | 4.9 ± 0.9 | 11.1 ± 9.0 | 13.5 ± 15.2 | 15.4 ± 1.8 |
| 2-h insulin (mU/l) | 28.7 ± 23.4 | 29.3 ± 54.4 | 31.5 | 41.1 ± 44.3 | 47.3 ± 54.3 | 19.4 ± 9.3 | 54.9 ± 53.2 | 40.6 ± 35.1 | 31.3 ± 40.0 |
| Total cholesterol (mmol/l) | 5.7 ± 0.9 | 6.0 ± 1.0 | 4.4 | 5.7 ± 1.2 | 5.2 ± 1.0 | 6.1 ± 0.1 | 5.7 ± 1.1 | 5.9 ± 1.1 | 6.1 ± 1.9 |
| HDL cholesterol (mmol/l) | 1.4 ± 0.3 | 1.4 ± 0.5 | 1.1 | 1.3 ± 0.4 | 1.2 ± 0.4 | 1.4 ± 0.4 | 1.1 ± 0.3 | 1.1 ± 0.3 | 1.0 ± 0.2 |
| Triglycerides (mmol/l) | 1.3 ± 0.3 | 1.4 ± 0.6 | 1.1 | 1.5 ± 0.8 | 1.5 ± 0.9 | 1.6 ± 0.2 | 2.2 ± 1.7 | 2.3 ± 2.6 | 3.6 ± 4.3 |

Data are means ± SD, unless otherwise indicated, and were adjusted for age. **P* < 0.05 vs. KK; †*P* < 0.001 vs. KK. FP, fasting plasma; FS, fasting serum.

siblings with the KK genotype. Systolic blood pressure was also higher in diabetic siblings with the QK genotype than in diabetic siblings with the KK genotype (*P* = 0.02).

The data obtained from the association, as well as from the study of genotype-discordant sib-pairs, indicate that the

Q allele in the glycoprotein PC-1 gene is associated with elevated plasma glucose and serum insulin levels and higher blood pressure. This association was seen in both type 2 diabetic subjects and their relatives, but the allele frequency was not increased in the patients with type 2 diabetes. The

TABLE 2
Clinical characteristics of nondiabetic and type 2 diabetic sibling-pairs discordant for the KK and QK genotypes of the glycoprotein PC-1 gene

| Subjects | Nondiabetic sib pairs (30/52) | | | <i>P</i> * | Type 2 diabetic sib pairs (49/94) | | | <i>P</i> * |
|---------------------------------|-------------------------------|-----------------|-------|----------------------|-----------------------------------|-----------------|--------|------------|
| | Sib KK (n = 24) | Sib QK (n = 28) | OSD | | Sib KK (n = 46) | Sib QK (n = 48) | OSD | |
| Age (years) | 33 ± 15 | 42 ± 2 | -34 | | 59 ± 12 | 57 ± 12 | -40 | |
| BMI (kg/m ²) | 21.5 ± 1.5 | 23.9 ± 3.0 | 67.3 | 0.07 | 31.3 ± 6.7 | 31.0 ± 5.5 | -10.36 | |
| Waist-to-hip ratio | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.01 | | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.22 | |
| Systolic blood pressure (mmHg) | 122.7 ± 13.7 | 128.6 ± 15.5 | 170 | | 140.0 ± 18.7 | 147.0 ± 18.0 | 369 | 0.02 |
| Diastolic blood pressure (mmHg) | 75.5 ± 10.9 | 80.4 ± 13.5 | 143 | | 81.3 ± 11.0 | 84.4 ± 13.5 | 155 | |
| FP glucose (mmol/l) | 5.4 ± 0.6 | 6.1 ± 2.0 | 37.7 | 8 × 10 ⁻⁵ | 8.8 ± 2.8 | 9.9 ± 3.0 | 50.9 | 0.02 |
| 2-h glucose (mmol/l) | 6.9 ± 2.9 | 7.9 ± 3.2 | 23.0 | | 12.9 ± 4.2 | 17.3 ± 8.5 | 100.6 | 0.03 |
| FS insulin (mU/l) | 4.8 ± 2.6 | 7.0 ± 3.6 | 47.3 | 0.02 | 10.3 ± 7.4 | 12.2 ± 8.2 | 89.8 | 0.08 |
| 2-h insulin (mU/l) | 23.0 ± 17.6 | 28.9 ± 19.9 | -99.5 | | 41.0 ± 39.0 | 47.6 ± 50.8 | 119.2 | |
| Total cholesterol (mmol/l) | 4.4 ± 0.1 | 5.0 ± 0.2 | -8.8 | | 5.9 ± 1.2 | 6.0 ± 1.3 | 3.8 | |
| HDL cholesterol (mmol/l) | 1.3 ± 0.1 | 1.4 ± 0.3 | -9.9 | | 1.1 ± 0.3 | 1.2 ± 0.3 | 4.8 | |
| Triglycerides (mmol/l) | 0.8 ± 0.04 | 0.7 ± 0.1 | 8.0 | | 2.0 ± 1.1 | 2.0 ± 1.2 | 2.4 | |

Data are means ± SD, unless otherwise indicated. *Two-tailed *P* values estimated from a large random sample (10⁷) of all possible permutations. FP, fasting plasma; FS, fasting serum; OSD, observed sum of differences.

results are consistent with a previous study showing that the carriers of the Q allele were more insulin resistant than carriers of the K allele (11). As previously suggested, the mechanism could include impaired IR-TK activity. In contrast, no association was observed between the K121Q variant and measures of insulin resistance (S_I derived from an intravenous glucose tolerance and the homeostasis assessment model) in type 2 diabetic and nondiabetic Danish subjects (12). Population-based association studies will always suffer from problems related to the stratification of case patients and control subjects (13). To circumvent this problem, family-based approaches like the transmission disequilibrium test (14) or the current measured genotype approach can be used (15,16). We have previously shown that this is a sensitive and powerful method to detect subtle differences in phenotypic variables (17). Despite an association with features of the insulin-resistance syndrome, the Q allele was not increased in the patients with type 2 diabetes. Therefore, the question arises of whether insulin resistance alone is sufficient to increase susceptibility to type 2 diabetes.

RESEARCH DESIGN AND METHODS

A total of 922 subjects from Finland and Sweden were included in the population-based association study. From 156 nuclear families with 1 or more affected sibs, 383 nondiabetic first-degree relatives and 392 type 2 diabetic patients were selected. In addition, 147 nondiabetic unrelated spouses served as control subjects. Concentrations of plasma glucose, serum insulin, total cholesterol, HDL-cholesterol, and triglycerides were measured, as previously described (18). All participants gave informed consent and the study was approved by the local ethics committees.

Genomic DNA was extracted from peripheral blood using standard methods. The c.533A>C (K121Q) variant in the glycoprotein PC-1 gene was detected using a polymerase chain reaction (PCR)-restriction fragment length polymorphism (11). A 238-bp amplified PCR product was digested with *Ava*II restriction enzyme (New England BioLabs). The wild-type genotype (KK) was not digested, whereas the mutated homozygous genotype (QQ) was cut as a doublet of 148 and 90 bp. The heterozygous genotype (QK) was represented as 3 fragments of 238, 148, and 90 bp. Samples were analyzed by electrophoresis using 2.5% high-strength agarose gels (Appligene, Illkirch, France) and precasted DNA retardation gels containing 6% polyacrylamide (Novex, Frankfurt, Germany) and were stained with ethidium bromide (0.3 μ g/ml). The significance of differences in allele and genotype frequencies between randomly selected subjects was tested by the χ^2 test. To correct for nonindependence due to multiple members from the same family pedigrees, we applied the measured genotype approach, which corrects for nonindependence by using the residual genetic covariance among the family members (19). The mean difference between the carriers of the KK and QK genotypes was estimated using maximal likelihood ratios. We also performed a permutation test of the family association study in siblings discordant for the Q allele using a modified program (15,16). The 2-tailed *P* values were estimated using 10^7 random samples from all of the possible permutations (if $2^n > 10^7$). The difference between pairs was considered statistically significant if the observed sum of differences was located within the 5% region of rejection.

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REFERENCES

- DeFronzo RA: Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. *Diabetes Rev* 5:177-269, 1997
- Groop LC: Etiology of non-insulin-dependent diabetes mellitus: a general overview. In *Molecular Pathogenesis of Diabetes Mellitus*. Leslie RGD, Ed. Basel, Karger, 1997, p. 131-156
- Maddux BA, Sbraccia P, Kumakura S, Sasson S, Youngren J, Fisher A, Spencer 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* 373:448-451, 1995
- Kahn CR: Causes of insulin resistance. *Nature* 373:384-385, 1995
- Goldfine ID, Maddux BA, Youngren JF, Frittitta L, Trischitta V, Dohm GL: Membrane glycoprotein PC-1 and insulin resistance. *Mol Cell Biochem* 182:177-184, 1998
- Frittitta L, Youngren J, Vigneri R, Maddux BA, Trischitta V, Goldfine ID: PC-1 content in skeletal muscle of non-obese, non-diabetic subjects: relationship to insulin receptor tyrosine-kinase activity and whole body insulin sensitivity. *Diabetologia* 39:1190-1195, 1996
- Youngren J, Maddux BA, Sasson S, Sbraccia P, Tapscott EB, Swanson MS, Dohm LG, Goldfine ID: Skeletal muscle content of membrane glycoprotein PC-1 in obesity. *Diabetes* 45:1324-1328, 1996
- Frittitta L, Youngren J, Sbraccia P, D'Adamo M, Buongiorno A, Vigneri R, Goldfine ID, Trischitta V: Increased adipose tissue PC-1 protein content but not tumor necrosis factor- α gene expression, is associated with a reduction of both whole body insulin sensitivity and insulin receptor tyrosine-kinase activity. *Diabetologia* 47:1095-1110, 1997
- Frittitta L, Spampinato D, Solini A, Nosadini R, Goldfine ID, Vigneri R, Trischitta V: Elevated PC-1 content in cultured skin fibroblasts correlates with decreased in vivo and in vitro insulin action in nondiabetic subjects: evidence that PC-1 may be an intrinsic factor in impaired insulin receptor signaling. *Diabetes* 47:1095-1110, 1998
- Funakoshi I, Kato H, Horie K, Yano T, Hori Y, Kobayashi H, Inoue T, Suzuki H, Fukui S, Tsukahara M, Kajii T, Yamashina Y: Molecular cloning of cDNAs for human fibroblast nucleotide pyrophosphatase. *Arch Biochem Biophys* 295:180-187, 1992
- 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* 48:1881-1884, 1999
- Rasmussen SK, Urhammer SA, Pizzuti A, Echwald SM, Ekstrom CT, Hansen L, Hansen T, Borch-Johnsen K, Frittitta L, Trischitta V, Pedersen O: The K121Q variant of human PC-1 gene is not associated with insulin resistance or type 2 diabetes among Danish Caucasians. *Diabetes*. In press
- Altshuler D, Kruglyak L, Lander E: Genetic polymorphisms and disease. *N Engl J Med* 338:1626, 1998
- Spielman RS, McGinnis RE, Ewens WJ: Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus. *Am J Hum Genet* 52:506-516, 1993
- Sidney S, Castellan NJ Jr: The McNemar change test. In *Nonparametric Statistics for the Behavioral Sciences*. 2nd ed., New York, McGraw-Hill, 1988, p. 75-80
- Orho-Melander M, Almgren P, Kanninen T, Forsblom C, Groop LC: A paired sibling analysis of the *Xba*I polymorphism in the muscle glycogen synthase gene. *Diabetologia* 42:1138-1145, 1999
- Widen E, Lehto M, Kanninen T, Walston J, Shuldiner AR, Groop LC: Association of a polymorphism in the β_3 -adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. *N Engl J Med* 333:348-351, 1995
- Groop LC, Forsblom C, Lehtovirta M, Tuomi T, Karanko S, Nissen M, Ehrström BO, Forsen B, Isomaa B, Snickars B, Taskinen MR: Metabolic consequences of a family history of NIDDM (the Botnia Study): evidence for sex-specific parental effects. *Diabetes* 45:1585-1593, 1996
- Boerwinkle E, Chakraborty R, Sing CF: The use of measured genotype information in the analysis of quantitative phenotypes in man. *Ann Hum Genet* 50:181-194, 1986