Brief Genetics Report

The K121Q Variant of the Human PC-1 Gene Is Not Associated With Insulin Resistance or Type 2 Diabetes Among Danish Caucasians

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The human plasma-cell membrane differentiation antigen-1 (PC-1) has been shown to inhibit insulin receptor tyrosine kinase activity. Recently, a K121Q polymorphism in the human PC-1 gene was found in a Sicilian population and was shown to be strongly associated with insulin resistance. The objectives of the present investigation were to examine in the Danish Caucasian population whether the K121Q variant was associated with type 2 diabetes or, in glucose-tolerant subjects, with impaired whole-body insulin sensitivity. We genotyped 404 Danish type 2 diabetic patients and found that the allele frequency of the variant was 0.14 (95% CI 0.12–0.16), whereas the allele frequency was 0.16 (95% CI 0.13–0.19) among 237 matched glucose-tolerant control subjects (P = 0.6). In the control subjects, there were no significant differences among wild-type, heterozygous, or homozygous subjects in regard to *1*) serum insulin and plasma glucose levels at fasting, 60 min, or 120 min during an oral glucose tolerance test (OGTT) or *2*) the estimates of insulin resistance obtained from the homeostasis model assessment (HOMA). Furthermore, we investigated the impact of the variant in 2 other Danish population samples that comprised 356 young healthy subjects and 226 glucose-tolerant offspring of type 2 diabetic probands, respectively. In all of the study populations, the polymorphism was not associated with an altered insulin sensitivity index as estimated from an intravenous glucose tolerance test in combination with an intravenous injection of tolbutamide. In addition, among the 226 offspring, the variations in serum insulin and serum C-peptide responses measured during an OGTT were not related to the PC-1 genotype. In conclusion, the K121Q polymorphism of the human PC-1 gene is not associated with type 2 diabetes or insulin resistance among Danish Caucasians. *Diabetes* 49:1608–1611, 2000

Insulin resistance, which may be caused by a combination of genetic and environmental factors, is a major component of the pathogenesis of type 2 diabetes (1). The putative genetic background of insulin resistance is largely unknown, but rare mutations in the insulin receptor gene have been found in patients with extreme insulin resistance and acanthosis nigricans (1,2). Furthermore, a polymorphism at codon 972 in the insulin receptor substrate 1 (IRS-1) has recently been shown to be associated with decreased insulin signaling and to aggravate obesity-associated insulin resistance (3,4). Insulin receptor kinase activity is impaired in muscle and other insulin-sensitive tissues of many type 2 diabetic patients (5), and a potential inhibitor of the insulin receptor tyrosine kinase has been purified from a patient with marked insulin resistance and type 2 diabetes (6). This inhibitor was identified as the plasma-cell membrane differentiation antigen-1 (PC-1), and PC-1 activity was further found to be increased in skin fibroblasts from 7 of 9 type 2 diabetic patients when compared with control subjects (6). PC-1 has multiple biochemical functions and was originally described as an enzyme with phosphodiesterase I and nucleotide pyrophosphatase activities (7). Expression levels of PC-1 in human breast cancer cell lines were found to be negatively correlated with insulin receptor tyrosine kinase activity (8). However, the interpretation of results from this study was recently challenged by transduction experiments in 3T3-L1 adipocytes in which no effects were observed from overexpression of PC-1 by an adenoviral approach (9). On the other hand, human studies have shown inverse correlations between the expression levels of PC-1 and both whole-body insulin sensitivity and insulin receptor tyrosine kinase activity in skeletal muscle (10), adipose tissue (11), and skin fibroblasts (12), thereby providing a possible link to insulin resistance. Recently, an amino acid polymorphism (K121Q) was identified in the human PC-1 gene (13). This polymorphism is not associated with altered expression of PC-1, but skin fibroblasts derived from heterozygous carriers of the poly-

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HOMA, homeostasis model assessment; IRS-1, insulin receptor substrate 1; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test; PC-1, plasma-cell membrane differentiation antigen-1; PCR, polymerase chain reaction.

TABLE 1
Clinical and biochemical data of 237 glucose-tolerant middle-aged control subjects classified in accordance with the K121Q polymorphism of the PC-1 gene.

	Wild-type	Heterozygous	Homozygous	*P*
M/F	81/84	34/33	3/2	
Age (years)	52.9 ± 13.2	49.1 ± 14.0	65.0 ± 12.4	
BMI (kg/m^2)	25.5 ± 3.7	24.9 ± 3.9	26.2 ± 6.4	
Fasting values				
Plasma glucose (mmol/l)	5.1 ± 0.5	5.1 ± 0.5	4.9 ± 0.3	0.17
Serum insulin (pmol/l)	42 ± 22	39 ± 20	41 ± 18	0.78
Serum-C-peptide (pmol/l)	571 ± 161	537 ± 139	520 ± 93	0.45
Values at 60 min during OGTT				
Plasma glucose (mmol/l)	7.3 ± 2.0	7.3 ± 2.3	7.3 ± 1.5	0.73
Serum insulin (pmol/l)	326 ± 219	285 ± 250	377 ± 165	0.31
Serum C-peptide (pmol/l)	2,250 ± 730	2,109 ± 810	2,443 ± 597	0.47
Values at 120 min during OGTT				
Plasma glucose (mmol/l)	5.5 ± 1.2	5.2 ± 1.2	5.8 ± 1.2	0.15
Serum insulin (pmol/l)	179 ± 123	136 ± 107	278 ± 184	0.01
Serum C-peptide (pmol/l)	1,943 ± 798	1,695 ± 729	2,325 ± 822	0.18
HOMA insulin resistance (10^{-5} × [mmol/l]^2)	0.99 ± 0.59	0.90 ± 0.51	0.89 ± 0.37	0.58

Data are *n* or means ± SD. Comparisons among data from wild-type, heterozygous, and homozygous carriers were performed using a generalized linear model. *P* values refer to results after analyses that included sex and genotype status as fixed factors and BMI and age as covariate factors. For analysis of plasma glucose, serum insulin, or serum C-peptide levels at 60 min during an OGTT, the corresponding basal levels were included as covariates in the model and basal and 60-min values were included in the model for analysis of the corresponding 120-min levels during the OGTT. HOMA insulin resistance was calculated as HOMA = (fasting serum insulin × fasting plasma glucose)/22.5.

morphism had reduced insulin receptor tyrosine kinase activity when compared with carriers of the wild-type allele (13). In addition, a significant association was found between this polymorphism and whole-body insulin sensitivity in a Sicilian population (13).

The objective of the present study was to test whether the K121Q variant was associated with insulin resistance or type 2 diabetes among Danish Caucasians. The impact of the polymorphism was investigated in 4 different Danish study populations constituting 3 independent studies: *1*) a case-control study that was performed in 404 type 2 diabetic patients and 237 aged-matched glucose-tolerant control subjects; *2*) a population-based study of 356 unrelated young healthy subjects to further investigate an effect of the K121Q polymorphism on insulin sensitivity; and *3*) a study comprising 226 glucose-tolerant offspring of type 2 diabetic probands from 62 families.

The allele frequencies of the K121Q variant in the case-control study were 0.14 (95% CI 0.12–0.16) and 0.16 (95% CI 0.13–0.19), respectively. The observed genotype frequencies were in Hardy-Weinberg equilibrium, and there was no significant difference in allele frequencies between the 2 groups (Fisher's exact test, $P = 0.6$). Among the 237 middle-aged control subjects, the homeostasis model assessment (HOMA) was used as an estimate of insulin resistance (14). There were no differences among the wild-type, heterozygous, and homozygous carriers of the K121Q variant in regard to HOMA values (Table 1). The plasma glucose and serum insulin levels (at 0, 60, and 120 min) during an oral glucose tolerance test (OGTT) did not differ between the genotype groups after correction for multiple testing. Moreover, we did not observe any significant differences in plasma glucose and serum insulin levels during an OGTT or in the insulin sensitivity index in the offspring of type 2 diabetic probands after genotype stratification (Table 2). Finally, there were no differences among wild-type, heterozygous, or homozygous carriers of the K121Q polymorphism among young healthy subjects regarding fasting levels of plasma glucose and serum insulin or the insulin sensitivity index (Table 3).

The overall allele frequency obtained from all of the subjects included in this study is 0.16 (95% CI 0.14–0.18), and this finding is in accordance with the allelic frequency previously described in the Sicilian population (13). In the Sicilian population, by examining 121 nonobese glucose-tolerant subjects, significantly higher levels of fasting plasma glucose and significantly higher plasma insulin levels at 120 min during an OGTT were found when combined heterozygous and homozygous carriers of the K121Q variant ($n = 41$) were compared with wild-type carriers ($n = 80$). In addition, carriers of the K121Q variant had lower levels of insulin-stimulated glucose disposal as measured by an euglycemic-hyperinsulinemic clamp performed in a subgroup of 73 healthy nonobese glucose-tolerant individuals (13). In the present study, we identified 2 *P* values <0.05: serum insulin values at 120 min during an OGTT in glucose-tolerant middle-aged control subjects ($P = 0.01$) and fasting serum insulin values ($P = 0.03$) in glucose-tolerant offspring of a diabetic proband (Tables 1 and 2, respectively). In both cases, heterozygous carriers of the K121Q variant had lower insulin concentrations compared with wild-type carriers, contrary to the findings of the previously reported study (13). In addition, we have not corrected for multiple testing and, therefore, we consider these differences as statistical type 1 errors. In summary, among Danes, we were not able to detect differences between groups with respect to any of the measured variables.

The power of the present study to detect an effect of the variant of ~10% on basal plasma glucose and 70% on plasma

TABLE 2
Clinical and biochemical data of 226 glucose-tolerant offspring of 62 type 2 diabetic patients classified in accordance with the K121Q polymorphism of the PC-1 gene

	Wild-type	Heterozygous	Homozygous	*P*
M/F	72/75	30/40	3/6	
Age (years)	40.1 ± 8.8	38.4 ± 7.7	35.1 ± 10.0	
BMI (kg/m^2)	25.9 ± 4.3	25.7 ± 4.8	23.6 ± 1.9	
Fasting values				
Plasma glucose (mmol/l)	5.2 ± 0.6	5.0 ± 0.5	5.1 ± 0.4	0.32
Serum insulin (pmol/l)	42 ± 28	35 ± 22	29 ± 6	0.03
Serum C-peptide (pmol/l)	516 ± 173	466 ± 176	459 ± 106	0.23
Values at 60 min during OGTT				
Plasma glucose (mmol/l)	7.9 ± 2.1	7.7 ± 1.9	6.9 ± 2.0	0.50
Serum insulin (pmol/l)	388 ± 256	331 ± 192	277 ± 121	0.86
Serum C-peptide (pmol/l)	2,095 ± 666	1,908 ± 606	1,882 ± 542	0.15
Values at 120 min during OGTT				
Plasma glucose (mmol/l)	5.6 ± 1.1	5.8 ± 1.2	5.8 ± 1.2	0.51
Serum insulin (pmol/l)	213 ± 200	192 ± 119	181 ± 98	0.78
Serum C-peptide (pmol/l)	1,732 ± 600	1,623 ± 600	1,711 ± 558	0.60
Insulin sensitivity index ($10^{-5} \times$ [min $\times$ pmol/l]$^{-1}$)	10.5 ± 6.1	11.1 ± 6.6	15.3 ± 7.1	0.16

Data are *n* or means ± SD. Comparisons among data from wild-type, heterozygous, and homozygous carriers were performed using a variance component model. *P* values refer to results after analyses that included sex and genotype status as fixed factors, BMI and age as covariate factors, and family as a random factor. For analysis of plasma glucose, serum insulin, or serum C-peptide levels at 60 min during an OGTT, the corresponding basal levels were included as covariates in the model and basal and 60-min values were included in the model for analysis of the corresponding 120-min levels during the OGTT.

insulin at 120 min during an OGTT, as previously reported (13), was >99% for both variables in the 2 study populations, which consisted of 356 young healthy subjects and 226 glucose-tolerant control subjects, respectively. Thus, there was low risk of reporting false negative results.

In the present study, we did not apply the euglycemic-hyperinsulinemic clamp for studies of whole-body insulin sensitivity. However, whole-body insulin sensitivity as estimated during an intravenous glucose tolerance test (IVGTT) and during an euglycemic-hyperinsulinemic clamp is highly correlated (15). The population comprising 226 glucose-tolerant offspring was closely matched to the reported Sicilian population (13) with respect to age, sex, and BMI. The discrepancy between results derived from the Sicilian and the Danish populations might therefore be due to differences in genetic background. For example, the K121Q polymorphism may need to interact with mutations of other genes to end up in a phenotype of insulin resistance (i.e., genetic epistasis), and such interacting mutations may be absent in the Danish population. Alternatively, the K121Q polymorphism could be in linkage disequilibrium with mutations in other genes that are, in fact, responsible for insulin resistance, and the degree of linkage disequilibrium may be different in the Danish population. Although possible, a different genetic background is unlikely to be the major cause of the discrepancy observed between the present study and the study in Sicilians because of the data obtained in a recent study in Swedish populations (Gu H.F, Almgren P., Lindholm E., Frittatta L., Pizzuti A., Trischitta V., and Groop L.C., in press). In this study, Gu et al. found that among type 2 diabetic patients, as well as their first degree relatives, those carrying the Q allele had increased fasting plasma glucose as com-

TABLE 3
Clinical and biochemical data of 356 healthy young subjects classified in accordance with the K121Q polymorphism of the PC-1 gene

	Wild-type	Heterozygous	Homozygous	*P*
M/F	127/136	45/41	1/6	
Age (years)	25.4 ± 3.5	24.7 ± 3.2	25.4 ± 3.8	
BMI (kg/m^2)	23.5 ± 3.8	23.7 ± 3.7	22.9 ± 3.5	
Fasting values				
Plasma glucose (mmol/l)	5.0 ± 0.5	5.0 ± 0.5	4.9 ± 0.6	0.90
Serum insulin (pmol/l)	37 ± 21	38 ± 24	33 ± 20	0.51
Serum C-peptide (pmol/l)	467 ± 154	488 ± 160	428 ± 134	0.50
Insulin sensitivity index ($10^{-5} \times$ [min $\times$ pmol/l]$^{-1}$)	15.4 ± 9.1	14.7 ± 10.0	17.2 ± 9.4	0.76

Data are *n* or means ± SD. Comparisons among data from wild-type, heterozygous, and homozygous carriers were performed using a generalized linear model. *P* values refer to results after analyses that included sex and genotype status as fixed factors and BMI and age as covariate factors.

pared with wild-type carriers. This finding could not be replicated in nondiabetic control subjects with no family history of type 2 diabetes. In addition, in a family-based association study performed in sib-pairs discordant for the K121Q polymorphism, individuals carrying the Q allele compared with wild-type carriers showed several abnormalities related to insulin resistance, including higher fasting plasma glucose and serum insulin concentrations among nondiabetic pairs and higher fasting and postglucose load plasma glucose and blood pressure among type 2 diabetic pairs.

In conclusion, the present study clearly indicates that the pathophysiological impact of the PC-1 K121Q variant on insulin sensitivity and type 2 diabetes is insignificant in the Danish population. The reason for the discrepancy on the impact of this polymorphism in various populations is unknown. Transfection and functional studies in an insulin-sensitive cell line would certainly help to understand the potential implications of this polymorphism on cellular insulin action and whole-body insulin resistance.

RESEARCH DESIGN AND METHODS

Subjects. A total of 404 type 2 diabetic patients recruited from the outpatient clinic at Steno Diabetes Center and 237 age-matched glucose-tolerant control subjects living in the same area of Copenhagen as the patients and recruited through the Danish Central Population Register were enrolled in the case-control study. Diabetes was diagnosed in accordance with the 1985 World Health Organization criteria. The patients had a mean age of 55 years (range 30–84), a BMI of 29.0 kg/m^2 (range 16.5–52.3), and a reported average duration of diabetes of 6 years (range 0–28). Of the patients, 27% were treated with diet alone, 60% were treated with oral hypoglycemic agents, and 13% were treated with insulin. The control subjects underwent a 2-h OGTT. A population-based sample of 356 young healthy subjects was used for further examination of the impact of the K121Q polymorphism. The subjects underwent a tolbutamide-modified IVGTT for measurements of the insulin sensitivity index. Physiological characterization of this population has been presented previously (16). Additionally, 226 glucose-tolerant offspring of type 2 diabetic probands from 62 families were recruited from the Danish family resource bank at the Department of Human Genetics of the University of Copenhagen and from the outpatient clinic at the Steno Diabetes Center. Physiological characterization of this population has been reported previously (17). The offspring underwent both an OGTT and an IVGTT. Informed consent was obtained from all of the studied subjects before participating in the study. The study was approved by the ethical committee of Copenhagen and was in accordance with the principles of the Declaration of Helsinki II. The plasma glucose, serum insulin, and serum C-peptide levels and the insulin sensitivity index were measured as previously reported (16).

DNA analysis. Genotyping was carried out on genomic DNA isolated from human leukocytes. The genotype was determined by using *Ava*II restriction enzyme digestion of polymerase chain reaction (PCR) products obtained with the forward primer 5′-CTGTGTTCACTTTGGACATGTTG-3′ and the reverse primer 5′-GAGGTTGGAAGATACCAGGTTG-3′ followed by agarose electrophoresis of the digested PCR products.

Statistical analysis. Fisher's exact test was applied to examine differences in allele frequencies. Differences between the genotype groups among the control subjects and the young healthy subjects were tested with a generalized linear model that included sex and genotype as fixed factors and BMI and age as covariate factors using the Statistical Package of Social Science version 9.0 for Windows. For analysis of data obtained from offspring of diabetic probands, a variance component model was used as described (17). When analyzing plasma glucose, serum insulin, or serum C-peptide levels at 60 min during an OGTT, the corresponding basal levels were included as covariates in the model. Similarly, basal and 60-min values were included in the model for analysis of the corresponding 120-min levels during an OGTT.

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