RESEARCH NOTE

K121Q *ENPP1/PC-1* gene polymorphism is associated with insulin resistance in a north Indian population

JAI PRAKASH[1], BALRAJ MITTAL[2], SHALLY AWASTHI[3], C. G. AGARWAL[4] and NEENA SRIVASTAVA[5]*

[1]*Department of Physiology,* [3]*Department of Pediatrics,* [4]*Department of Medicine and* [5]*Department of Physiology, King George's Medical University, Lucknow 226 003, India*
[2]*Department of Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226 014, India*

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Introduction

Obesity is commonly associated with insulin resistance and hyperinsulinaemia and is often associated with various metabolic abnormalities, such as dyslipidaemia and elevated plasma glucose. A total of 642 study participants, 309 obese and 333 nonobese individuals were included in this study. Insulin, glucose and lipid levels were estimated using standard protocols. The degree of insulin resistance was calculated according to the homeostasis model assessment. All study participants were genotyped by PCR restriction fragment length polymorphism method. The K121Q polymorphism of *ENPP1/PC-1* gene showed no association with obesity. The K121Q polymorphism showed association with different obesity associated phenotypes like percentage body fat, fat mass, insulin and HOMA-IR (homeostasis model assessment for insulin resistance). The strongest associations were observed in percentage body fat, fat mass, insulin and HOMA-IR under recessive and dominant models. The genetic association of *ENPP1/PC-1* K121Q polymorphism with insulin resistance has been established in other population (Bacci *et al*. 2005). We have replicated the genetic association of *ENPP1/PC-1* K121Q polymorphism with obesity and its association with plasma insulin, insulin resistance, per cent body fat and fat mass in a north Indian population.

Obesity is related with significantly increased morbidity (Eckel and Krauss 1998) and mortality (Calle *et al.* 1999). Obesity is commonly associated with insulin resistance and hyperinsulinaemia and is often associated with high blood pressure and various metabolic abnormalities, such as dyslipidaemia and elevated plasma glucose (Thomas *et al*. 2004). Obesity is associated with insulin resistance and ectoenzyme nucleotide pyrophosphate phosphodiesterase 1 (ENPP1), also known as plasma cell membrane glycoprotein 1 (PC-1) plays an important role in insulin resistance (Pizzuti *et al*. 1999). ENPP1/PC-1 is an inhibitor of the insulin receptor, and is either overexpressed or overactive in muscle, adipose tissue, fibroblasts and other tissues of insulin-resistant individuals, both nondiabetic and diabetic (Goldfine *et al*. 2008). ENPP1 interacts with α-subunit of the insulin receptor to interrupt signalling (Maddux and Goldfine 2000). ENPP1/PC-1 polymorphism (rs1044498) has a glutamine substitution for lysine at codon 121 (Keshavarz *et al*. 2006).

However, outcomes of human studies of the K121Q variant have been conflicting. The initial findings indicating association of the *Q* allele with insulin resistance (Pizzuti *et al*. 1999; Gu *et al*. 2000; Bacci *et al*. 2005) were not confirmed in subsequent studies (Rasmussen *et al*. 2000; Gonzalez-Sanchez *et al*. 2003). To further clarify the role of this polymorphism, we determined whether the K121Q variant was associated with either obesity or obesity-associated phenotypes in the north Indian population.

Methods

All individuals were of north Indian origin and the population was homogeneous with regard to ethnic background. A total of 821 study participants were enrolled initially from the outpatient department of King George's Medical University, Lucknow, India (formerly Chatrapati Shahuji Maharaj Medical University, Lucknow) and volunteers from general population of Lucknow. Out of these, 88.20% were Hindu north Indians (Hindi speaking, residing in Lucknow) while 11.80% represented other religions or language (Muslims (7.2%), Sikh (3.2%), Christian (1.4%)). Only 642 Hindu individuals were included in the present study while other religions

*For correspondence. E-mail: neenasrivastavakgmc@gmail.com.

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were excluded. Every individual has been classified as Hindu north Indian (Hindi speaking) depending on selfreported family origin from two generations. A previous study provides strong evidence for two ancient populations, genetically divergent, that are ancestral to most Indians today. One, the 'ancestral north Indians' (ANI), while the other, the 'ancestral south Indians' (ASI) (Reich *et al*. 2009). A process of disproportionate stratified and systematic sampling was used to select individuals aged between 19 and 60 years, oversampling of the majority groups to ensure that prevalence estimates for the majority groups were reliable and to allow statistical comparison.

Out of these study participants, only 309 obese (body mass index (BMI) > 30 kg/m^2) and 333 nonobese (BMI ≤ 30 kg/m^2) individuals were selected on the basis of strict inclusion criteria. In all individuals, body height, body weight, waist circumferences and hip circumferences were measured for calculation of BMI and waist–hip ratio (WHR). Hypertension was diagnosed when the systolic or diastolic blood pressure was $\geq 140/\geq 90$ mm Hg on repeated single-day measurements or when the individual was a known hypertensive. Diabetes was diagnosed when a subject provided history of previously diagnosed diabetes or the fasting blood glucose was ≥ 126 mg/dL. Study participants with established diabetes mellitus, coronary artery disease, congestive heart failure and pregnant women were excluded. Informed consent was obtained from each participant and the study was carried out in accordance with the local ethics committee. All study participants were subjected to a thorough screening programme that included assessment of a detailed personal and family history, physical examination, determination of anthropometric indices and measurement of various biochemical parameters.

In general, smokers (12.6%) included present smokers and persons with history of smoking or any other tobacco use. Of the participants, 87.4% were nonsmokers, 88.6% were nonalcoholic, 88.3% did not have tobacco chewing habit and about 56.1% were vegetarians. We also classified our study participants according to their physical activity: about 63.5% were sedentary workers, 30.6% were moderate worker and 5.9% were classified as heavy workers.

Laboratory measurements

Venous blood was collected after an overnight fast, and plasma and serum samples were either used immediately for analysis or were stored frozen at -80°C. Commercial enzymatic test kits were used for determining total cholesterol, high-density lipoprotein (HDL) and triglyceride concentrations but low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol were calculated by the formula of Friedewald (LDL-cholesterol = total cholesterol – HDL cholesterol – triglyceride/5 mg/dL). The interassay coefficient of variation was less than 5.0% for HDL-cholesterol, less than 2.5% for triglycerides. Insulin levels were determined by enzyme-linked radioimmunosorbent assay (RIA) (Linco Research, St Charles, USA). The interassay coefficients of variation for the insulin assay were 5.7%. The degree of insulin sensitivity/resistance was calculated according to the homeostasis model assessment (HOMA) which is a good index for assessing insulin sensitivity/resistance. Insulin resistance (IR) was calculated as follows: IR = FI $\times$ g/22.5; where FI, fasting insulin (μU/mL); g, fasting glucose (mmol/L) (Matthews *et al*. 1985).

The fasting glucose concentration was measured by glucose oxidase-peroxidase (GOD-POD) method (Young *et al*. 1975), the interassay coefficient of variation was less than 5.0% and systolic (SBP) and diastolic (DBP) blood pressure were measured twice on the right arm, after a 15-min rest, using a mercury sphygmomanometer (Parker *et al*. 1988). All protocols were approved by the Institutional Review Board or Ethical Committee at KGMU, Lucknow, India (formerly CSM Medical University, Lucknow) and all the study participants gave informed consent.

Estimation of body fat composition

Body fat composition was assessed (bioelectrical impedance) using Tanita-TBF-310, Tokyo, Japan) to obtain percentage body fat, fat mass (FM) and fat free mass (FFM). The interassay coefficient of variation was 5.7% for per cent body fat, 3.5% for FM (kg) and 4.3% for FFM.

Genotyping

The genomic DNA was extracted from peripheral blood leukocyte pellet using the standard salting out method. K121Q polymorphism was genotyped by PCR-based restriction fragment length polymorphism analysis as previously described (Rasmussen *et al*. 2000).

Quality control

Quality control and assessment was done at every step of the study. The amount of isolated DNA was of good quality (absorbance 260 nm/280 nm ratio > 1.75). One sample with known genotype and a reagent blank were included after every 20 samples in the PCR. A 50-bp marker was included during electrophoresis. Twenty per cent of samples from patients and controls including samples of each genotype were regenotyped by other laboratory personnel and no discrepancy was found.

Statistical analysis

Mean values of continuous traits were compared using the Student's *t*-test according to nonobese (BMI ≥ 30) and obese (BMI < 30) groups; $P < 0.05$ (two-tailed) was considered significant. Genotype and allele distribution was compared between obese and nonobese study participants using X^2 test. The independent segregation of alleles was tested for the Hardy–Weinberg equilibrium (HWE), comparing the observed genotype frequencies with those expected (X^2 test).

Binary logistic regression analysis was used to adjust odds ratio for age and gender. The results for continuous variables are given as the mean ± SD. The differences among the three groups were assessed by one-way ANOVA for continuous variables and variables are given as the mean ± SD. Association of genotype with obesity-associated phenotypes was performed assuming additive, dominant and recessive models to detect the association of different genotype of *ENPP1/PC-1* K121Q like KK, KQ and QQ with the obesity-associated phenotype like waist-to-hip ratio, fasting insulin, HOMA-IR and others in different statistical model, to ensure that the effect of the genotype with condition are due to the genetic variation or due to the other factors. The statistical power of the study was >80%.

Results

Clinical characteristics of the 642 participants of the present study are significantly different between obese and nonobese study participants. WHR, systolic blood pressure, diastolic blood pressure, per cent body fat, FM, insulin, HOMA-IR and lipid profile showed significant difference between obese and nonobese study participants while glucose are not significantly different between the two groups (table 1).

Genotype frequencies of K121Q polymorphism (rs17817449) were in HWE in nonobese (BMI < 30, *P* value of HWE = 0.411). Obese cases had similar minor allele frequencies (18.3%), as compared to nonobese individuals (17.7%). In the present study K121Q polymorphism was not associated with obesity (table 2).

The K121Q polymorphism showed association with different obesity associated phenotypes like percentage body fat, FM, insulin and HOMA-IR. The strongest associations were observed with fasting percentage body fat, FM, insulin and HOMA-IR under recessive and dominant models (table 3).

Discussion

The results of different studies between *ENPP1/PC-1* K121Q variants and obesity in numerous races are conflicting. We

Table 1. Clinical characteristics of study participants.

Variable	BMI ≥ 30 (333)*	BMI < 30 (309)*	*P* value
Waist-to-hip ratio	0.95 ± 0.08	0.97 ± 0.09	0.015
Sys. BP (mm Hg)	120.51 ± 11.68	128.39 ± 15.19	<0.001
Dias. BP (mm Hg)	80.76 ± 7.68	86.23 ± 8.05	0.027
Per cent body fat	27.86 ± 6.12	37.28 ± 6.16	<0.001
FM (kg)	20.60 ± 8.16	30.60 ± 8.33	<0.001
Fasting insulin (μU/mL)	10.27 ± 6.01	14.99 ± 9.73	<0.001
HOMA-IR	2.83 ± 1.83	4.15 ± 2.87	<0.001
Fasting glucose (mg/dL)	109.23 ± 15.94	109.64 ± 18.62	0.065
T cholesterol (mg/dL)	161.71 ± 44.69	213.54 ± 35.72	0.003
HDL cholesterol (mg/dL)	46.30 ± 10.16	42.82 ± 7.13	0.001
Triglyceride (mg/dL)	107.12 ± 19.57	130.28 ± 28.88	0.008
LDL cholesterol (mg/dL)	99.68 ± 37.08	151.28 ± 30.44	<0.001
VLDL cholesterol (mg/dL)	21.42 ± 3.91	26.06 ± 5.78	0.008

*Total number of nonobese subjects (333) and obese subjects (309). Data are presented as mean ± SD. Sys. BP, systolic blood pressure; dias. BP, diastolic blood pressure; FM, fat mass; HOMA-IR, homeostasis model assessment of insulin resistance; T cholesterol, total cholesterol; HDL cholesterol, high density lipoprotein cholesterol; LDL cholesterol, low density lipoprotein cholesterol; VLDL cholesterol, very low density lipoprotein cholesterol.

Table 2. Genotype and allele frequency of *ENPP1/PC-1* K121Q (rs1044498) gene polymorphism in nonobese and obese subjects.

	BMI ≥ 30 (333)	BMI < (309)	*P* value	OR (95% CI)
Genotype				
KK	229 (68.8%)	217 (70.2%)	Reference	Reference
KQ	90 (27.0%)	71 (23.0%)	0.321	0.833 (0.579–1.196)
QQ	14 (4.2%)	21 (6.8%)	0.199	1.583 (0.785–3.192)
Allele				
K	548 (82.3%)	505 (81.7%)	Reference	Reference
Q	118 (17.7%)	113 (18.3%)	0.792	1.039 (0.782–1.382)

Table 3. Phenotypes and genotypic classes for *ENPP1/PC-1* K121Q (rs1044498) polymorphism.

	KK (446)	*KQ* (161)	*QQ* (35)	Additive *P* value	Recessive *P* value*	Dominant *P* value*
Waist-to-hip ratio	0.95 ± 0.09	0.97 ± 0.09	0.98 ± 0.09	0.164	0.285	0.064
Sys. BP (mm Hg)	124.73 ± 14.09	123.16 ± 12.01	124.06 ± 20.67	0.474	0.916	0.241
Dias. BP (mm Hg)	83.73 ± 8.42	81.79 ± 7.57	86.46 ± 9.08	0.003	0.025	0.120
Per cent body fat	31.53 ± 6.51	33.55 ± 9.45	38.04 ± 10.20	0.0001	0.0001	0.0001
FM (kg)	24.73 ± 8.63	25.80 ± 10.82	32.38 ± 13.00	0.0001	0.0001	0.006
Fasting insulin (μU/mL)	7.89 ± 8.03	11.87 ± 10.23	13.15 ± 7.47	0.001	0.001	0.005
HOMA-IR	2.18 ± 2.29	3.20 ± 2.78	3.67 ± 2.33	0.001	0.002	0.002
Fasting glucose (mg/dL)	110.53 ± 17.62	106.61 ± 16.65	108.34 ± 14.11	0.044	0.704	0.015
T cholesterol (mg/dL)	186.89 ± 49.06	186.59 ± 44.99	183.97 ± 51.79	0.942	0.735	0.852
HDL cholesterol (mg/dL)	45.00 ± 9.01	43.66 ± 8.76	44.34 ± 9.72	0.267	0.844	0.115
Triglyceride (mg/dL)	118.56 ± 28.23	117.49 ± 22.78	118.17 ± 30.80	0.913	0.982	0.685
LDL cholesterol (mg/dL)	124.18 ± 43.12	125.58 ± 40.46	123.98 ± 48.23	0.936	0.939	0.761
VLDL cholesterol (mg/dL)	23.71 ± 5.65	23.50 ± 4.56	23.63 ± 6.16	0.913	0.982	0.685

Data are mean ± SD for genotypic classes based on unrelated individuals, $n = 642$.
*Recessive indicates GG vs GT and TT, dominant indicates GG and GT vs TT.

carried out this study to investigate the association of K121Q variants with obesity and obesity associated phenotypes like insulin resistance in a north Indian population. In a previous study, polymorphism in *ENPP1* K121Q carriers and/or *Q* allele was associated with obesity (Wan *et al*. 2006), whereas some studies observed no association between the K121Q allele and obesity in European (Matsuoka *et al*. 2006; Prudente *et al*. 2007) and African populations (Matsuoka *et al*. 2006). Similarly, no effect of *ENPP1/PC-1* gene variants was reported on body weight (Weedon *et al*. 2006). In the present study, there was no difference in distribution of K121Q between obese and nonobese individual.

Further, we investigated the role of *ENPP1/PC-1* K121Q polymorphism with obesity related various phenotypes like systolic, diastolic blood pressure, WHR, per cent body fat, FM, fasting insulin, HOMA-IR, fasting glucose and lipid profile. The association was observed for per cent body fat, FM, insulin, HOMA-IR but no significant associations were detected with systolic, diastolic blood pressure, WHR, fasting glucose and lipid profile in the present study. Insulin resistance is frequently associated with obesity. In the present study, the risk allele of Q121 PC-1 variant was positively associated with fasting insulin and insulin-resistant traits, HOMA-IR (table 3). The influence of this Q121 *PC-1* variant on insulin resistant appears to be mediated through adiposity. The Q121 *PC-1* variant has been associated with insulin resistance in several studies (Pizzuti *et al*. 1999; Gu *et al*. 2000), but in another study, this polymorphism was not associated with insulin resistance (Rasmussen *et al*. 2000).

Previous studies using cDNAs of the *PC-1* variants transfected in cultured cells showed that the Q121 *PC-1* variant was stronger inhibitor of insulin signalling than the wild-type *PC-1* (Costanzo *et al*. 2001). It is speculated that the Q121 allele may have an additive affect on *ENPP1/PC-1* gene expression such as increasing mRNA half life. Thus higher expression of *ENPP1/PC-1* would downregulate the signalling pathway of insulin. These data indicate that the Q121 *PC-1* variant plays a role in causing insulin resistance.

The mechanism through which *ENPP1/PC-1* might influence BMI has not been investigated. It is feasible that the Q121 allele carriers develop insulin resistance in hypothalamic neurons, where insulin has effective anorectic roles (Bruning *et al*. 2000). On the other hand, this resistance, increases the appetite and body weight while impairing insulin inhibition of hepatic glucose production. The difference in results of genotype–phenotype association studies is frequent in the assessment of complex disorders (Lyon *et al*. 2006). The results of any study depend on the differences in the genetic and/or environmental background of the population, thus, difference in findings between studies do not mean false results.

Plentiful confirmation in human tissues for insulin, indicates that *PC-1* overexpression (i.e., due to the presence of the Q121 allele) negatively modulates insulin sensitivity. In humans, *PC-1* increased function in muscle and fat most likely 'inactivates' the IR and causes insulin resistance. The mechanism(s) is unknown till date, although data have suggested that, it may be genetic and due to augmented gene expression. Functional data point out that the Q121 allele is a 'gain of function' mutation that associated with IR inactivation in the deficiency of protein overexpression. Moreover, in several animal models, overexpression of *PC-1* causes insulin resistance and hyperglycaemia (Calle *et al.* 1999). While the association of increased *PC-1* expression with insulin resistance and diabetes has been established, but its role on body weight is controversial (Goldfine *et al*. 2008).

Our findings are in agreement with the leading hypothesis that insulin resistance recognizes multiple etiopathogenetic factors, including environmental determinants and a variety

of genes that may be involved, each one having a small effect (i.e. polygenic background) (Beck-Nielsen and Groop 1994). Our results are also well-matched with the prospect that obesity and the Q121 *PC-1* variant (Costanzo *et al*. 2001) impair insulin signalling and action through different molecular mechanisms. It is believed that, obesity associated with insulin resistance through the overproduction of metabolites, cytokines and hormones that obstruct with insulin action at different postreceptor levels (Kahn and Flier 2000).

On the basis of the present study, although performed in a relatively smaller study sample of ethnically homogeneous people, we conclude that the *ENPP1/PC-1* K121Q polymorphism does not seem to influence the risk of obesity but suggests a potential role in obesity associated insulin resistance in north Indian population. These results are well-suited with impaired insulin signalling projected for the 121Q allele variant (Costanzo *et al*. 2001).

In summary, we have replicated the findings of genetic association of *ENPP1/PC-1* K121Q polymorphism in a north Indian population and, to our knowledge; this is the first such study in Indian population. We have also established that the *ENPP1/PC-1* K121Q polymorphism is strongly associated with plasma insulin, insulin resistance, per cent body fat and fat mass. Functional studies would certainly help to understand the potential implications of this polymorphism on cellular insulin action and whole-body insulin resistance. Therefore, a more comprehensive appraisal of genetic variation in and approximately *ENPP1* would be essential to fully assess the role of this gene in obesity and obesity associated phenotypes like insulin resistance are required.

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