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Obesity risk associated with the K121Q polymorphism of the glycoprotein *PC-1* gene

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Background: Obesity is considered to be a multifactorial trait resulting from the combined influence of genetic and environmental determinants. Insulin resistance plays an important role in the development of obesity. Plasma-cell membrane differentiation antigene-1 (PC-1) inhibits insulin receptor signalling when overexpressed and thus causes insulin resistance. PC-1 gene polymorphism might be associated with adipocyte metabolism disturbance and energy imbalance. The purpose of this study was to determine whether K121Q polymorphism in PC-1 gene is involved in obesity susceptibility in Chinese Han population.
Methods: The genotype of the polymorphism was determined using polymerase chain reaction-restriction fragment length polymorphism analysis for 338 unrelated subjects of Beijing, China. Their Body mass index (BMI), plasma glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL), free fatty acid (FFA) and insulin level were measured. Chi-square analyses were applied to test the significance differences in genotypic and allelic frequencies. Association studies were undertaken using the *t*-test and logistic regression analyses.
Results: The obese had significantly higher frequency of KQ/QQ genotype or Q allele than non-obese in females (26.7% vs. 10.9%, $p = 0.014$ and 13.3% vs. 5.5%, $p = 0.021$). Significant elevation of insulin amongst the Q121 carrier women in obesity individuals and higher FFA level of Q121 carrier men in non-obese controls (BMI $\leq$ 23 kg/m^2) were observed. Binary logistic regression analysis revealed that PC-1 genotype together with higher glucose, total cholesterol, triglyceride and serum HDL were independently associated with the presence of obesity.
Conclusions: The observed genotype distributions revealed a significant association of PC-1 K121Q with obesity. PC-1 Q121 carriers are more likely to be insulin-resistant or get fatter in respect to KK subjects and carriers of the Q allele are at higher risk for the development of obesity in female.
Keywords: insulin resistance, obesity, plasma-cell membrane differentiation antigene-1, polymorphism

Introduction

Obesity is a most common and rapidly growing health problem, by dramatically raising mortality and the risk of morbidity from hypertension, diabetes mellitus and cardiovascular diseases. Although obesity aetiology is poorly understood, both genetic background and environmental factors are considered to be highly related with the disease development.

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Obesity is a major contributor to the elevated plasma insulin concentration, insulin resistance and hyperlipidaemia [1]. Impairment in insulin signalling activity has been demonstrated in obesity subjects. Obesity and the risk of type 2 diabetes is in a linear association. A population study indicated that the risk of diabetes in women increased fivefold for those with BMI of 25 kg/m^2 compared to controls with BMI $\leq$ 21 kg/m^2 [2]. As for insulin resistance, it also associates with lipid abnormalities, and promotes fat storage by the conversion of excessive amounts of sugar in the bloodstream sugar to fat and manufacturing more fat cells. One could speculate therefore that obesity and insulin resistance may share some common genetic determinants. Common variants in a number of candidate genes affecting fat and glucose metabolism, in combination with environmental triggers, might increase susceptibility to the obesity syndrome.

The glycoprotein PC-1 is a deeply studied inhibitor of the tyrosine-kinase activity of insulin receptor and associates with human insulin resistance. PC-1 is a membrane glycoprotein that is expressed in several human tissues, including fat, skeletal muscle, liver, pancreas and testis. Several evidences have proven a negative modulation of PC-1 on insulin signalling and a significant role in insulin resistance and its related abnormalities. *PC-1* gene in human is organized in 24 exons on chromosome 6q22–q23, a region been linked to insulin resistance [3,4]. A K121Q polymorphism in exon 4 was found in a Sicilian population and shown to be strongly associated with insulin resistance [5]. As compared to the more common K allele, the Q variant has a greater inhibitory activity on insulin receptor function and action, by interacting more strongly with the insulin receptor and significantly reducing receptor autophosphorylation [6]. As an essential factor in insulin signalling, the *PC-1* polymorphism may affect lipid metabolism and energy imbalance. Therefore, the present study was designed to examine whether the polymorphism of *PC-1* gene is associated with insulin resistance and other features of obesity in Chinese Han population.

Subjects and Methods

Subjects

According to specific classifications of obesity in Asians, totally 221 unrelated obesity subjects (106 male and 105 female, age 56.7 $\pm$ 12.6) and 127 non-obese control individuals (63 male and 64 female, age 60.0 $\pm$ 14.3) were recruited from a randomly selected population. We adopted here specific classifications of obesity in Asians, 18.5 $kg/m^2 \leq$ BMI $\leq$ 23 kg/m^2 for normal weight and BMI $\geq$ 27 kg/m^2 for obesity [7]. All subjects recruited were Chinese Han in origin and all from Beijing city. The obese and control groups were matched in terms of sex and age distributions. All subjects were proven healthy by physician of Beijing Hospital, the Ministry of Public Health. Written informed consent approved by the local Ethical Committee of Human Genetic Resources was obtained from all participated subjects.

Phenotype Measurements

Body height and body weight were measured in all subjects by general way. BMI was calculated as weight in kilograms divided by the square of height in metres (kg/m^2). The blood samples were taken from above subjects under the condition of overnight fasting. Plasma glucose, total cholesterol, triglyceride, HDL and FFA level were determined using a biochemical autoanalyser (Hitachi 7170, Japan). Plasma insulin was determined with method of chemiluminescence by Automated Chemiluminescences Systemm (Biorad, Hercules, CA, USA).

Genotype Determination by Restriction Fragment Length Polymorphism Analysis

Genomic DNA was extracted and purified from peripheral blood using salting-out method [8]. The *PC-1* K121Q polymorphism was detected by polymerase chain reaction-restriction fragment length polymorphism with *Ava*II restriction enzyme as previously described [5]. The K allele resulted in a fragment of 238-bp and the Q allele gave fragments of 148-bp and 90-bp.

Data Presentation and Statistical Analysis

Because insulin and triglyceride did not show normal distribution, the data were analysed after logarithmic transformation. Results were expressed as mean $\pm$ s.d. Chi-square analysis was applied to test the significance of differences in genotypic and allelic frequencies. Association studies were undertaken by comparing the mean values of different groups using analysis of *t*-test. A binary logistic regression analysis, with the states of BMI (obese or non-obese) as the dependent variable and plasma insulin, glucose, total cholesterol, triglyceride, HDL, FFA level in addition to *PC-1* genotype as covariates, was performed with forward: conditional selection. All the p-values <0.05 (two-tailed) were considered as

Table 1 Comparison of mean values for continuous variables in the Beijing population of obese and non-obese subjects

	Obese	Non-obese	p-value
Number	211	127	
Sex ration (F/M)	105/106	64/63	
Age (years)	56.7 ± 12.6	60.0 ± 14.3	
Body-mass Index (kg/m^2)	30.6 ± 3.5	21.2 ± 1.2	
Total cholesterol (m mol/l)	5.4 ± 3.6	4.8 ± 1.3	0.098
Triglyceride (μ mol/l)*	31.51 ± 2.18	29.64 ± 2.30	<0.001
HDL cholesterol (m mol/l)	48.0 ± 30.4	53.7 ± 16.5	0.052
Glucose (m mol/l)	5.5 ± 1.1	5.0 ± 0.8	<0.001
FFA (μ m mol/l)	679.0 ± 211.9	569.0 ± 236.8	<0.001
Insulin (m U/l)*	10.08 ± 2.17	7.35 ± 2.60	<0.001

Results are the mean ± s.d.
HDL, high-density lipoprotein cholesterol; FFA, free fatty acid.
*Log transformed.

significant difference. Statistical analysis was performed using the Statistical Package of Social Sciences (SPSS) for Windows, version 10.0 (SPSS Inc., 1999).

Results

The clinical characteristics of obese and non-obese controls were presented in table 1. As expected, significant correlations were observed in each obesity related anthropometric measurement between obese and non-obese groups except total cholesterol and HDL cholesterol.

Genotype frequencies in obese and non-obese groups were in accordance with the Hardy–Weinberg equilibrium. Due to the only one QQ individual (male) existed in the present population, we combined it with KQ heterozygote to form Q carrier group. In female, 77 (73.3%) obese subjects had the KK genotype and 28 (26.7%) the KQ/QQ genotype. Fifty-seven (89.1%) non-obese female had the KK genotype and seven (10.9%) the KQ/QQ genotype (table 2). As compared with non-obese controls, significant higher frequencies for both KQ/QQ genotype (OR = 2.961, OR 95% CI: 1.208–7.256, p = 0.014) and Q allele (OR = 2.659, OR 95% CI: 1.126–6.282, p = 0.021) in obese female were observed. There was no significant difference in the genotypic or allelic distribution between obese and non-obese males (p = 0.977 and p = 0.909, respectively). The genotype frequencies of the KK, KQ and QQ were 77.3, 22.3 and 0.5% in obese group, respectively, while 85.0, 15.0 and 0% in non-obese group. The genotype distribution and allele frequency did not show statistical difference between obese and control group in the total population.

In order to evaluate the possible association between K121Q and features of obesity, including BMI, total cholesterol, triglyceride, HDL, glucose, FFA and plasma insulin levels, we adopted analysis of *t*-test in obese and non-obese groups separately. Biochemical data were compared by genotype and gender. Q121 carriers and K121 homozygotes were used as analysis groups. When obese individuals were studied, significant elevation of insulin amongst the Q121 carrier women was observed. However, no significant correlations were found amongst men (table 3). When non-obese individuals were studied, FFA was shown to be significantly higher

Table 2 Frequencies of PC-1 K121Q genotypes according to BMI in female

	genotype		allele	
	KK	KQ/QQ	K	Q
Obese	77 (73.3%)	28 (26.7%)	182 (86.7%)	28 (13.3%)
Non-obese	57 (89.1%)	7 (10.9%)	121 (94.5%)	7 (5.5%)
Chi-Square	5.991		5.299	
p	0.014		0.021	
OR	0.338	2.961	0.376	2.659
OR 95% CI	0.138~0.828	1.208~7.256	0.159~0.888	1.126~6.282

Table 3 Association of C-2747T polymorphism with metabolic characteristics in obese subjects

	Male			Female		
	KK ($n = 86$)	KQ/QQ ($n = 20$)	p-value	KK ($n = 77$)	KQ/QQ ($n = 28$)	p-value
Body-mass Index (kg/m^2)	30.53 ± 2.29	30.33 ± 1.63	0.719	30.49 ± 4.63	31.05 ± 4.14	0.579
Total cholesterol (m mol/l)	4.96 ± 0.89	5.17 ± 0.84	0.323	5.94 ± 5.74	5.30 ± 1.13	0.561
Triglyceride (μ mol/l)*	31.82 ± 2.20	30.89 ± 1.67	0.082	31.29 ± 2.22	31.63 ± 2.24	0.493
HDL cholesterol (m mol/l)	48.28 ± 45.23	46.27 ± 9.19	0.844	49.41 ± 13.09	44.87 ± 13.09	0.119
Glucose (m mol/l)	5.41 ± 0.75	5.32 ± 0.38	0.620	5.38 ± 0.59	5.89 ± 2.67	0.325
FFA (μ m mol/l)	667.92 ± 212.31	640.20 ± 182.58	0.592	689.04 ± 224.61	715.00 ± 198.58	0.598
Insulin (m U/l) *	10.56 ± 2.16	9.76 ± 2.26	0.141	9.48 ± 2.09	10.47 ± 2.02	0.033

Results are the mean ± s.d.
HDL, high-density lipoprotein cholesterol; FFA, free fatty acid.
*Log transformed.

amongst Q121 carrier men. Amongst non-obese women, there were no significant differences between two kinds of genotypes (table 4).

To determine the relationship between the incidence of the obese disease and *PC-1* genotype or different clinical variables, a binary logistic regression analysis was carried out in all individuals. Significant correlations were observed in *PC-1* genotype ($p < 0.001$), HDL ($p < 0.001$), glucose ($p < 0.001$), total cholesterol ($p < 0.001$) and triglyceride ($p = 0.002$). The analysis performed suggests that all the variables tested are independently associated to the presence of obesity and that altogether explain 33.9% of the BMI variance [R^2 (adjusted) = 0.339].

Discussion

In order to define the role of the *PC-1* gene polymorphisms in insulin resistance and other metabolic features of obesity, we genotyped 338 individuals with 211 obesity and 127 non-obesity cohort. The frequency of the Q variant carriers was significantly higher in obesity patients (26.7%) than that in control subjects (10.9%) in females ($p = 0.014$). The risk of obesity for Q carrier subjects was estimated to be 2.961 times than for non-carriers (95% CI, 1.208–7.256). We also found a significant association between the Q allele and the onset of obesity in females ($p = 0.021$) (table 2). As compared with KK individuals, Q carriers had significantly higher plasma insulin level in obese females ($p = 0.033$), which is in concert with the leading hypothesis that the Q121 variant associates with whole-body insulin resistance [5,9–11]. The mechanism of the association between Q121 variant and insulin resistance was explained by an observed significant increase in haplotype-specific mRNA half-life and a more strong interaction of the Q121 type protein with the insulin receptor, as assessed by transfection experiment in MCF-7 and HEK-293 cells [6]. When cDNAs of the two PC-1 variants were transfected in cultured cells, the Q121 variant turned out to be a stronger inhibitor of insulin signalling than the wild-type protein. Individuals from Sicily, Italy carrying this variant were at higher risk ($p < 0.01$) for

Table 4 Association of C-2747T polymorphism with metabolic characteristics in non-obese subjects

	Male			Female		
	KK ($n = 51$)	KQ/QQ ($n = 12$)	p-value	KK ($n = 57$)	KQ/QQ ($n = 7$)	p-value
Body-mass Index (kg/m^2)	21.18 ± 1.21	20.91 ± 1.74	0.521	21.18 ± 1.09	21.39 ± 1.29	0.649
Total cholesterol (m mol/l)	4.78 ± 0.96	4.85 ± 1.44	0.844	4.96 ± 1.47	4.27 ± 1.06	0.231
Triglyceride (μ mol/l)*	29.56 ± 1.93	29.92 ± 2.28	0.571	29.78 ± 2.60	28.69 ± 2.47	0.296
HDL cholesterol (m mol/l)	51.60 ± 16.63	47.50 ± 17.64	0.450	57.03 ± 16.65	53.21 ± 5.76	0.231
Glucose (m mol/l)	5.10 ± 0.74	5.05 ± 1.00	0.833	4.95 ± 0.64	4.66 ± 1.28	0.573
FFA (μ m mol/l)	495.84 ± 185.91	624.90 ± 149.28	0.044	631.98 ± 267.87	517.00 ± 300.72	0.329
Insulin (m U/l)*	7.41 ± 2.77	5.94 ± 2.99	0.137	7.51 ± 2.38	7.74 ± 2.26	0.811

Results are the mean ± s.d.
HDL, high-density lipoprotein cholesterol; FFA, free fatty acid.
*Log transformed.

insulin resistance and had higher plasma glucose and insulin levels during an oral glucose tolerance test.

Binary logistic regression also supported that K121Q was a determine predictor variable affecting the incidence of obesity. *PC-1* genotype together with elevated glucose, total cholesterol, triglyceride and serum HDL are independent risk factors for higher BMI. Combining the observation of Q allele at higher risk of obesity and Q allele being associated with insulin resistance in female, this study suggests a deleterious effect of the *PC-1* Q121 polymorphism on lipid metabolism in Chinese female. The PC-1 Q121 allele might be used for identifying individuals at high risk of developing obesity.

Our results are in contrast with two previous studies in Sicily and Dominican Republic populations [12,13], which failed to find higher allele frequency in obese subjects. In addition, a great difference of allele frequencies was observed among the populations. The ethnic differences may have population specific effects to modify the risk. Another possible explanation might lie in plenty of environmental factors, especially for food structure, also involving in the aetiology of obesity. The same genetic determinants might have different contribution to the aetiology of a complex disease when interacting with different environmental factors. Gender difference could also complicate the genetic influence to of obesity in separated researches.

The frequency of PC-1 Q allele in the non-obese Chinese, 7.5%, was much lower than that in Europeans and Americans, 12.9–21.5% [5,9,12,14–16]. In a black children population Q allele frequency was 77%, showing highly significant difference with most other populations [17]. Given this low Q allele prevalence, there are limited amount of KQ/QQ individuals in the present population. It is worthy to collect a larger sample to detect the possible impact of PC-1 gene on human lipid metabolism in future study. Our finding of an absolutely low Q allele frequency in Chinese Han population is intriguing, which suggests a high differentiation of allelic frequencies among different ethnic continents. As such, K121Q genotyping should be useful in forensic epidemiology and population genetics.

In summary, the present study suggests that the subjects carrying the Q allele are more likely to get fatter in respect to subjects with K121K genotype in female. K121Q polymorphism of *PC-1* gene may be a useful molecular marker reflecting obesity susceptibility. Large, prospective studies are needed to confirm this preliminary observation, especially in Chinese, because there are few data in Asian population. Furthermore, transfection and functional studies in cell line would certainly help to understand the potential implications of this polymorphism on cellular insulin action and lipid metabolism.

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