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Genetic Polymorphism PC-1 K121Q and Ethnic Susceptibility to Insulin Resistance

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Genetic susceptibility may be responsible for high prevalence of insulin resistance in Asian Indians. This study was carried out in samples of local Asian Indians and Caucasians to determine whether plasma cell membrane glycoprotein (PC)-1 K121Q and insulin receptor substrate-1 (IRS-1) G972A polymorphisms contribute significantly to susceptibility to insulin resistance in Asian Indians. The frequency of carrying at least one copy of the PC-1 121Q variant in Asian Indians was significantly higher than that in Caucasians ($P = 0.01$), but the frequency was similar for IRS-1 972A (6% and 7%). A significantly higher insulin area under the curve during oral glucose tolerance testing ($P < 0.0001$) and lower insulin sensitivity during hyperinsulinemic-euglycemic clamps ($P = 0.04$) were found in Asian Indians with PC-1 121Q variant compared with Asian Indians with wild-type PC-1 and with Caucasians with or without the polymorphism. IRS-1 972A was not associated with any change in insulin sensitivity. We conclude that the PC-1 K121Q polymorphism associates with primary insulin resistance in migrant Asian Indians. A relatively high frequency of this polymorphism thus may be one factor contributing to insulin resistance susceptibility in Asian Indians. This finding indicates the need for expanded studies on the association between PC-1 K121Q and insulin resistance in a representative sample of the Asian Indian population. (*J Clin Endocrinol Metab* 88: 5927–5934, 2003)

INSULIN RESISTANCE IS a major component of the metabolic syndrome and is a risk factor for both type 2 diabetes and coronary heart disease (CHD) (1–10). Obesity, particularly truncal obesity, is strongly associated with insulin resistance (11, 12). The rising prevalence of obesity worldwide and obesity-related insulin resistance are largely responsible for the increasing incidence of type 2 diabetes as well as the metabolic syndrome, a cardiovascular risk factor. However, many studies show that there is a strong genetic component to insulin resistance. Some populations, such as those of South Asia, *e.g.* Asian Indians, seemingly have a high susceptibility to insulin resistance (13–15), type 2 diabetes (16–19), and cardiovascular disease (20–24). A small increase in body fat content translates into a significant increase in risk for diabetes and possibly cardiovascular disease in this susceptible population.

One potential mechanism for insulin resistance may be genetic defects that impair the insulin-signaling pathway. Several candidate genes for defects in insulin signaling have been postulated, and a few studies report positive associations between polymorphisms of genes in the insulin-signaling pathway and insulin resistance (25). Two of the latter are insulin receptor substrate-1 (IRS-1) G972A (26–29) and plasma cell membrane glycoprotein (PC-1) K121Q (30–39). The present study was carried out to determine whether these two polymorphisms contribute to the ethnic susceptibility to insulin resistance of Asian Indians. To accomplish this goal we assessed the frequency of IRS-1 G972A and PC-1 K121Q in Asian Indians and Caucasians. In addition, insulin responsiveness to an oral glucose load and glucose disposal rates during euglycemic hyperinsulinemia (insulin sensitivity index) were measured in subjects with IRS-1 G972A and PC-1 K121Q *vs.* wild-type in these two populations.

Abbreviations: AUC, Area under the curve; CHD, coronary heart disease; GCRC, General Clinical Research Center; HDL, high density lipoprotein; IRS, insulin receptor substrate; LDL, low density lipoprotein; OGTT, oral glucose tolerance test; PC-1, plasma cell membrane glycoprotein; Rd, rate of glucose disposal; VLDL, very low density lipoprotein.

Subjects and Methods

Subjects and study protocol

To determine whether the frequencies of the two candidate genetic polymorphisms were different in the two ethnic groups, we first recruited Asian Indians and Caucasians by public advertisement and offering free screening for cardiovascular risk factors at University of Texas Southwestern Lipid and Heart Disease Risk Management Clinic (Dallas, TX). Informed consent was obtained from all participants. The study was approved by the institutional review board at University of Texas Southwestern Medical Center (Dallas, TX). Each of these participants was administered a health questionnaire. Personal and family history of coronary artery disease included any history of angina, myocardial infarction, and bypass surgery. Height and weight were measured by standard procedures. Blood pressure was measured after about 5 min of rest in the sitting position, using an automated sphygmomanometer. A blood sample was then drawn from each participant and immediately refrigerated. After separation of plasma and serum, aliquots were frozen at −80 C. Blood samples were collected for biochemistries and genetic studies. To further evaluate the impact of the studied mutations on insulin resistance, we invited the volunteers to participate in the second part of the study, which included more detailed anthropometric measurements (skinfolds, waist and hip circumferences, and underwater weighing) and an oral glucose tolerance test (OGTT). To further explore the role of genetics on insulin resistance, we invited young individuals (<40 yr of age) to have hyperinsulinemic-euglycemic clamps as well.

A total of 1376 subjects volunteered for the first part of the study. Of those, 638 originated from the Asian Indian subcontinent (India, Pakistan, and Bangladesh), and 738 subjects were of European ancestry. The

Asian Indian group included both subjects who had recently immigrated to U.S. and those who were born in U.S. In Table 1 the general characteristics of Asian Indians and Caucasians are compared. The 2 ethnic groups had similar ratios of male to female participants, but the percentage of menopausal women was lower in the Asian Indian group, probably as a reflection of their younger age. Despite lower average age and body mass index, systolic blood pressure, diastolic blood pressure, and fasting plasma insulin concentrations were significantly higher in the Asian Indians compared with the Caucasians. Plasma concentrations of total cholesterol and low (LDL) and high (HDL) density lipoprotein cholesterol were significantly lower in the Asian Indians, whereas plasma triglycerides concentrations were higher. Personal and family histories in the 2 ethnic groups are compared in Table 2. The Asian Indians had twice the prevalence of personal history for diabetes mellitus as the Caucasians ($P = 0.01$). The family history of coronary artery disease was significantly lower in the Asian Indians ($P < 0.0001$). The data on family history were obtained by each subject's recall. It is unclear why a lower frequency of coronary artery disease was detected in the Asian Indians despite higher family history of diabetes mellitus. A similar frequency of smoking and the remainder of personal and family history were reported in the 2 ethnic groups.

Subjects with diabetes mellitus or other endocrine disorders, coronary artery disease, renal insufficiency, or liver test abnormalities and those receiving any form of therapy were excluded from the metabolic studies. All volunteers were weight stable before entering the body composition and metabolic studies. A group of 158 Asian Indians and 152 Caucasians volunteered for the body composition and OGTT studies. A group of 23 Asian Indians and 28 Caucasians also volunteered for hyperinsulinemic-euglycemic clamps. Those who had OGTT and body composition studies were admitted for 1 d to the General Clinical Research Center (GCRC) of University of Texas. Those who also volunteered for the clamp studies were admitted for 4 d to the GCRC and were provided with an isocaloric diet (calculated from height, weight, and age; containing 30% of calories from fat, 55% from carbohydrate, 15% from protein, and 300 mg cholesterol) as out-patients for 3 d. They were then admitted to the in-patient unit of the GCRC and had a hyperinsulinemic-euglycemic clamp study on the morning of d 4.

Body composition studies

All participants in the OGTT and clamp studies had anthropometric measurements made during admission at the GCRC. Waist and hip circumferences were measured, using a flexible measuring tape with a tension caliper at the extremity (Gulick-creative Health Product, Inc., Plymouth, MI), midway between xyphoid and umbilicus during the midexpiratory phase, and at the maximum circumference in the hip area, respectively. Skin folds thickness was measured at nine different anatomical sites (subscapular diagonal and vertical, chest, midaxillary, abdominal horizontal and vertical, suprailiac diagonal and vertical, triceps, biceps, thigh, and calf) using a Lange skinfold caliper (Cambridge Scientific Instruments, Inc., Cambridge, MD), as previously reported (12). Body density was calculated from measurements of body volume determined by hydrostatic weighing, with adjustment for residual volume measured by helium dilution during the underwater weighing. Percent body fat was calculated using the Siri equation (40), assuming a fat-free density of 1.10 g/cc for men and 1.097 g/cc for women.

Glucose tolerance testing

A standard oral glucose tolerance test with 75 g glucose (Tru-Glu100, Fisher Scientific, Pittsburgh, PA) was conducted after 12-h overnight fasting. An iv catheter was placed in a forearm vein, and blood was collected for determination of glucose and insulin concentrations before glucose administration and at 30-min intervals thereafter for 180 min.

Hyperinsulinemic-euglycemic clamps

On the morning of study d 4, breakfast was withheld, and the euglycemic-hyperinsulinemic clamp procedure was performed after an overnight fast. Two polyethylene catheters were placed under local anesthesia. One catheter was placed in a dorsal hand vein for blood sampling, and the hand was kept in a heated box at 70 C for arterialization of venous blood. At 0800 h, a primed-continuous infusion of regular insulin (Humulin, Squibb-Novo, Princeton, NJ) was started at a rate of 80 mU/m^2 (body surface area)·min and continued for 2 h. The insulin infusion rate of 80 mU/m^2·min is expected to assure complete suppression of hepatic glucose output during the hyperinsulinemic phase of study. A 20% glucose infusion was started after 4 min of insulin infusion at a rate calculated to maintain the plasma glucose concentration at the fasting level throughout the clamp procedure, according to the method described by DeFronzo *et al.* (41). Blood for plasma glucose concentration measurements was drawn every 5 min for the entire duration of the study. Blood for the determination of insulin levels was

TABLE 2. General characteristics of the two ethnic study groups: personal history and family history

	Asian Indians	Caucasians	*P* value
Personal history			
Diabetes mellitus (%)	6	3	0.01
Coronary artery disease (%)	6	7	0.4
Stroke (%)	0.5	0.5	1
Hypertension (%)	11	11	0.9
Hyperlipidemia (%)	11	14	0.07
Smoking (%)	5	7	0.1
Family history			
Diabetes mellitus (%)	41	25	<0.0001
Coronary artery disease (%)	27	38	<0.0001
Stroke (%)	16	19	0.1
Hypertension (%)	36	33	0.2
Hyperlipidemia (%)	17	21	0.07

Data are expressed as the percentage of subjects who reported positive history within the same ethnic group. *P* values were determined using Fisher's exact test.

TABLE 1. General characteristics of the two ethnic study groups

	Asian Indians	Caucasians	*P* value
Number (male/female)	638 (377/261)	738 (347/391)	
Age (yr)	41 ± 14	45 ± 15	<0.0001
Postmenopausal status (% of women)	15	26	0.001
BMI (kg/m^2)	24.5 ± 4.1	25.3 ± 6.5	0.008
Systolic blood pressure (mm Hg)	127 ± 19	120 ± 16	<0.0001
Diastolic blood pressure (mm Hg)	80 ± 12	74 ± 11	<0.0001
Glucose (mg/dl)	91 ± 31	90 ± 19	0.7
Insulin (μU/ml)	16 ± 25	10 ± 11	<0.0001
Total cholesterol (mg/dl)	181 ± 37	187 ± 42	0.02
LDL cholesterol (mg/dl)	110 ± 31	115 ± 37	0.007
HDL cholesterol (mg/dl)	45 ± 12	47 ± 15	0.03
Triglycerides (mg/dl)	154 ± 118	139 ± 122	0.0003

Results are expressed as the mean ± SD, unless otherwise specified. *P* values were determined using the Mann-Whitney *U* test for comparison of means and using the Fisher's exact test for comparison of frequency. Systeme International conversion factors are: 0.0555 mmol/liter for glucose, 0.6945 pmol/liter for insulin, 0.0259 mmol/liter for cholesterol, and 0.0113 mmol/liter for triglycerides.

drawn every 10 min from −30 to 0 min (baseline phase) and from 80–120 min (hyperinsulinemic phase, after 80 min of equilibration time).

The rate of glucose infusion was calculated based on glucose concentration obtained at various times during the clamp study. The rate of glucose disposal (Rd) was calculated by subtracting the urinary glucose excretion from the rate of glucose appearance and using space correction. The Rd data were computed in milligrams per minute per kilogram of lean body mass. To account for differences in plasma insulin levels during hyperinsulinemia, the insulin sensitivity index was obtained for the study groups by dividing Rd by plasma insulin concentrations in milliunits per milliliter × 100.

Biochemistries

Cholesterol and triglycerides were measured with enzymatic methods. Very low density lipoprotein (VLDL; density, <1.006 g/ml) was removed by preparative ultracentrifugation, and cholesterol was measured in the VLDL fraction and infranatant. HDL cholesterol was determined in the supernatant after precipitating apolipoprotein B-containing lipoproteins using heparin-manganese chloride. LDL cholesterol was calculated as the difference between the cholesterol content of the 1.006 g/ml infranatant and HDL cholesterol. Insulin was measured by RIA at Linco Research, Inc. (St. Charles, MO).

DNA amplification by PCR

Fasting blood samples were drawn into a 10-ml vacuum tube containing EDTA. Plasma was separated by centrifugation and stored at 4 C until analysis. Genomic DNA was isolated from whole blood using commercial DNA isolation kits from Qiagen (Chatsworth, CA). The genomic DNA of each subject was amplified in a final volume of 10 μl containing 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 75 ng of each primer, 100 μM deoxy-NTP, and 1 U *Taq* polymerase.

Assay of PC-1 and IRS-1 polymorphisms

PC-1 and IRS-1 polymorphisms were detected by PCR-restriction fragment length polymorphism analysis. The PC-1 K121Q polymorphism creates an *Ava*II restriction site (5′-GGACC-3′). To assay this polymorphism, a 238-bp fragment encompassing the region of interest was amplified by PCR using oligonucleotides 5′-CTGTGTTCACTTTG-GACATGTTG-3′ and 5′-GACGTTGGAAGATACCAGGTTG-3′. The PCR-amplified DNA fragment was digested by adding 10 U *Ava*II restriction enzyme in 30 μl buffer 4 (New England BioLabs, Inc., Beverly, MA) to the PCR product, and then it was run on a 2% agarose gel. The K alleles are displayed as a single uncut band of 238 bp, whereas the Q alleles are shown as a doublet of 148- and 90-bp bands. The IRS-1 G972A polymorphism creates an *Sma*I restriction site (5′-CCCGGG-3′). To assay the IRS-1 G972 polymorphism, a 200-bp fragment was PCR-amplified using the primers 5′-CTTTCCACAGCTCACCTTCTGTCA-3′ and 5′-CCGGGTAGGCCTGCAAATGCTAGC-3′. Digestion was obtained by adding 10 U *Sma*I in 30 μl buffer 4 (New England Biolabs, Inc.) to the PCR product. The digestion products were then run on a 2% agarose gel.

Statistical analysis

Continuous demographic variables were compared between Asian Indians and Caucasians using the Mann-Whitney U test. The frequencies of mutations and of personal or family history variables were compared between ethnic groups using Fisher's exact test. Two-way ANOVA models were used to assess the effects of ethnicity, polymorphisms, and the interaction between ethnic group and polymorphisms. Multiple comparisons of these group means were made with the least squares contrasts of the ANOVA models. Due to skewness, triglycerides were log-transformed before analysis. Statistical analysis was performed using SAS version 8.02 (SAS Institute, Inc., Cary, NC).

Results

Polymorphism frequency

Thirty-three percent of Asian Indians had at least one copy of PC-1 121Q; the frequency in Caucasians was significantly less at 27% (Fig. 1). The prevalence of carrying at least one copy of IRS-1 972A was much lower (6% in Asian Indians and 7% in Caucasians). There were 3% of Asian Indians and 2% of Caucasians with homozygosity of the PC-1 121 Q polymorphism. The allele frequency of PC-1 121Q was 14.3% in the Caucasian group and 17.9% in the Asian Indian group (by χ^2, $P = 0.01$). The results are in Hardy-Weinberg equilibrium. Individuals homozygous and heterozygous for PC-1 121Q were grouped together for analysis and identified as a study subgroup carrying the PC-1 121Q variant. Similarly, individuals homozygous and heterozygous for IRS-1 972A were grouped together for analysis.

OGTT

A total of 158 Asian Indians and 152 Caucasians underwent OGTT (Tables 3 and 4). Although the 2 groups were of similar age and body mass index, Asian Indians generally had a higher thickness of truncal skin folds than Caucasians. Systolic blood pressure tended to be lower in Asian Indians. Figure 2 depicts the changes in plasma glucose and plasma insulin concentrations during OGTT and the overall glucose and insulin area under the curve (AUC) during the OGTT. Plasma glucose concentrations and glucose AUC were similar during the OGTT for the 4 subgroups. The plasma insulin AUC was higher for the Asian Indians as a group than for Caucasians. However, the most striking difference was present in Asian Indians with PC-1 121Q variant. In this

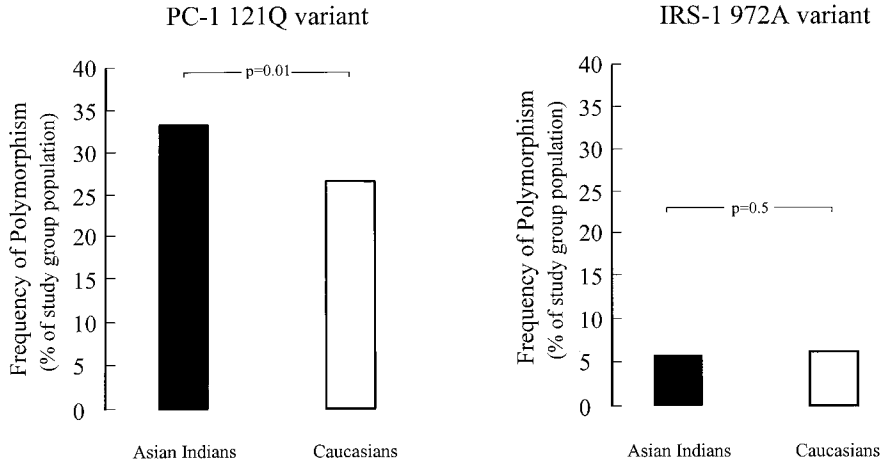

FIG. 1. Frequency of PC-1 121Q and IRS-1 972A variants in Asian Indians and Caucasians.

TABLE 3. General characteristics of Asian Indians and Caucasians who underwent OGTT

	Asian Indians		Caucasians	
	Wild-type PC 1	PC-1 121Q carriers	Wild-type PC 1	PC-1 121Q carriers
No. (male/female)	110 (73/37)	48 (30/18)	108 (42/66)	44 (21/23)
Age (yr)	33 ± 11	33 ± 11[a]	30 ± 8	27 ± 7[b]
Postmenopausal status (% of women)	8	6	6	9
BMI (kg/m^2)	24.3 ± 4.0	24.3 ± 3.1	25.5 ± 5.5	24.5 ± 4.8
Systolic blood pressure (mm Hg)	112 ± 13	110 ± 14[a]	114 ± 13	118 ± 11[b]
Diastolic blood pressure (mm Hg)	70 ± 11	69 ± 9	69 ± 10	70 ± 10
Total cholesterol (mg/dl)	165 ± 35	159 ± 30	165 ± 36	163 ± 39
LDL cholesterol (mg/dl)	103 ± 32	98 ± 26	97 ± 30	95 ± 38
HDL cholesterol (mg/dl)	42 ± 11[c]	42 ± 12[a]	50 ± 14[d]	50 ± 13[b]
Triglycerides (mg/dl)	107 ± 81	111 ± 62	101 ± 88	94 ± 55

Results are expressed as the mean ± SD unless otherwise specified. *P* values were determined using ANOVA for multiple comparisons of means and using the Fisher's exact test for comparison of frequency. Systeme International conversion factors are: 0.0259 mmol/liter for cholesterol and 0.0113 mmol/liter for triglycerides.

[a] $P < 0.05$ for mean difference *vs.* the group of Caucasians with PC-121Q variant.
[b] $P < 0.05$ for mean difference *vs.* the group of Asian Indians with PC-1 121Q variant.
[c] $P < 0.05$ for mean difference *vs.* the group of Caucasians with wild-type PC 1.
[d] $P < 0.05$ for mean difference *vs.* the group of Asian Indians with wild-type PC 1.

TABLE 4. Body composition and fat distribution Asian Indians and Caucasians who underwent OGTTs

	Asian Indians		Caucasians	
	Wild-type PC 1	PC-1 121Q carriers	Wild-type PC 1	PC-1 121Q carriers
Body fat (% of total body weight)	26.1 ± 8.4	28.0 ± 7.0	27.3 ± 9.7	26.7 ± 8.7
Waist circumference (cm)	83 ± 1	82 ± 10	83 ± 13	83 ± 15
Hip circumference (cm)	94 ± 10[a]	94 ± 8	99 ± 11[b]	98 ± 11
Truncal skinfold thickness (cm)	123 ± 44	133 ± 42[c]	114 ± 46	105 ± 45[d]
Peripheral skinfold thickness (cm)	69 ± 32	73 ± 36	79 ± 37	71 ± 29

Results are expressed as the mean ± SD unless otherwise specified. *P* values were determined using ANOVA for multiple comparisons of means.

[a] $P < 0.05$ for mean difference *vs.* the group of Caucasians with wild-type PC 1.
[b] $P < 0.05$ for mean difference *vs.* the group of Asian Indians with wild-type PC 1.
[c] $P < 0.05$ for mean difference *vs.* the group of Caucasians with PC-1 121Q variant.
[d] $P < 0.05$ for mean difference *vs.* the group of Asian Indians with PC-1 121Q variant.

subgroup, the insulin AUC was strikingly higher than in the subjects with wild-type PC-1. In contrast, although statistically different, the insulin AUC was only marginally higher in Asian Indians with wild-type PC-1 than in Caucasians. Of interest, no difference was found between Caucasians subgroups with the PC-1 121Q variant and PC-1 wild-type. The IRS-1 polymorphism was not associated with any difference in either glucose AUC or insulin AUC during the OGTT among any of the study subgroups (data not shown).

Hyperinsulinemic-euglycemic clamp studies

Clamp studies were carried out in 23 Asian Indians, including 15 men and 3 women with wild-type PC-1 and 5 men with the 121Q variant. Plasma glucose levels were comparable between the 2 subgroups both at baseline [88 ± 4 mg/dl (4.9 ± 0.2 mmol/liter) and 88 ± 11 mg/dl (4.9 ± 0.06 mmol/liter), respectively] and during hyperinsulinemia [88 ± 4 mg/dl (4.9 ± 0.2 mmol/liter) and 86 ± 4 mg/dl (4.8 ± 0.2 mmol/liter), respectively]. On the other hand, baseline insulin and insulin concentrations during the last 40 min of the clamps were significantly higher in Asian Indians carrying the PC-1 121Q variant [200 ± 34 μU/ml (1389 ± 236 pmol/liter)] compared with those in Asian Indians with the wild-type PC-1 [167 ± 21 μU/ml (1160 ± 146 pmol/liter)]. There were no differences among any of the parameters listed in Tables 3 and 4 for the 2 subgroups selected for glucose clamp studies (data not shown). In those subjects with PC-1 121Q, insulin sensitivity was significantly reduced compared with that in subjects with wild-type PC-1 (Fig. 3).

Identical studies were performed in 28 Caucasians, including 19 subjects with wild-type PC-1 (10 males and 9 females) and 9 others with the 121Q variant (6 males and 3 females). During the hyperinsulinemic-euglycemic clamp, the levels of glucose were comparable in these 2 subgroups at baseline [86 ± 5 mg/dl (4.8 ± 0.3 mmol/liter) and 87 ± 7 mg/dl (4.8 ± 0.4 mmol/liter), respectively] and during hyperinsulinemia [88 ± 4 mg/dl (4.9 ± 0.2 mmol/liter) and 86 ± 4 mg/dl (4.8 ± 0.2 mmol/liter), respectively]. On the other hand, baseline insulin and insulin concentrations during the last 40 min of the clamps were similar in the Caucasians with wild-type PC-1 [172 ± 30 μU/ml (1195 ± 208 pmol/liter)] and Caucasians with PC-1 121Q [177 ± 22 μU/ml (1229 ± 153pmol/liter)]. No differences were observed between these 2 subgroups in any of the measurements listed in Tables 3 and 4. Moreover, the difference in insulin sensitivity index was not statistically significant between the 2 subgroups (Fig. 3), nor was the insulin sensitivity index for Asian Indians with wild-type PC-1 different from that for the Caucasian subgroups. Finally, the IRS-1 polymorphism was not associated with any difference in insulin sensitivity index among the study subgroups (data not shown).

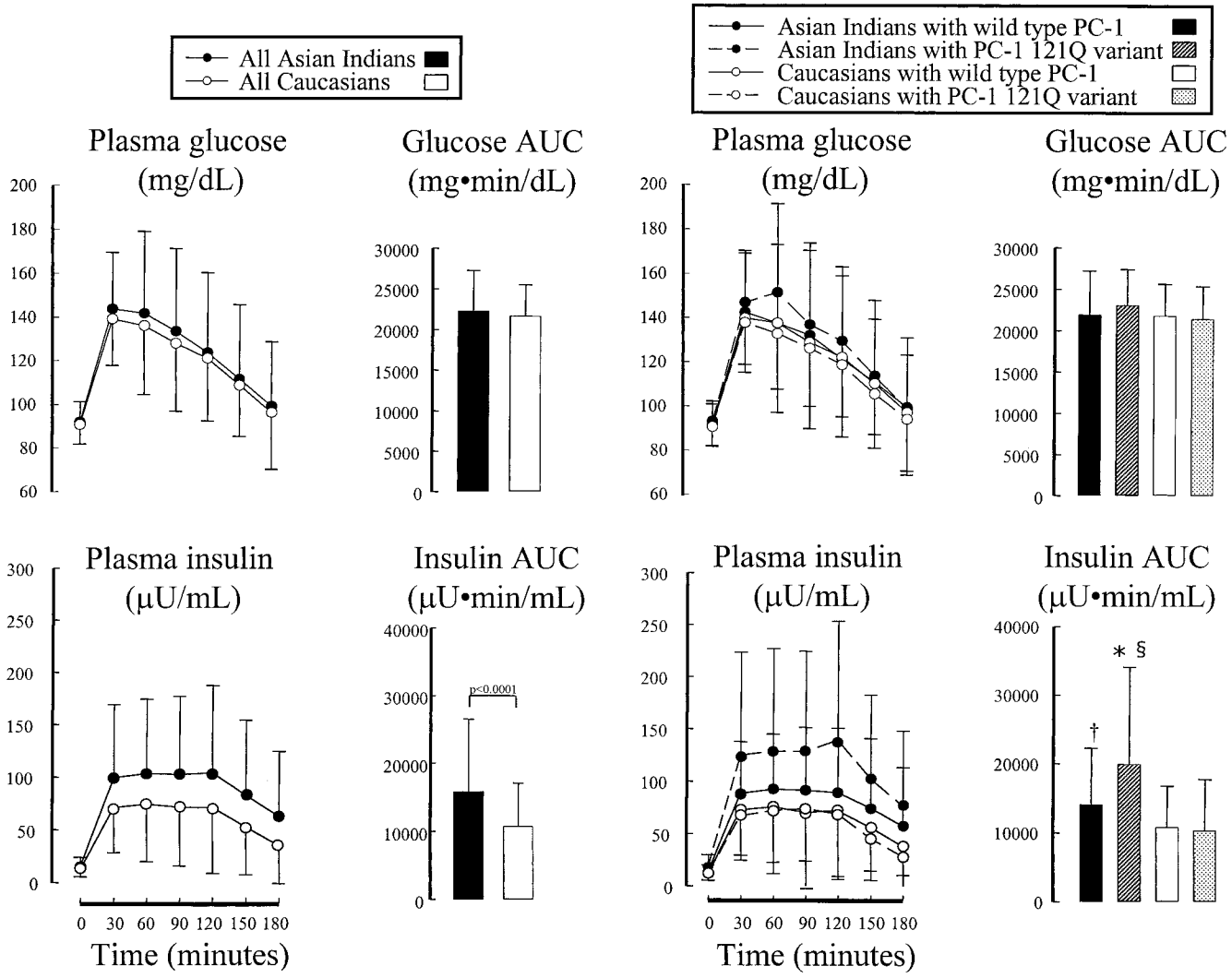

FIG. 2. Plasma glucose and insulin concentrations during the OGTT. *P* values were determined using ANOVA for multiple comparisons of means. *, $P < 0.0001$ for mean difference between Asian Indians with wild-type PC 1 and Asian Indians with the PC-1 121Q variant. §, $P < 0.0001$ for mean difference between Asian Indians with the PC-1 121Q variant and Caucasians with the PC-1 121Q variant. †, $P = 0.02$ for mean difference between Asian Indians with wild-type PC-1 and Caucasians with wild-type PC-1. The Systeme International conversion factor is 0.0555 pmol/liter for glucose and 0.695 pmol/liter for insulin.

Discussion

The major finding of this study is that the presence of a common genetic polymorphism of PC-1, the 121Q variant, is strongly associated with primary insulin resistance among migrant Asian Indians. This association was demonstrated by two independent methods on separate occasions: the insulin response to OGTT and the glucose disposal rate during hyperinsulinemic clamp. This finding accords with reports of a positive association between PC-1 121Q variant and insulin resistance in both southern and northern European populations (30, 31, 42, 43). Maddux *et al.* (32, 33) previously suggested that membrane glycoprotein PC-1 has an important modulating effect on cellular insulin signaling. Overexpression of membrane-bound PC-1 has been shown to inhibit insulin receptor tyrosine kinase activity in cell cultures (32, 34). Furthermore, the PC-1 content has been reported to be increased in skeletal muscle cells, adipocytes, and fibroblasts of insulin-resistant subjects (35–37). Although the specific mechanisms involved in the effects of the PC-1 K121Q polymorphism on insulin signaling is largely unknown, Costanzo *et al.* (38) showed that the presence of PC-1 K121Q is associated with a stronger inhibitory effect on insulin receptor than is wild-type PC-1. Decreased activation of tyrosine phosphorylation then results in reduced signaling for glucose transport and utilization in skeletal muscle cells and adipocytes (35, 36, 39).

If the PC-1 121Q allele associates with insulin resistance and confers increased risk for type 2 diabetes, it is of importance to determine the frequency of this polymorphism in various populations and the associated risk for type 2 diabetes. Previous studies in Caucasian subjects revealed a variable allele frequency in different populations. The lowest prevalences (9.8% and 10.5%) were reported in two unrelated nondiabetic Finnish groups by Kubszek *et al.* (43). Another study that included Finnish and Swedish subjects (42) reported a PC-1 121Q allele frequency of 13.8%, very similar to

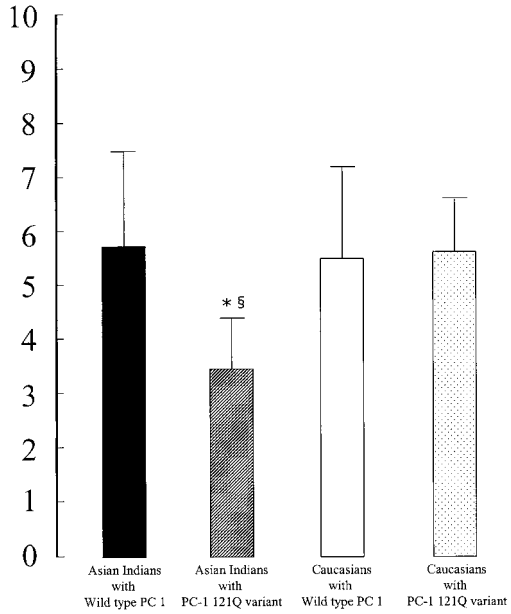

FIG. 3. Insulin sensitivity index from the hyperinsulinemic-euglycemic clamp studies. The insulin sensitivity index was computed from the Rd in milligrams per kilogram of lean body mass per minute and mean plasma insulin concentrations (microunits per milliliter) during the last 40 min of the clamp, as follows: (Rd/insulin) × 100. *P* values were determined using ANOVA for multiple comparisons of means. *, $P = 0.006$ for mean difference from the group of Asian Indians with wild-type PC 1. §, $P = 0.02$ for mean difference from the group of Caucasians with the PC-1 121Q variant.

the 14% reported in Spanish subjects (44). Pizzuti *et al.* (30) reported the highest PC-1 121Q allele frequency in a Sicilian population sample (17.8%). The finding of a PC-1 121Q allele frequency of 14.3 in the Caucasians of our study is consistent with the reported allele frequency in the European population. Interestingly, we detected a significantly higher PC-1 121Q allele frequency in our Asian Indian population. The allele frequency of 17.9% in Asian Indians is comparable to the reported frequency in the Sicilian population (30), which also manifests a strong association between PC-1 121Q and insulin resistance.

In the current study, Caucasians from our geographical region with the PC-1 121Q variant revealed no evidence of increased insulin resistance compared with the control group with wild-type PC-1. In previous reports, the finding of a positive association between PC-1 121Q and insulin resistance in other Caucasian populations has been inconsistent. Positive associations have been reported by Pizzutti *et al.* (30) and Frittitta *et al.* (31) for Sicilians, by Gu *et al.* (42) in intrafamily association studies in subjects from Finland and Sweden, and by Kubaszek *et al.* (43) in Finnish subjects. In contrast, Rasmussen *et al.* (45) and Gonzalez-Sanchez *et al.* (44) found no association among Danish and Spanish Caucasians, respectively.

The results of our study do not elucidate the reasons for the observed differences in allele frequencies of PC-1 121Q between Asian Indians and Caucasians or the molecular basis for the positive association between PC-1 121Q and insulin resistance in Asian Indians, but not in some Caucasian groups. Whether the difference in frequencies of the polymorphism is present in the whole South Asian population and the whole Caucasian population is unknown. Both Asian Indian and Caucasians in this study were samples of convenience and cannot be considered representative of the whole population. However, the difference in frequency is suggestive enough to justify a more extensive study and more representative sampling.

There is little doubt that Asian Indians as a group are more insulin resistant than Caucasians. This difference has been documented in several studies (14, 15, 46) and is consistent with a high prevalence of type 2 diabetes throughout India and in migrant Indians (16–19). These findings suggest that insulin resistance in Asian Indians has a strong genetic component. If so, Asian Indians could have a combination of independent genes, each affecting insulin sensitivity. Alternatively, there could be multiple interacting genes that are required to produce a high prevalence of insulin resistance. We cannot rule out the possibility that migrant Indians may not be representative of the whole South Asian population with respect to gene clustering. Migration may be more likely to occur in certain segments of the population, which could differ in genetic architecture from other segments. If so, the group prone to migration may be enriched in certain genetic patterns that influence insulin sensitivity. In the current study, details of immigrant status in the Asian Indian cohort, including recent *vs.* first generation and length of stay in the United States, were not documented sufficiently to draw a firm conclusion. Thus, replication of the current findings in different regions of the Indian subcontinent will be necessary to define the scope of the association between PC-1 121Q variant and insulin resistance in all Asian Indians.

A perplexing observation is the failure to observe an association between PC-1 121Q and insulin resistance in some Caucasian populations (44, 45). This failure raises the possibility that the positive association in Asian Indians is spurious. However, several findings support a true association. Positive associations have been reported in other Caucasian cohorts (30, 31, 42, 43) and were independently confirmed by both OGTT and glucose clamp studies in current Asian Indians. If the association is real, it still does not prove that PC-1 121Q *per se* can cause insulin resistance. It could be an associated marker for a particular genetic architecture that is responsible for insulin resistance. Such a genetic marker might occur in some populations, but not in others. Alternatively, PC-1 121Q acting alone may not be responsible for insulin resistance, but may interact with one or more other genes that are required to produce detectable insulin resistance. In our view this latter possibility seems the more likely mechanism to explain the inconsistent relationship between PC-1 121Q and insulin resistance in different populations.

The results of our study suggest that the PC-1 121Q polymorphism is a key factor for the development of insulin resistance in Asian Indians even if it does not act alone. Of interest, plasma AUCs during OGTT and insulin sensitivity indexes in Asian Indian PC-1 wild types were not different from those in Caucasians. Our study used two different techniques to assess insulin resistance: measurement of the insulin AUC of an OGTT and measurement of Rd during a hyperinsulinemic-euglycemic clamp. Although a smaller number of subjects participated in the clamp studies, the

findings from the OGTT on a much larger sample size were virtually identical those from the clamps. However, before it can be concluded that the PC-1 121Q variant is a dominant factor associated with insulin resistance in Asian Indians, the study would have to be greatly expanded to include a larger number of study participants in the hyperinsulinemic-euglycemic clamps. Given the relatively low and unequal prevalence of PC-1 121Q in different ethnic groups, future investigations should be designed to assure equal distribution of PC-1 subgroups within the clamp studies. In addition, to attempt a relative quantification of the impact of the PC-1 121Q polymorphism on the excessive insulin resistance of Asian Indians, more detailed information about environmental factors involved in the pathogenesis of insulin resistance is needed. It seems unlikely that a single polymorphism in one gene can entirely explain the propensity of South Asians to insulin resistance. Nonetheless, it is of interest that the frequency of the PC-1 121Q variant is higher among Asian Indians than Caucasians. This finding raises the possibility that PC-1 121Q confers a biological advantage, such as that postulated in the thrifty gene hypothesis (47).

Of interest are the results we obtained on IRS-1 972A. This polymorphism has also been associated with insulin resistance in previous studies (26, 27). However, the prevalence of the IRS-1 972A variant has been reported to be very low in the Caucasian population (48). Pima Indians, another ethnic group with excessive insulin resistance, have been shown not to have excessive prevalence of the IRS-1 972A mutation (28). The lack of association between the prevalence of IRS-1 972A polymorphisms and the prevalence of diabetes has been shown in both Caucasian and Asian Indian populations (29). Our findings confirm the low prevalence of this polymorphism in nondiabetic Caucasians and Asian Indians. Furthermore, we found no relationship between IRS-1 972A and excessive insulin resistance in the Asian Indian population. Although not all ethnic groups have been studied, the low prevalence of the mutation and the absence of an ethnic difference in prevalence suggest that the IRS-1 G972A polymorphism is unlikely to play a major role in the interethnic variability in insulin resistance susceptibility.

In summary, we conclude that PC-1 121Q polymorphism associates strongly with primary insulin resistance in Asian Indians and significantly contributes to the insulin resistance susceptibility of this population. Insulin resistance is almost certainly the result of a complex interaction of environmental/acquired factors and genetic factors. Obesity, fat distribution, and physical activity are related to insulin resistance, and they influence the variability of insulin sensitivity in the population. Nonetheless, identification of individuals who are genetically susceptible to insulin resistance may provide a useful tool for intervention strategies to reduce the risk for type 2 diabetes and cardiovascular disease in our population.

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References

1. **Fontbonne A. M., Eschwege, E. M** 1991 Insulin and cardiovascular disease. Paris Prospective Study. Diabetes Care 14:461–469
2. **Pyorala, M., Miettinen, H., Laakso, M., Pyorala, K** 1998 Hyperinsulinemia predicts coronary heart disease risk in healthy middle-aged men: the 22-year follow-up results of the Helsinki Policemen Study. Circulation 98:398–404
3. **Pyorala K, Savolainen E, Kaukola S, Haapakoski J** 1985 Plasma insulin as coronary heart disease risk factor: relationship to other risk factors and predictive value during 9 1/2-year follow-up of the Helsinki Policemen Study population. Acta Med Scand 701:(Suppl)38–52
4. **Despres JP, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S, Lupien PJ** 1996 Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med 334:952–957
5. **Howard G, O'Leary DH, Zaccaro D, Haffner S, Rewers M, Hamman R, Selby JV, Saad MF, Savage P, Bergman R** 1996 Insulin sensitivity and atherosclerosis. The Insulin Resistance Atherosclerosis Study (IRAS) Investigators. Circulation 93:809–817
6. **Reaven GM** 1988 Role of insulin resistance in human disease. Diabetes 37: 1595–1607
7. **Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C** 1993 Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. N Engl J Med 329:1988–1992
8. **Haffner SM, Stern MP, Mitchell BD, Hazuda HP, Patterson JK** 1990 Incidence of type II diabetes in Mexican Americans predicted by fasting insulin and glucose levels, obesity, and body-fat distribution. Diabetes 39:283–288
9. **Bergstrom RW, Newell-Morris LL, Leonetti DL, Shuman WP, Wahl PW, Fujimoto WY** 1990 Association of elevated fasting C-peptide level and increased intra-abdominal fat distribution with development of NIDDM in Japanese-American men. Diabetes 39:104–111
10. **Charles MA, Fontbonne A, Thibult N, Warnet JM, Rosselin GE, Eschwege E** 1991 Risk factors for NIDDM in white population. Paris prospective study. Diabetes 40:796–799
11. **Bogardus C, Lillioja S, Mott DM, Hollenbeck C, Reaven G** 1985 Relationship between degree of obesity and in vivo insulin action in man. Am J Physiol 248:286–291
12. **Abate N, Garg A, Peshock RM, Stray-Gundersen J, Grundy SM** 1995 Relationships of generalized and regional adiposity to insulin sensitivity in men. J Clin Invest 96:88–98
13. **UK Prospective Diabetes Study Group** 1994 UK Prospective Diabetes Study. XII. Differences between Asian, Afro-Caribbean and white Caucasian type 2 diabetic patients at diagnosis of diabetes. Diabetic Med 11:670–677
14. **McKeigue PM, Shah B, Marmot MG** 1991 Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. Lancet 337:382–386
15. **Chandalia M, Abate N, Garg A, Stray-Gundersen J, Grundy S. M** 1999 Relationship between generalized and upper body obesity to insulin resistance in Asian Indian men. J Clin Endocrinol Metab 84:2329–2335
16. **Dowse GK, Gareeboo H, Zimmet PZ, Alberti KG, Tuomilehto J, Fareed D, Brissonnette LG, Finch CF** 1990 High prevalence of NIDDM and impaired glucose tolerance in Indian, Creole, and Chinese Mauritians. Mauritius Noncommunicable Disease Study Group. Diabetes 39:390–396
17. **Ramachandran A, Snehlatha C, Dharmaraj D, Viswanathan M** 1992 Prevalence of glucose intolerance in Asian Indians. Urban-rural difference and significance of upper body adiposity. Diabetes Care 15:1348–1355
18. **Ramachandran A, Jali MV, Mohan V, Snehalatha C, Viswanathan M** 1988 High prevalence of diabetes in an urban population in south India. Br Med J 297:587–90
19. **Mather HM, Keen H** 1985 The Southall Diabetes Survey: prevalence of known diabetes in Asians and Europeans. Br Med J 291:1081–1084
20. **McKeigue PM, Miller GJ, Marmot MG** 1989 Coronary heart disease in south Asians overseas. J Clin Epidemiol 42:597–609
21. **Sarvotham SG, Berry JN** 1968 Prevalence of coronary heart disease in an urban population in northern India. Circulation 37:939–953
22. **Dewan BD, Malhotra KC, Gupta SP** 1974 Epidemiological study of coronary heart disease in rural community in Haryana. Indian Heart J 26:68–78
23. **Chadha SL, Radhakrishnan S, Ramachandran K, Kaul U, Gopinath N** 1990 Epidemiological study of coronary heart disease in urban population of Delhi. Indian J Med Res 92:424–430
24. **Beckles GL, Miller GJ, Kirkwood BR, Alexis SD, Carson DC, Byam NT** 1986 High total and cardiovascular disease mortality in adults of Indian descent in Trinidad, unexplained by major coronary risk factors. Lancet 1:1298–1301

25. **Groop L** 2000 Genetics of the metabolic syndrome. Br J Nutr 83:39–48
26. **Almind K, Inoue G, Pedersen O, Kahn CR** 1996 A common amino acid polymorphism in insulin receptor substrate-1 causes impaired insulin signaling. Evidence from transfection studies. J Clin Invest 97:2569–2575
27. **Hribal ML, Federici M, Porzio O, Lauro D, Borboni P, Accili, D, Lauro R, Sesti G** 2000 The Gly→Arg^{972} amino acid polymorphism in insulin receptor substrate-1 affects glucose metabolism in skeletal muscle cells. J Clin Endocrinol Metab 85:2004–2013
28. **Celi FS, Silver K, Waltson J, Knowler WC, Bogardus C, Shuldiner AR** 1995 Lack of IRS-1 codon 513 and 972 polymorphism in Pima Indians. J Clin Endocrinol Metab 80:2827–2829
29. **Hitman GA, Hawrami K, McCarthy MI, Viswanathan M, Snehalatha C, Ramachandran A, Tuomilehto J, Tuomilehto-Wolf E, Nissinen A, Pedersen O** 1995 Insulin receptor substrate-1 gene mutations in NIDDM; implications for the study of polygenic disease. Diabetologia 38:481–486
30. **Pizzutti A, Frittitta L, Argiolas A, Baratta R, Goldfine ID, Bozzali M, Ercolino T, Scarlato G, Iacoviello L, Vigneri R, Tassi V, Trischitta V** 1999 A polymorphism (K121Q) of the human glycoprotein PC-1 gene coding region is strongly associated with insulin resistance. Diabetes 48:1881–1884
31. **Frittitta L, Baratta R, Spampinato D, Di Paola R, Pizzuti A, Vigneri R, Trischitta V** 2001 The Q121 PC-1 variant and obesity have additive and independent effects in causing insulin resistance. J Clin Endocrinol Metab 86:5888–5891
32. **Maddux BA, Sbraccia P, Kamakura S, Sasson S, Youngren J, Fisher A, Spencer S, Grupe A, Henzel W, Stewart TA** 1995 Membrane glycoprotein PC-1 and insulin resistance in non-insulin-dependent diabetes mellitus. Nature 373:448–451
33. **Maddux BA, Goldfine ID** 2000 Membrane glycoprotein PC-1 inhibition of insulin receptor function occurs via direct interaction with the receptor α-subunit. Diabetes 49:13–19
34. **Kumakura S, Maddux BA, Sung CK** 1998 Overexpression of membrane glycoprotein PC-1 can influence insulin action at a post-receptor site. J Cell Biochem 3:366–377
35. **Youngren J, Maddux BA, Sasson S, Sbraccia P, Tapscott EB, Swanson MS, Dohm GL, Goldfine ID** 1996 Skeletal muscle content of membrane glycoprotein PC-1 in obesity. Relationship to muscle glucose transport. Diabetes 45:1324–1328
36. **Frittitta L, Youngren J, Sbraccia P, D'Adamo M, Buongiorno A, Vigneri R, Goldfine ID, Trischitta V** 1997 Increased adipose tissue PC-1 protein content, but not tumour necrosis factor-α gene expression, is associated with a reduction of both whole body insulin sensitivity and insulin receptor tyrosine-kinase activity. Diabetologia 40:282–289
37. **Frittitta L, Spampinato D, Solini A, Nosadini R, Goldfine ID, Vigneri R, Trischitta V** 1998 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 47:1095–1100
38. **Costanzo BV, Trischitta V, Di Paola R, Spampinato D, Pizzuti A, Vigneri R, Frittitta L** 2001 The Q allele variant (GLN121) of membrane glycoprotein PC-1 interacts with the insulin receptor and inhibits insulin signaling more effectively than the common K allele variant (LYS121). Diabetes 50:831–836
39. **Frittitta L, Youngren J, Vigneri R, Maddux BA, Trischitta V, Goldfine ID** 1996 PC-1 content in skeletal muscle of non-obese, non-diabetic subjects: relationship to insulin receptor tyrosine kinase and whole body insulin sensitivity. Diabetologia 39:1190–1195
40. **Siri WE** 1961 Techniques for measuring body composition. Washington DC: National Academy of Sciences; 223–224
41. **DeFronzo RA, Tobin JD, Andres R** 1979 Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 237:E214–E223
42. **Gu HF, Almgren P, Lindholm E, Frittitta L, Pizzuti A, Trischitta V, Groop LC** 2000 Association between the human glycoprotein PC-1 gene and elevated glucose and insulin levels in a paired-sibling analysis. Diabetes 49:1601–1603
43. **Kubaszek A, Pihlajamaki J, Karhapaa P, Vauhkonen I, Laakso M** 2003 The K121Q polymorphism of the PC-1 gene is associated with insulin resistance but not with dyslipidemia. Diabetes Care 26:464–467
44. **González-Sánchez JL, Martínez-Larrad MT, Fernández-Pérez C, Kubaszek A, Laakso M, Serrano-Ríos M** 2003 K121Q PC-1 gene polymorphism is not associated with insulin resistance in a Spanish population. Obes Res 11: 603–605
45. **Rasmussen SK, Urhammer SA, Pizzuti A, Echwald SM, Ekstrom CT, Hansen L, Hansen T, Borch-Johnsen K, Frittitta L, Trischitta V, Pedersen O** 2000 The K121Q variant of the human PC-1 gene is not associated with insulin resistance or type 2 diabetes among Danish Caucasians. Diabetes 49:1608–1611
46. **Kalhan R, Puthawala K, Agarwal S, Amini SB, Kalhan SC** 2001 Altered lipid profile, leptin, insulin, and anthropometry in offspring of South Asian immigrants in the United States. Metabolism 10:1197–1202
47. **Neel JV** 1962 Diabetes mellitus: a thrifty genotype rendered detrimental by progress. Am J Hum Genet 14:353–362
48. **Clausen JO, Hansen T, Bjorbaek C, Echwald SM, Urhammer SA, Rasmussen S, Andersen CB, Hansen L, Almind K, Winther K, Haraldsdottir J, Borch-Johnsen K** 1995 Insulin resistance: interactions between obesity and a common variant of insulin receptor substrate-1. Lancet 346:397–402