ENPP1/PC-1 K121Q Polymorphism and Genetic Susceptibility to Type 2 Diabetes

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Genetic susceptibility modulates the impact of obesity on risk for type 2 diabetes. The present study evaluates the role of ENPP1 K121Q polymorphism in prediction of type 2 diabetes in three populations that differ in susceptibility to diabetes and environmental exposure. The three cohorts included 679 nonmigrant South Asians living in Chennai, India (223 with type 2 diabetes); 1,083 migrant South Asians living in Dallas, Texas (121 with type 2 diabetes); and 858 nonmigrant Caucasians living in Dallas, Texas (141 with type 2 diabetes). Patients with type 2 diabetes were included in these cohorts if they had diabetes onset before the age of 60 years. The prevalence of subjects carrying the polymorphic ENPP1 121Q allele was 25% in the nondiabetic group and 34% in the diabetic group of South Asians living in Chennai ($P = 0.01$). The prevalence in the nondiabetic and diabetic groups were 33 and 45% ($P = 0.01$) for the South Asians living in Dallas and 26 and 39% ($P = 0.003$) for the Caucasians. Although further replication studies are necessary to test the validity of the described genotype-phenotype relationship, our study supports the hypothesis that ENPP1 121Q predicts genetic susceptibility to type 2 diabetes in both South Asians and Caucasians. *Diabetes* 54:1207–1213, 2005

Type 2 diabetes has reached epidemic proportions in the U.S. and has become a major public health problem of worldwide dimensions. Escalating rates of type 2 diabetes are likely attributable to changes in the environment favoring the onset of obesity, a major risk factor for type 2 diabetes. However, not all obese persons develop diabetes. Furthermore, a large variation in the prevalence of type 2 diabetes is observed among different ethnic groups, even when they are exposed to similar environmental conditions (1). These observations support the notion that genetic factors predispose susceptible individuals and populations to be at excessive risk for type 2 diabetes in the context of the current worldwide epidemic of obesity. An example of a susceptible population is given by the South Asians. People originating from India, Pakistan, or Bangladesh have been shown to be excessively insulin resistant even in the absence of obesity (2). These findings may help explain why the prevalence of diabetes among South Asians in various areas of the world is increased (3–7), and they suggest that susceptibility to diabetes may derive from genetic determinants of insulin resistance. Therefore, we recently took the approach of evaluating the role of candidate gene variants in determining ethnic differences in insulin resistance, and we have shown that a single polymorphism in exon 4 of the ENPP1 gene (K121Q), also known as PC-1 K121Q, largely explains the obesity-independent excess of insulin resistance of South Asians (8). However, the role of the ENPP1 121Q allele on the pathogenesis of "primary" forms of insulin resistance in other ethnic groups remains controversial (9–14). Moreover, the role of the ENPP1 121Q allele on predisposition to type 2 diabetes has not been firmly established (11,14–17). In the current study, we evaluated the role of the ENPP1 121Q variant on prediction of type 2 diabetes in three different cohorts characterized by different "susceptibility" to diabetes and living in different environmental conditions: South Asians living in Chennai, India; South Asians who migrated to Dallas, Texas; and Caucasians living in Dallas, Texas.

RESEARCH DESIGN AND METHODS

Diabetic and nondiabetic South Asians and Caucasians living in Dallas were recruited by public advertisement and by offering free screening for cardiovascular risk factors at the University of Texas Southwestern Lipid and Heart Disease Risk Management Clinic. Subjects with onset of diabetes at age <60 years were selected for this study. The South Asians enrolled in the Dallas cohort were new immigrants or first generation from India, Pakistan, or Bangladesh. The Caucasians were non-Hispanic whites of European descent. The subjects in the South Asian group living in Chennai were participants of the Chennai Urban Rural Epidemiological Study (CURES) (18). This study is being conducted in a representative population (aged ≥20 years) of Chennai (formerly Madras), the fourth largest city in India, with a population of ~4.2 million. The nondiabetic subjects were chosen randomly from the CURES population. Informed consent was obtained from all the participants. The study was approved by the institutional review boards of the University of Texas Southwestern Medical Center at Dallas and by the ethical committee of the Madras Diabetes Research Foundation, Chennai.

Each of the participants was administered a health questionnaire. Personal and family history of diabetes was obtained. Height and weight were measured by standard procedures. Blood pressure was measured after ~5 min rest in the sitting position, using an automated sphygmomanometer. A blood

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AUC, area under the curve; CURES, Chennai Urban Rural Epidemiological Study; HOMA-IR, homeostasis model assessment of insulin resistance; OGTT, oral glucose tolerance test.

TABLE 1
General characteristics of South Asians living in Chennai

	Nondiabetic subjects		Diabetic subjects	
	ENPP1 wild type	ENPP1 121Q	ENPP1 wild type	ENPP1 121Q
n (M/F)	344 (112/232)	112 (49/63)	148 (64/84)	75 (36/39)
Age (years)	44 ± 15	44 ± 16	41 ± 6	43 ± 5
BMI (kg/m^2)	24 ± 5	23 ± 5	26 ± 5*†	25 ± 4
Systolic BP (mmHg)	122 ± 18	119 ± 14	124 ± 16†	120 ± 17
Diastolic BP (mmHg)	77 ± 11	74 ± 10	79 ± 10†	76 ± 11
Total cholesterol (mg/dl)	184 ± 39	179 ± 38	198 ± 41	207 ± 47
LDL cholesterol (mg/dl)	116 ± 33	112 ± 33	118 ± 38	125 ± 39
HDL cholesterol (mg/dl)	45 ± 10	45 ± 11	43 ± 9	44 ± 9
Triglycerides (mg/dl)	115 ± 62	113 ± 61	184 ± 162*†	193 ± 195*†
Fasting glucose (mg/dl)	85 ± 9	85 ± 9	162 ± 63*†	180 ± 77*†
HOMA-IR	1.9 ± 1.3	1.6 ± 0.9	NA	NA

Data are means ± SD unless otherwise specified. *P* values were determined using ANOVA for multiple comparisons of means. *$P < 0.05$ for mean difference with the subgroup of nondiabetic subjects with wild-type ENPP1. †$P < 0.05$ for mean difference with the subgroup of nondiabetic subjects with ENPP1 K121Q variant. BP, blood pressure.

sample was drawn after overnight fast from each participant for glucose determination and for the genetic studies. All biochemical investigations and genotyping of subjects from Chennai were carried out at the Madras Diabetes Research Foundation, Chennai, India.

Patients with diabetes were identified based on their history, use of hypoglycemic agents, fasting plasma glucose (≥126 mg/dl), and 2-h plasma glucose (≥200 mg/dl), when available. Only patients with diabetes onset before age 60 years were selected for this study to minimize age differences between case and control subjects. Of the diabetic subjects, 7% were on diet only, 83.3% were on sulfonylureas, and 9.8% were on insulin therapy for management of diabetes. For the subjects without history of type 2 diabetes and with fasting plasma glucose ≤126 mg/dl, an oral glucose tolerance test (OGTT) with 75 g of glucose was conducted after 12 h overnight fasting in all the subjects from the Chennai cohort and in a subgroup of 306 volunteers from the Dallas cohorts.

DNA amplification by PCR and assay of ENPP1 polymorphism. As previously described (8), genomic DNA was isolated from whole blood using commercial DNA isolation kits from Qiagen (Chatsworth, CA). ENPP1 K121Q was detected by PCR restriction fragment–length polymorphism analysis using a PCR-amplified DNA fragment digested with *Ava*II restriction enzyme and then run on a 2% agarose gel. To assure that the genotyping was of sufficient quality, we performed random duplicates in ~10% of the samples, and we carried controls from carriers and noncarriers in each genotyping assay. The assays were performed by a technician who was blind to the phenotype. No genotype errors were detected in the random duplicates.

Statistical analysis. The cohort of study subjects recruited in Dallas was a sample of convenience that included 262 diabetic subjects (141 Caucasians and 121 South Asians) and 1,679 nondiabetic subjects (717 Caucasians and 962 South Asians). This sample had an observed difference in K121Q prevalence of 13% between case (diabetic) and control (nondiabetic) subjects for the Caucasians (39% for the case subjects and 26% for the control subjects) and an observed difference of 12% for the South Asians (45% for the case subjects and 33% for the control subjects). Based on these data, we planned to recruit 200 diabetic and 400 control subjects from Chennai to assure a power of 0.85. Continuous demographic variables were compared between diabetic and nondiabetic subjects using Student's *t* test. Frequencies of the mutation were compared between diabetic and nondiabetic subjects using Fisher's exact test. Logistic regression analysis was performed to compare frequencies after adjustment for age, BMI, and sex differences between groups of comparison. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula described by Matthews et al. (19): (fasting insulin [μU/ml] × fasting glucose [mmol/l])/22.5. The insulin area under the curve ($AUC_{insulin}$) during an OGTT was calculated using the trapezoidal method. Because of skewness, triglycerides, HOMA-IR, and $AUC_{insulin}$ were log-transformed before analysis. Hardy-Weinberg equilibrium was assessed with a χ^2 test of goodness of fit among the random population sample. Regression analysis was performed to assess the relationship between BMI and $\log^e AUC_{insulin}$ and to compare regression lines between carriers of ENPP1 K121Q and those with the wild-type allele. Statistical analysis was performed using SAS version 8.02 (SAS Institute, Cary, NC). A meta-analysis of case-control studies published in PubMed until October 2004 on frequency of ENPP1 X121Q was performed to estimate the risk difference between patients with diabetes and individuals without diabetes. The studies were combined using inverse variance weights, and the *Q* statistic was used to test for heterogeneity. These computations were made with Comprehensive Meta-Analysis version 1.0.23 (Biostat, Englewood, NJ).

RESULTS

Tables 1-3 summarize and compare the general characteristics of the three study cohorts. Within each study group (diabetic and nondiabetic subjects), subgroups were identified based on their genotype. The ENPP1 121Q subgroup includes both homozygous and heterozygous subjects. When compared with the nondiabetic subjects, the diabetic subjects were older in the Dallas cohorts but slightly younger in the Chennai cohort. Other differences between the diabetic and nondiabetic groups included higher BMI and variables of the metabolic syndrome, such as higher systolic blood pressure, higher plasma triglycerides concentrations, and lower HDL cholesterol in the diabetic Caucasians but not in the South Asians living in Dallas. Higher BMI, blood pressure, and plasma triglyceride concentrations were observed in the diabetic subgroups of South Asians living in Chennai who carried polymorphic ENPP1. Most of the studied variables, including HOMA-IR, were not significantly different between carriers of polymorphic ENPP1 121Q and the wild-type subgroup. However, in 306 nondiabetic subjects from the Dallas cohort, we had more accurate measures of insulin resistance obtained by calculating integrated $AUC_{insulin}$ (20). Within the South Asians living in Dallas, the comparison between 108 nondiabetic subjects with wild-type ENPP1 and 45 nondiabetic subjects with polymorphic ENPP1 revealed median $AUC_{insulin}$ values of 11,471 and 15,750 μU · min/ml, respectively ($P = 0.006$, *t* test for independent variables). $AUC_{insulin}$ was not different in 108 nondiabetic Caucasians with wild-type ENPP1 and 44 nondiabetic Caucasians with polymorphic ENPP1 (median 9,559 and 8,066 μU · min/ml, respectively). $AUC_{insulin}$ was not available in the Chennai cohort. A comparison of general characteristics and $AUC_{insulin}$ among the nondiabetic subjects who underwent OGTT for measure of integrated $AUC_{insulin}$ is reported in Table 4.

Figure 1 illustrates the relationship between BMI and $AUC_{insulin}$ for nondiabetic Caucasians and South Asians living in Dallas. BMI was significantly correlated with

TABLE 2
General characteristics of South Asians living in Dallas

	Nondiabetic subjects		Diabetic subjects	
	Wild-type ENPP1	121Q ENPP1	Wild-type ENPP1	121Q ENPP1
n (M/F)	646 (378/268)	316 (187/129)	67 (47/20)	54 (29/25)
Age (years)	43 ± 14	42 ± 14	56 ± 10*†	53 ± 129*†
BMI (kg/m^2)	25 ± 4	25 ± 4	26 ± 4	25 ± 4
Systolic BP (mmHg)	126 ± 18	128 ± 20	137 ± 18	134 ± 21
Diastolic BP (mmHg)	79 ± 11	81 ± 12	82 ± 12	80 ± 12
Total cholesterol (mg/dl)	186 ± 38	183 ± 35	187 ± 43	188 ± 34
LDL cholesterol (mg/dl)	112 ± 30	110 ± 29	107 ± 34	110 ± 30
HDL cholesterol (mg/dl)	45 ± 11	45 ± 11	42 ± 9	41 ± 10
Triglycerides (mg/dl)	161 ± 122	161 ± 110	189 ± 127	208 ± 116
Fasting glucose (mg/dl)	91 ± 13	91 ± 13	153 ± 57	170 ± 58*†‡
HOMA-IR	3.1 ± 5.2	3.5 ± 3.3	NA	NA

Data are means ± SD, unless otherwise specified. *P* values were determined using ANOVA for multiple comparisons of means. *$P < 0.05$ for mean difference with the subgroup of nondiabetic subjects with wild-type ENPP1. †$P < 0.05$ for mean difference with the subgroup of nondiabetic subjects with ENPP1 K121Q variant. ‡$P < 0.05$ for mean difference with the subgroup of diabetic subjects with wild-type ENPP1. BP, blood pressure.

$AUC_{insulin}$ in both ethnic groups. Multiple regression analysis revealed that increasing BMI was associated with larger increases in $AUC_{insulin}$ in the carriers of polymorphic ENPP1 than in those with the wild-type allele ($P = 0.03$ for interaction between BMI and polymorphism).

The frequencies of the ENPP1 121Q allele were 14, 19, and 16% in the cohort of South Asians living in Chennai, South Asians living in Dallas, and Caucasians living in Dallas, respectively. The results are in Hardy-Weinberg equilibrium. Figure 2 illustrates the findings on the prevalence of XQ and QQ in the diabetic and nondiabetic subjects. Because the prevalence of homozygosity was very low, the heterozygous and the homozygous were combined for statistical analysis. In all study cohorts, the prevalence of the XQ+QQ genotype was significantly higher in the diabetic than in the nondiabetic subjects. Fisher's exact test *P* values for differences in XQ+QQ prevalence between diabetic and nondiabetic groups were 0.01, 0.01, and 0.003 for the South Asians living in Chennai, the South Asians living in Dallas, and the Caucasians living in Dallas, respectively. When subgroups were identified based on homozygosity and heterozygosity, only heterozygosity was shown to be significantly more frequent in the diabetic groups compared with the nondiabetic groups of the three study cohorts. The prevalence of homozygosity was comparable in nondiabetic and diabetic groups, likely because of the low frequency of this condition. Logistic regression analysis revealed that potential confounding variables, including age, sex distribution, and BMI differences between the diabetic and the nondiabetic subjects, did not affect the reported differences. After adjustment for age, sex, and BMI, *P* values were 0.01, 0.01, and 0.04 for the South Asians living in Chennai, the South Asians living in Dallas, and the Caucasians living in Dallas, respectively. Odds ratios (ORs) after adjustment for age, sex, and BMI differences between diabetic and nondiabetic subjects were 1.62 (CI 1.12–2.32), 1.86 (1.21–2.87), and 1.90 (1.04–3.47) for the South Asians living in Chennai, the South Asians living in Dallas, and the Caucasians living in Dallas, respectively.

Figure 3 summarizes the results of a meta-analysis of published case-control studies on the role of the ENPP1 121Q allele in predicting type 2 diabetes. The test of heterogeneity was significant ($P = 0.02$) as evidence of differences among the various published studies. Compounding a total of 1,733 case and 3,666 control subjects, which include our cohorts, this meta-analysis shows an overall 5% higher frequency of diabetes in the carriers of

TABLE 3
General characteristics of Caucasians living in Dallas

	Nondiabetic subjects		Diabetic subjects	
	Wild-type ENPP1	121Q ENPP1	Wild-type ENPP1	121Q ENPP1
n (M/F)	530 (249/282)	187 (91/96)	86 (61/25)	55 (42/13)
Age (years)	46 ± 15	43 ± 16	59 ± 9*†	58 ± 9*†
BMI (kg/m^2)	26 ± 5	26 ± 5	33 ± 7*†	34 ± 17*†
Systolic BP (mmHg)	121 ± 17	121 ± 14	134 ± 17*†	134 ± 13*†
Diastolic BP (mmHg)	73 ± 10	75 ± 11	80 ± 11*	80 ± 11
Total cholesterol (mg/dl)	187 ± 41	182 ± 41	185 ± 37	184 ± 25
LDL cholesterol (mg/dl)	116 ± 37	113 ± 36	104 ± 32	99 ± 29
HDL cholesterol (mg/dl)	47 ± 15	47 ± 14	40 ± 10*†	43 ± 14
Triglycerides (mg/dl)	134 ± 101	122 ± 91	225 ± 186*†	206 ± 116*†
Fasting glucose (mg/dl)	90 ± 9	89 ± 10	176 ± 76*†	174 ± 71*†
HOMA-IR	2.7 ± 2.0	3.1 ± 3.2	NA	NA

Data are means ± SD, unless otherwise specified. *P* values were determined using ANOVA for multiple comparisons of means. *$P < 0.05$ for mean difference with the subgroup of nondiabetic subjects with wild-type ENPP1. †$P < 0.05$ for mean difference with the subgroup of nondiabetic subjects with ENPP1 K121Q variant. BP, blood pressure.

TABLE 4
General characteristics of nondiabetic Caucasians and South Asians who underwent OGTTs for determination of insulin resistance

	Caucasians		South Asians	
	Wild-type ENPP1	121Q ENPP1	Wild-type ENPP1	121Q ENPP1
n (M/F)	108 (42/66)	44 (21/23)	108 (70/38)	45 (29/16)
Age (years)	30 ± 8	27 ± 7	32 ± 11	31 ± 10
BMI (kg/m^2)	25 ± 6	25 ± 5	23 ± 4	24 ± 3
Systolic BP (mmHg)	114 ± 12	117 ± 11	114 ± 12	116 ± 13
Diastolic BP (mmHg)	69 ± 10	72 ± 13	70 ± 11	73 ± 9
Total cholesterol (mg/dl)	165 ± 36	162 ± 39	170 ± 35	169 ± 32
LDL cholesterol (mg/dl)	97 ± 31	94 ± 38	104 ± 32	101 ± 29
HDL cholesterol (mg/dl)	49 ± 14	50 ± 13	43 ± 11*†	43 ± 11*†
Triglycerides (mg/dl)	100 ± 87	95 ± 56	113 ± 86	136 ± 89
Fasting glucose (mg/dl)	91 ± 8	91 ± 9	91 ± 9	96 ± 8
$AUC_{insulin}$ (μU · min/ml)	9,559	8,066	11,471*†‡	15,750*†

Data are means ± SD, unless otherwise specified. *P* values were determined using ANOVA for multiple comparisons of means. *$P < 0.05$ for mean difference with the subgroup of Caucasians with wild-type ENPP1. †$P < 0.05$ for mean difference with the subgroup of Caucasians with ENPP1 K121Q variant. ‡$P < 0.05$ for mean difference with the subgroup of South Asians with wild-type ENPP1. BP, blood pressure.

polymorphic ENPP1 X121Q than in the carriers of wild-type ENPP1 K121K. The 95% CI was 2–8% ($P = 0.001$). Expressed as the OR, the overall effect of polymorphic ENPP1 K121Q/Q121Q was 1.30 (CI 1.13–1.50), associated with type 2 diabetes across various populations.

DISCUSSION

The results of this study show that polymorphic ENPP1/PC-1 K121Q is associated with type 2 diabetes in South Asians and in Caucasians. Because age is a powerful predictive variable for type 2 diabetes, in this study we attempted to decrease the confounding impact of age on the phenotypic expression of type 2 diabetes by selecting subjects with onset of type 2 diabetes before 60 years of age. A logistic regression analysis showed that the residual confounding effects of age and also of sex and BMI differences did not impact the strong predictive role of the ENPP1 121Q allele for type 2 diabetes.

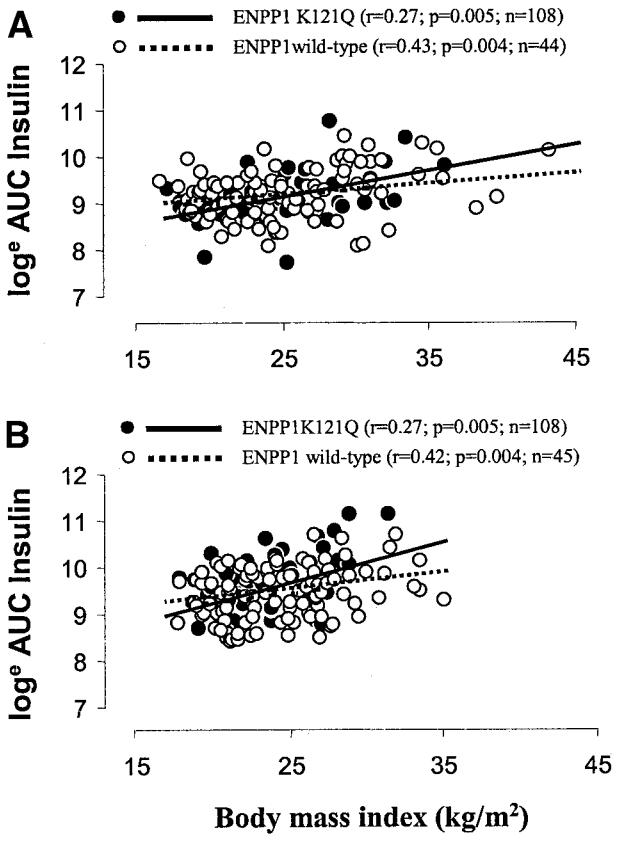

FIG. 1. Relationship of BMI to log^e of the integrated $AUC_{insulin}$ during OGTT in nondiabetic Caucasians (*A*) and South Asians (*B*) living in Dallas. Pearson correlation analysis is reported in parenthesis for each subgroup.

The findings of this study provide evidence for the potential role of a genetic marker in the identification of persons at risk for type 2 diabetes. It is recognized that associations between an allele and a phenotype may occur by a number of mechanisms, including the possibility that the allele may be in linkage disequilibrium with the studied variant or in association with some unmeasured confounding variables that define different subpopulations (population stratification). This is considered a small bias for non-Hispanic whites of European descent (21). The consistency of the prediction of the ENPP1 121Q allele's effect on type 2 diabetes we found in three different cohorts, together with the available mechanistic evidence for the role of ENPP1 K121Q polymorphism on insulin resistance (22–26), support the view that the ENPP1 121Q allele is the functional variant that affects the phenotype per se. However, further replication studies will be necessary to test the validity of this tentative genotype-phenotype relationship.

Maddux et al. (22) have reported that ENPP1 may inhibit the insulin receptor by interacting directly with a specific region in the α-subunit. The role of the ENPP1 121Q variant could be mediated by a functional change induced in insulin signaling transduction from the α- to the β-subunit of the insulin receptor. Although still a matter of debate, the hypothesis that ENPP1 modulates insulin signaling is supported by the findings that the expression levels of ENPP1 are increased in muscle and adipose tissue of insulin-resistant subjects (23–26) and that overexpression of ENPP1 in cultured breast cancer (MCF-7) cells impairs both insulin action and insulin receptor tyrosine kinase activity (26). In the current study, we did not adequately measure insulin sensitivity. However, data on $AUC_{insulin}$ during OGTT were available in a subgroup of 306 nondiabetic subjects of the Dallas cohort. As previ-

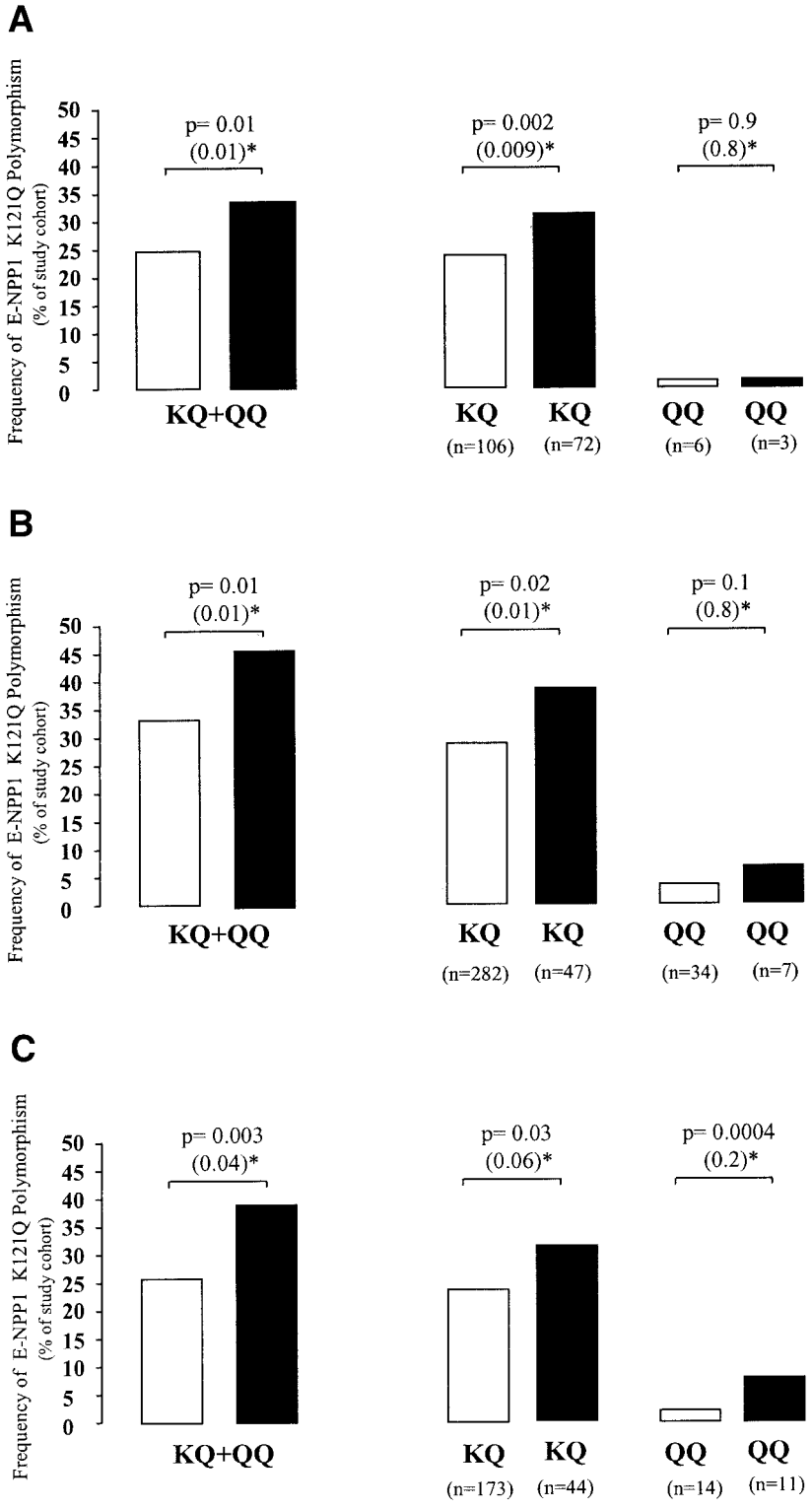

FIG. 2. Percentage of study subjects with ENPP1 121Q with normal glucose tolerance (□) and with diabetes (■) is reported for each study cohort. *A*: South Asians living in Chennai. *B*: South Asians living in Dallas. *C*: Caucasians living in Dallas. The groups labeled as KQ+QQ include combined data of heterozygous and homozygous subjects. Data are also reported for heterozygous (KQ) and homozygous (QQ) subjects separately. *P* values were determined using Fisher's exact test for comparisons of frequencies. *Data in parenthesis report *P* values of logistic regression after adjustment for age, BMI, and sex differences between nondiabetic and diabetic subjects.

ously shown, nondiabetic South Asians manifest insulin resistance even at a young age and with BMI in the nonobese range (2), and polymorphic ENPP1 K121Q was associated with a higher degree of insulin resistance than the wild-type ENPP1 L121Q in South Asians but not in Caucasians (8). The results shown in Fig. 1 confirmed a direct relationship between increasing BMI and increasing insulin resistance. This was true both for the carriers of the wild-type allele and carriers of polymorphic ENPP1. However, the presence of ENPP1 K121Q was associated with a steeper increase in insulin resistance in the more overweight subjects, suggesting the possibility of an interaction between ENPP1 K121Q and obesity.

The ENPP1 121Q allele has been associated with a higher degree of insulin resistance to peripheral glucose disposal in various human studies (8–12), but not in all (13,14). The reasons for these apparent discrepancies of results are not clear. It is possible that the magnitude of

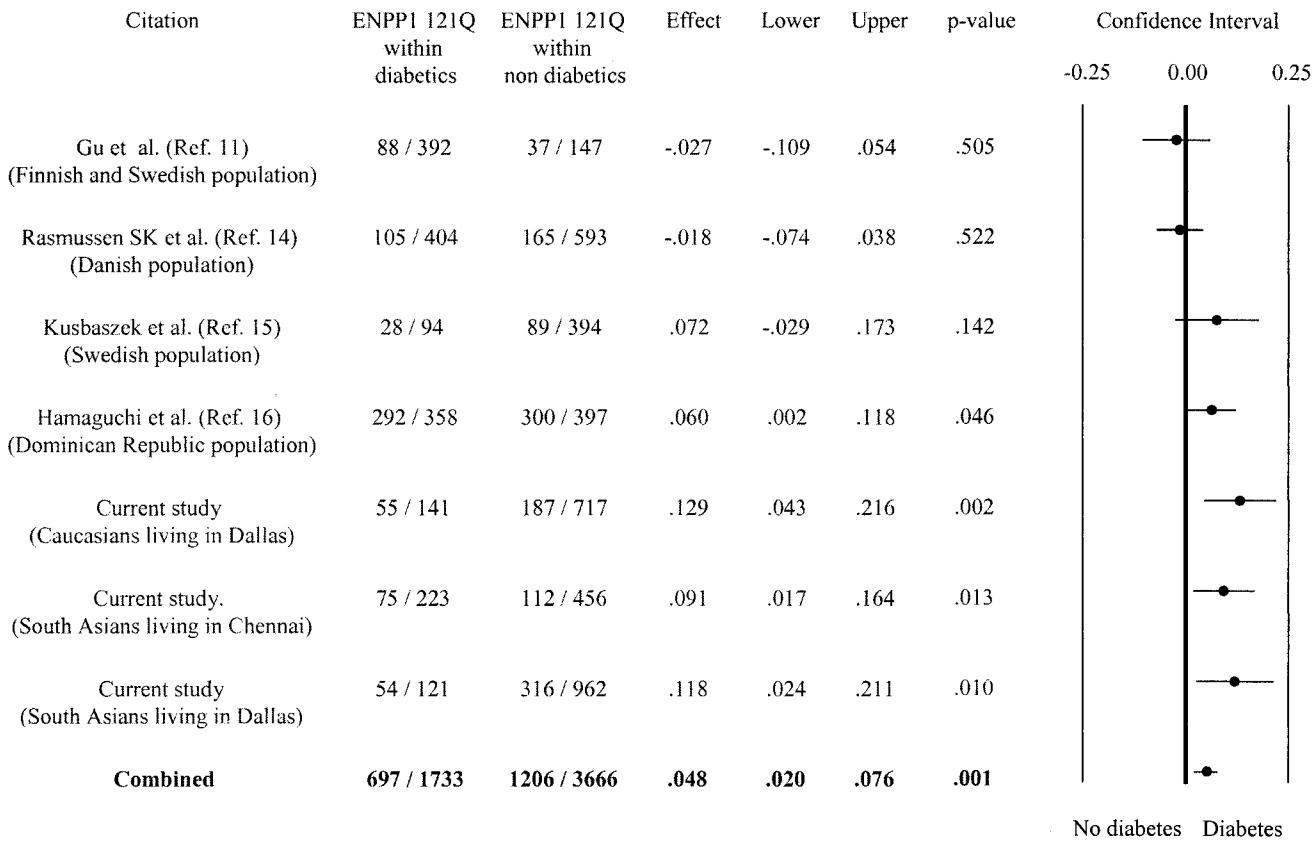

FIG. 3. Meta-analysis of case-control studies examining the frequency of polymorphic ENPP1 X121Q in subjects with and without type 2 diabetes (PubMed to October 2004). Data were computed using Comprehensive Meta-analysis version 1.0.23 (Biostat, Englewood, NJ). Data are shown for rate of diabetes and absence of diabetes among carriers of the polymorphism ENPP1 X121Q. Lines indicate the 95% CI.

insulin resistance induced by this single polymorphism may be significantly modulated by interaction with other genetic or acquired/environmental factors. Therefore, much larger studies will be required to adequately power the evaluation of the relative contribution of polymorphic ENPP1 K121Q on the insulin resistance phenotype in various populations. Similar considerations apply with the described associations between ENPP1 K121Q and type 2 diabetes. It is possible that lifetime exposure to even mild impairment in insulin signaling, often not detectable in clinical studies, mediates the increased prevalence of type 2 diabetes we observed in the ENPP1 121Q carriers in the current study. It is recognized that genotype-phenotype associations are often underpowered to reach firm conclusions in case-control studies (27–29). The *P* values for most association studies, including ours, are often considered not low enough to meet a conservative threshold for declaring significance that minimizes type I errors (false-positive studies). The meta-analysis shown in Fig. 3 includes both published case-control studies and our new findings. The results revealed that ~5% more patients with diabetes are seen in carriers of polymorphic ENPP1 compared with those with wild-type ENPP1. This translates into an overall OR of 1.3. Thus, a large number of case and control subjects will be required in replication studies to firmly establish the role of ENPP1 K121Q in type 2 diabetes. In fact, if we had an observed OR of 1.3, assuming ENPP1 K121Q prevalence of 25%, the power to detect an effect size in our studies would have been only 26% for the Caucasians living in Dallas, 31% for the South Asians living in Chennai, and 25% for the South Asians living in Dallas. Another potential source of error in association studies is population admixture (27,29). Because the frequency of ENPP1 K121Q appears variable, genetic admixture with populations at high prevalence for this polymorphism, such as those of African descent (30), may contribute to the heterogeneity of results observed in our meta-analysis (Fig. 3). This confounding factor could be taken into account by genotyping study groups for population admixture markers (31).

In conclusion, the findings of our study support the hypothesis that the ENPP1 121Q allele is associated with genetic susceptibility and may identify individuals at risk for type 2 diabetes in both Caucasians and South Asians. If our findings are confirmed in a larger sample and in other populations, the ENPP1 121Q variant may provide an important genetic marker to identify people at risk and focus treatment strategies for prevention of type 2 diabetes.

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