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ORIGINAL ARTICLE

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No evidence for association of the ENPP1 (PC-1) K121Q variant with risk of type 2 diabetes in a Japanese population

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Abstract Ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1, also known as PC-1) inhibits insulin signal transduction pathway(s). Previous studies have demonstrated the K121Q variant of the ENPP1 gene to have a significant functional role in determining susceptibility to insulin resistance and type 2 diabetes (T2D). To assess whether the K121Q variant has any impact on T2D in Japanese, we undertook an extensive case-control association study using a total of 911 unrelated Japanese T2D patients and 876 control subjects. No significant difference was observed in either genotype distribution ($P = 0.95$) or allele frequency ($P = 0.83$) between T2D and control groups. Notably, the frequency of the ancestral Q121 allele, which is also present in other primates, was quite high in African-Americans, and showed a marked ethnic variation (77.3% in African-Americans, 16.7% in European Americans, 10.5% in Japanese and 4.2% in Han Chinese). Consequently, the pairwise F_{ST} value (a classic measure of genetic distance between pairs of population) showed highly significant differentiations between African-American and non-African-American populations ($F_{ST} > 0.3$). Our results indicated that the K121Q variant of the ENPP1 gene has very little, if any, impact on T2D susceptibility in Japanese, but may play a role in the inter-ethnic variability in insulin resistance and T2D.

Keywords Type 2 diabetes · Ecto-nucleotide pyrophosphatase/phosphodiesterase 1 · K121Q missense variant · Genetic association study · Japanese population

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Introduction

Type 2 diabetes (T2D) is a complex group of disorders characterized by two distinct pathophysiological defects: impaired pancreatic β-cell function and insulin resistance in muscle, fat and the liver (Polonsky et al. 1996). There is growing evidence that genetic factors affect both of these components. Over several decades, intensive efforts have been made to identify the DNA sequence differences (polymorphisms/mutations) in gene(s) that encode protein(s) contributing to either insulin biosynthesis/secretion or insulin action, and several common genetic variants accounting for a substantial proportion of common T2D have been found (for review, see Almind et al. 2001; McCarthy 2004). These include the P12A variant of the peroxisome proliferator-activated receptor-γ (PPARG), the E23K variant of the ATP-sensitive potassium channel subunit Kir6.2 (KCNJ11) and the common single nucleotide polymorphisms (SNPs) (e.g., rs2144908) in the P2 promoter region of the hepatocyte nuclear factor-4α (HNF4A) gene. These may contribute to the risk of T2D by conferring insulin resistance in hepatic, muscle and fat tissues (PPARG, P12A) and a relative insulin secretory deficiency (KCNJ11, E23K; HNF4A, rs2144908). Undoubtedly, T2D is genetically more heterogeneous and many other important genes/variants that influence individual T2D

susceptibility await discovery and evaluation. Among such genes, the ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1, also known as PC-1) gene has attracted considerable attention because of its substantial contribution to insulin resistance (for a review, see Goldfine et al. 1998, 1999).

ENPP1 is one of five ecto-enzyme ENPP proteins with an extracellular active site hydrolyzing nucleoside 5′ triphosphates such as ATP, GTP, CTP, TTP and UTP to their corresponding monophosphates with release of pyrophosphate, and may also hydrolyze diadenosine polyphosphates and 3′, 5′-cAMP to AMP (Rebbe et al. 1991). ENPP family proteins consist of a short NH2-terminal cytoplasmic domain, a single transmembrane domain, two somatomedin β-like domains, a catalytic domain and a COOH-terminal nuclease-like domain. ENPP1 is the best characterized of the ENPP proteins, and is known to be expressed in many tissues, including muscle, fat, liver and kidney (Maddux et al. 1995). Although the precise physiological functions of ENPP1 in these tissues remain unknown, it has been shown that over-expression of ENPP1 in various cells inhibits insulin receptor (IR) tyrosine kinase activity and causes insulin resistance (Maddux et al. 1995; Maddux and Goldfine 2000; Dong et al. 2005). ENPP1 interacts directly with the IR α-subunit, thereby preventing insulin-induced conformational change of IR. The human ENPP1 gene, consisting of 25 exons, is located on chromosome 6q22–23. Recently, an SNP in exon 4 (rs1044498) that causes an amino acid change from lysine to glutamine at codon 121 (K121Q) was identified, and evidence for association of this variant with insulin resistance and related phenotypes in different ethnic groups was reported (Pizzutti et al. 1999; Gu et al. 2000; Frittitta et al. 2001; Endler et al. 2002; Abate et al. 2003, 2005; Kubaszek et al. 2004; Hamaguchi et al. 2004; Bacci et al. 2005; Meyre et al. 2005). In Sicilians and South Asians, subjects carrying the Q121 allele were reported to have significantly higher glucose and insulin levels during oral glucose tolerance tests (OGTT) and lower insulin sensitivity during glucose clamp studies (Pizzuti et al. 1999; Abate et al. 2003). Controversial results on the association between K121Q genotype frequency and the risk for T2D have also been reported (Rasmussen et al. 2000; González-Sánchez et al. 2003; Laukkanen et al. 2004), though a recent meta-analysis confirmed a modest but significant association with T2D (Bacci et al. 2005). In addition, Meyre et al. (2005) conducted a large study involving 6,147 subjects and found the variant to correlate strongly with childhood obesity, morbid or moderate obesity in adults and T2D. The K121Q variant is located within the second somatomedin β-like domain of the ENPP1 protein and may interfere with protein-protein interaction. When the ENPP1 Q121 allele was transfected into cultured cells, this allele was found to interact more strongly with IR and to more effectively inhibit insulin-stimulated IR autophosphorylation, IRS-1 (insulin receptor substrate-1) phosphorylation, PI (phosphatidylinositol) 3-kinase activation, glycogen synthesis and cell proliferation, as compared to the K121 allele (Costanzo et al. 2001). Moreover, transgenic mice overexpressing the human ENPP1 Q121 allele in muscle and liver became diabetic, and had higher glucose and insulin levels in the fed state, than did non-transgenic mice (Maddux et al. 2005).

If the ENPP1 K121Q variant is associated with insulin resistance and confers increased risk for T2D, it is important to determine the frequency of this variant in various populations, and the associated risk for T2D. Since no reports have been published to date on the K121Q variant in a Japanese population, we conducted a case-control association study using a large group of Japanese T2D patients.

Materials and methods

Subjects

This study was conducted in accordance with the tenets of the Declaration of Helsinki. All subjects consented to participate in the process approved by the Ethics Committee for Human Genome/Gene Research at the University of Tokushima. In this study, two separate case-control samples were collected: (1) 348 T2D patients (Case-1) and 369 control subjects (Control-1), both recruited through the Eye-clinic at Tokushima University Hospital and its affiliates; (2) 563 T2D patients (Case-2) from Kyoto Prefectural University Hospital and its affiliates, and 507 unrelated healthy volunteers (Control-2) collected by the Pharma SNP consortium (PSC). PSC samples were obtained through the Health Science Research Resources Bank (HSRRB) of the Japanese Collection of Research Bioresources (JCRB)/Japan Health Sciences Foundation (JHSF). All subjects were Japanese. The diagnosis of T2D was based either on the 1985 WHO criteria or on being treated with medications for diabetes. Genomic DNA samples were extracted from peripheral blood leucocytes or Epstein-Barr virus-immortalized B-lymphoblasts by standard techniques. Some genomic DNA samples were amplified prior to genotyping using a GenomiPhi DNA Amplification Kit (Amersham Biosciences, Uppsala, Sweden).

ENPP1 K121Q genotyping

The ENPP1 K121Q variant (SNP ID; rs1044498) was genotyped using a TaqMan 5′-nuclease assay with allele-specific fluorescent MGB probes. Sequences for the primer and probe sets were obtained from the JMDBase (Japan Metabolic Disease Data Base, ENPP1_6-AC, http://www.jmdbase.jp/gene_snp.asp?geneid=23101&chrnum=3), a database for metabolic disorder-related SNPs (Takeuchi et al. 2005), and purchased from Applied Biosystems. All reactions were performed according to the manufacturer's protocol using TaqMan Universal PCR Master Mix, No

AmpErase UNG (Applied Biosystems), with a 900 nM primer/200 nM probe final concentration and 5.0 ng genomic DNA, in a total reaction volume of 4 µl. Reactions were conducted in a 384-well format using pipetting robots (TECAN MiniPrep 75, BioMek FX, Beckman-Coulter, Munich, Germany). Reactions were carried out in an ABI GeneAmp PCR system 9700 (Applied Biosystems) under the following conditions: initial denaturation at 95°C for 10 min, followed by 45 cycles at 95°C for 15 sec and 60°C for 1 min, and a final soak at 15°C. After reaction, assay plates were transferred to Prism 7900HT instruments (Applied Biosystems) to read the fluorescence intensity in each well. Fluorescence data files from each plate were first analyzed using SDS2.1 software (Applied Biosystems), with an automated allele calling option under the "Quality Value" setting of 95.0, and were then independently reviewed by two skilled operators (P.K. and H.I.).

Genotyping accuracy and reliability of the TaqMan assay were validated in two ways: (1) reproducibility was demonstrated by genotyping replicate DNA samples (>10% of total samples), which provided 100% concordance of genotype data; (2) 12 DNA samples (4 of each genotype) were randomly chosen and analyzed by the PCR-RFLP method. Briefly, a 238 bp genomic fragment containing the ENPP1 K121Q SNP site was PCR amplified using ENPP1 gene-specific primers, the sequences of which having been previously described (Rasmussen et al. 2000). PCR was performed using AmpliTaq Gold DNA polymerase (Perkin Elmer, Boston, MA) in a final volume of 20 µl, under the following conditions: initial denaturation at 95°C for 10 min, followed by 35 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and a final extension at 72°C for 10 min. One-half of the PCR product was digested with 10 units of *Ava*II (NEB) for 1 h at 37°C. The genotypes were determined by electrophoresing PCR products on a 2% agarose gel and staining with ethidium bromide. The absence of an *Ava*II site resulted in an uncut band of 238 bp (K121 allele), while its presence resulted in the two additional bands on the gel of 148 and 90 bp (Q121 allele). There was 100% concordance between genotypes obtained by the PCR-RFLP method and the TaqMan assay results.

Frequencies of the ENPP1 K121Q variant among different populations

Genotype data for the ENPP1 K121Q variant in 22 African-Americans (10 males/12 females), 24 European Americans (13 males/11 females) and 24 Han Chinese (no gender information was available) were obtained from the Perlegen Genotype Browser (Perlegen SNP ID; afd0359226, http://genome.perlegen.com). The statistical significance and the genetic distance, indicated by differences in allele frequencies between the two populations, were assessed by Fisher's exact test and Wright's F_{ST}, respectively (Weir 1996).

Statistical analysis

All analyses were performed using either SPSS for Windows (ver.12.0, http://www.spss.com/) or SNPAlyze software (ver.5.0, Dynacom, Japan). Genotype and allele frequencies of the ENPP1 K121Q variants in the case and control groups were compared, and tested using a Pearson χ^2-statistic. Deviations from Hardy-Weinberg equilibrium (HWE) were tested using a χ^2 goodness-of-fit test. Analyses were also performed assuming dominant, co-dominant (additive) and recessive genetic models and the crude odds ratios (ORs), their 95% CI ranges and corresponding *P*-values were calculated using the Web-Assotest program (available at http://www.ekstroem.com/). Logistic regression was used to estimate both unadjusted and age-, gender- and body mass index (BMI)- adjusted ORs. Analysis of variance (ANOVA) or the unpaired two-tailed Student's *t* test was performed to quantitatively compare clinical data among populations or genotypes. The significance level for statistical tests was chosen to be 0.05. Statistical power to detect an association was determined with the PS power and sample-size program (available at http://www.mc.vanderbilt.edu/prevmed/ps). The present study (a total sample size of 911 cases and 876 controls) had a statistical power of more than 80% to detect an association with an OR of 1.5 at $P = 0.05$, for alleles with >10% frequency.

Results

In this study, we analyzed two cases and two control groups. The clinical characteristics of each group are presented in Table 1. The Case-1 and Control-1 groups were recruited from the Tokushima region of Japan (located in the eastern part of Shikoku island) and, therefore, were geographically matched. The Case-2 and Control-2 groups were recruited mainly from the Kyoto region (located in central Honshu, the main island of Japan) and from the Tokyo metropolitan area (located in the Kanto region), respectively, and thus were geographically separated from the Tokushima populations. There were significant differences in the distributions of age at blood sampling and gender between the case and control groups. The mean ages for the case groups (62.5 ± 9.7 years and 64.1 ± 10.0 years, for Case-1 and Case-2, respectively) were significantly higher ($P < 0.0001$) than those of the corresponding control groups (36.4 ± 12.1 years and 38.0 ± 11.3 years, for Control-1 and Control-2, respectively). The difference in mean age remained significant for the combined case and control groups (Case-3 vs Control-3, $P < 0.0001$). Gender distribution also differed significantly between the Case-1 and Control-1 groups, and between the Case-2 and Control-2 groups, but not between the combined Case-3 and Control-3 groups. The mean BMI and HbA1c levels did not differ significantly between the two control groups (Control-1 vs. Control-2). Case-1

Table 1 Clinical characteristics of the study subjects. Data are means ± SD. The statistical significance of differences between any two groups was analyzed by Student's *t* test. *T2D* Type 2 diabetes

	Study 1		Study 2		Study 3 (1 + 2)	
	Control-1	Case-1	Control -2	Case -2	Control-3	Case-3
Number of subjects (females/males)	369 (253/116)	348 (174/174)	507 (193/314)	563 (278/285)	876 (446/430)	911 (452/459)
Age (years)	36.4 ± 12.1 (n = 368)	62.5 ± 9.7 (n = 348)[a]	38.0 ± 11.3 (n = 507)	64.1 ± 10.0 (n = 563)[b]	37.3 ± 11.7 (n = 875)	63.5 ± 9.9 (n = 911)[c]
Body mass index (kg/m^2)	22.0 ± 3.0 (n = 336)	23.6 ± 3.2 (n = 339)[a]	22.3 ± 2.8 (n = 505)	23.4 ± 3.4 (n = 532)[b]	22.2 ± 2.9 (n = 841)	23.5 ± 3.3 (n = 871)[c]
Plasma glucose (mg/dl)	NA[d]	155.2 ± 56.3 (n = 313)	NA	145.7 ± 44.6 (n = 558)	NA	149.1 ± 49.4 (n = 871)
HbA1c (%)	4.7 ± 0.3 (n = 316)	7.1 ± 1.3 (n = 346)[a]	4.9 ± 0.3 (n = 507)	7.4 ± 1.4 (n = 562)[b]	4.8 ± 0.3 (n = 823)	7.3 ± 1.4 (n = 908)[c]
Age at onset						
< 50 years (%)	–	231 (66.4)	–	210 (37.3)	–	441 (48.4)
≥50 years (%)	–	115 (33.0)	–	350 (62.2)	–	465 (51.0)
Unknown (%)	–	2 (0.6)	–	3 (0.5)	–	5 (0.5)
Positive T2D family history (1st-degree relatives, %)	–	202 (58.0)	–	270 (48.0)	–	472 (51.8)
With insulin therapy (%)	–	132 (39.7)	–	124 (22.0)	–	256 (28.1)
With retinopathy (%)	–	297 (85.3)	–	91 (16.2)	–	388 (40.4)

[a] P-value < 0.0001 versus Control-1
[b] P-value < 0.0001 versus Control-2
[c] P-value < 0.0001 versus Control-3
[d] Not Assessed

patients showed significantly greater disease severity, in terms of a longer duration of T2D, more subjects on insulin therapy and a higher prevalence of diabetic retinopathy, as compared to Case-2 patients, presumably due to the nature of patient recruitment.

In the first case-control analysis (Study 1), we genotyped the ENPP1 K121Q variant in 348 unrelated T2D patients (Case-1) and 369 control subjects (Control-1). The genotype and allele distribution for this variant were similar and did not differ significantly between Case-1 and Control-1 groups (P = 0.17 and 0.46, respectively, Table 2). However, we noted a significant deviation from HWE within the Case-1 group (P = 0.008), while no deviation was observed in the Control-1 group. In

Table 2 Frequency of the ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) K121Q variant in Japanese T2D and control subjects. Data are number of subjects with each genotype and number of alleles (frequency in %). *OR* Odds ratio, *CI* confidence interval. ORs for different modes of inheritance were calculated using the Web-Assotest program (available at http://www.ekstroem.com)

	Genotype (%)			Allele (%)		*P*-value		Dominant model (KQ/QQ vs KK)		Co-dominant model (QQ vs KQ) = (KQ vs. KK)		Recessive model (QQ vs KK/KQ)	
	K K	K Q	Q Q	K	Q	Genotype	Allele	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Study 1													
Control-1	300 (81.3)	66 (17.9)	3 (0.8)	666 (90.2)	72 (9.8)	0.17	0.46	1.04 (0.72–1.51)	0.84	1.13 (0.81–1.57)	0.47	3.25 (0.87–12.1)	0.059
Case-1	280 (80.7)	58 (16.7)	9 (2.6)	618 (89.0)	76 (11.0)								
Study 2													
Control-2	403 (79.8)	94 (18.6)	8 (1.6)	900 (89.1)	110 (10.9)	0.39	0.74	1.00 (0.74–1.35)	0.99	0.95 (0.72–1.26)	0.74	0.45 (0.13–1.49)	0.18
Case-2	447 (79.8)	109 (19.5)	4 (0.7)	1,003 (89.6)	117 (10.4)								
Study 3 (1 + 2)													
Control-3	703 (80.4)	160 (18.3)	11 (1.3)	1,566 (89.6)	182 (10.4)	0.95	0.83	1.02 (0.81–1.29)	0.88	1.02 (0.83–1.26)	0.83	1.14 (0.51–2.56)	0.75
Case-3	727 (80.2)	167 (18.4)	13 (1.4)	1,621 (89.4)	193 (10.6)								

addition, homozygosity for Q121 (QQ) tended to be more common in Case-1 than in Control-1 (2.6 vs 0.8%), and there was suggestive evidence of an association in a recessive model (QQ vs KK/KQ; OR 3.25, 95% CI 0.87–12.1, $P = 0.059$). In the hope of obtaining further evidence for an association between the K121Q variant and T2D, we next genotyped an independent set of case-control groups for the second comparison (Study 2); Case-2 ($n = 563$) and Control-2 ($n = 507$). In Study 2, neither the genotype nor the allele distribution of the K121Q variant differed between the two groups ($P = 0.39$ and 0.74, respectively), and no deviation from HWE was observed (Table 2). Furthermore, we found no indication of a recessive mode of action of the Q121 variant (OR 0.45, 95% CI 0.13–1.49, $P = 0.18$), as was seen in Study 1. The same was observed for the association test between the combined groups (Study 3), including a total of 911 T2D patients (Case-3) and 876 controls (Control-3). It is noteworthy that the deviation from HWE among Case-1 patients was no longer significant for the Case-3 group. The lack of an association was confirmed to be independent of age, gender and BMI in the logistic regression analysis (data not shown). We also performed a subanalysis to test the significance of a possible association between the K121Q variant and body weight status. We divided each T2D (Case-3) and control (Control-3) group into "normal-weight" and "overweight/obese" groups, as defined by BMI values of < 25 and ≥ 25 kg/m^2, respectively, according to the recommendations of the Japan Society for the Study of Obesity (JASSO), and compared the K121Q genotype distribution and allele frequency between the two weight subgroups. There were no significant differences between the subgroups in either the Case-3 or the Control-3 group (Case-3: $P = 0.53$ and 0.39, for genotype and allele, respectively; Control-3: $P = 0.38$ and 0.69). In addition, ANOVA was used to test the significance of associations between the K121Q variant and BMI, and other available clinical data including blood pressure and HbA1c levels. No significant K121Q genotype-dependent differences were observed (data not shown).

The frequency of the ENPP1 Q121 allele in our Japanese sample was considerably lower than those reported in other populations. We therefore compared the K121Q genotype distribution in Japanese (JPN) with those in three different populations (AER, African-American; EUR, European; CHN, Han Chinese), for which data are publicly available through the Perlegen Genotype Browser. As shown in Table 3, the frequencies of the Q121 allele in AER, EUR, CHN and JPN populations differed in magnitude, being 77.3, 16.7, 4.2 and 10.5%, respectively. No significant deviations from HWE were observed among these populations. Consequently, the pairwise F_{ST} values, a classic measure of genetic distance between pairs of population, showed very significant differentiations ($F_{ST} > 0.3$) between AER and non-AER populations (pairwise F_{ST}; 0.369, 0.554 and 0.452 for EUR, CHN and JPN, respectively). Within non-AER populations, there was no evidence of a significant differentiation with this sample size ($P > 0.05$, pairwise $F_{ST} < 0.05$). Since different numbers of subjects were used to calculate F_{ST} for the Japanese ($n = 1{,}781$) and Perlegen data sets ($n = 22$ or 24), we randomly selected 24 Japanese subjects and repeated the analysis. The pair-wise F_{ST} values in the data set were extremely similar and were nearly identical to those observed in the full Japanese set (data not shown).

Discussion

Positive associations between the ENPP1 K121Q variant and T2D have previously been reported in United States Caucasians and South Asians (Abate et al. 2005), a Dominican Republic population (Hamaguchi et al. 2004) and a French population (Meyre et al. 2005). In contrast, no significant association was found in a Sicilian population (Pizzutti et al. 1999), a Finnish population (Kubaszek et al. 2004), a Finnish and Swedish mixed population (Gu et al. 2000), a Danish population (Rasmussen et al. 2000) or an Italian and United States Caucasian population (Bacci et al. 2005). The results of such analyses are intriguing but difficult to interpret because, in some studies, a sampling effect due to relatively small sample sizes is undeniable. In addition, insulin resistance is not necessarily a prerequisite for the development of T2D. The results of a recent meta-analysis involving a total of 2,834 T2D patients

Table 3 ENPP1 K121Q genotypic frequencies among different ethnic groups and pairwise population differentiation (F_{ST}). *AFR* African American , *EUR* European American, *CHN* Han Chinese, *JPN* Japanese, *HWE* Hardy–Weinberg equilibrium

	K121Q genotype			HWE (*P*-value)	Q121 allele frequency (no. of chromosomes)	Pairwise F_{ST} value[c]/*P*-value[d]			
	K K	K Q	Q Q			vs AFR	vs EUR	vs CHN	vs JPN
AFR[a]	1	8	13	0.869	0.773 (22)	–	0.369/< 0.0001	0.554/< 0.0001	0.452/< 0.0001
EUR[a]	16	8	0	0.327	0.167 (24)		–	0.042/0.091	0.008/0.160
CHN[a]	22	2	0	0.831	0.042 (24)			–	0.015 / 0.230
JPN[b]	1,430	327	24	0.544	0.105 (3,562)				–

[a]Data derived from the Perlegen Genotype Browser (ss23404875, PERLEGEN ID; afd0359226)
[b]Genotype distribution in the total Japanese population (907 T2D and 874 control subjects combined)
[c]F_{ST}, a fixation index
[d]Fisher's exact test (two-sided) *P*-value

and 4,425 controls, however, showed a modest but significant association of the K121Q polymorphism of the ENPP1/PC-1 gene with T2D (Bacci et al. 2005). Notably, the reported OR in the meta-analysis was 1.29 (95% CI 1.09–1.53, $P = 0.003$), which is in the same range as those for well-established T2D susceptibility variants, such as PPARG P12A and KCNJ11 E23K. These findings prompted us to initiate the present study to elucidate the role of the K121Q variant in a Japanese population. In the current study, we found no evidence for association of the ENPP1 K121Q polymorphism with T2D in our population. Unfortunately, because clinical measures, such as plasma insulin levels and homeostasis model assessment of insulin resistance (HOMA-IR), were not available for our population, we were unable to assess the relationship between the Q121 variant and insulin resistance in detail. Thus, it remains possible that the Q121 variant is among the factors influencing insulin resistance in Japanese.

Results of the present and previous studies have suggested an inter-population variance in the frequency of the ENPP1 Q121 allele according to ethnicity and geographic location. The reported prevalence of the Q121 allele in Caucasian populations were 10% in Finns (Kubaszek et al. 2003), 13.8% in a Finnish–Swedish mixed population (Gu et al. 2000), 12.3% in Spanish subjects (González-Sánchez et al. 2003), 14.3% in an European American population (Bacci et al. 2005), 16.1% in Danish subjects (Rasmussen et al. 2000), 16.9% in a French population (Meyre et al. 2005) and 17.8% in a Sicilian population (Pizzutti et al. 1999). The allele frequency in South Asian immigrants living in the United States is reported to be 17.9%, i.e., comparable to that in the Sicilian population (Abate et al. 2005). Furthermore, a significantly higher 121Q allele frequency of 54.2% was reported in the Dominican Republic population with a mixed genetic background including indigenous Caribbean peoples, African blacks and Hispanic whites (Hamaguchi et al. 2004). In the current study, we found a relatively low 121Q allele frequency (10.5%) in our Japanese population, as compared to the reported allele frequency in Caucasians. In addition, a web-accessible database (Perlegen Genotype Browser) allows us to access ENPP1 Q121 allele frequency data for a diverse set of population samples. It is noteworthy that an exceptionally high prevalence of the Q121 allele (77.3%) was observed in African-Americans. In Han Chinese, conversely, the frequency of the Q121 allele was the lowest reported to date (4.2%). Consequently, the pairwise F_{ST} values showed very significant differentiations ($F_{ST} > 0.3$) between African-American and non-African-American populations. Although the frequency in the Perlegen European population (16.7%) was consistent with the reported allele frequency, because of the small sample size ($n = 22$ or 24), the Perlegen frequency data may not be representative of the whole population. However, these results are sufficiently encouraging to warrant more extensive population studies.

Given the lack of an association in our Japanese study population and the difference in allele frequencies among population groups, we speculate that the ENPP1 K121Q polymorphism might play a major role in the inter-ethnic variability in insulin resistance and subsequent development of T2D. Supporting this hypothesis, the Japanese population, as an ethnic group, is thought to be more genetically prone to developing pancreatic β-cell dysfunction rather than insulin resistance (Chen et al. 1995), although there has been debate about the relative importance of insulin resistance versus β-cell dysfunction in the development of T2D. On the other hand, populations with a high prevalence of the 121Q allele (African-Americans, Dominicans and South Asians) are reportedly more insulin resistant than Caucasians (Jensen et al. 2002; Abate and Chandalia 2003). Interestingly, the Chimpanzee Genome Project has recently revealed that ENPP1 Q121 is indeed the ancestral allele (Chimpanzee Sequencing and Analysis Consortium 2005). Taken together, these observations raise the possibility that Q121 confers a biological and selective advantage, which may be compatible with the "thrifty genotype hypothesis" proposed by Neel (1962). One such example has recently been shown. Two common polymorphisms of the angiotensinogen (AGT) gene, the −6A/G promoter variant and T235M, are in tight linkage disequilibrium with each other (Nakajima et al. 2002, 2004). The ancestral allele, −6A/T235, has repeatedly been shown in multiple populations to be associated with an increased risk of essential hypertension, a condition that is pathophysiologically related to insulin resistance. The frequency of the −6A/T235 allele varies widely across populations, with lower frequencies outside of Africa, and recent comprehensive analyses have revealed the genetic evidence of natural selection for these variants. Accordingly, it is speculated that the −6A/T235 allele may be associated with increased salt retention and thus be beneficial in environments near the equator where salt is scarce, while the −6G/M235 allele probably rises in frequency as distance from the equator increases, as salt retention becomes less important. Because of this adaptation, populations with a higher −6A/T235 allele frequency may have an increased susceptibility to hypertension in modern times. To determine whether ENPP1 K121Q allele-frequency differentiation among populations has been driven by natural selection, a much more comprehensive understanding of the evolutionary history as well as the geographic and ethnic distributions of this variant is required.

In conclusion, it is unlikely that the K121Q variant in the ENPP1 gene has a major effect on susceptibility to T2D in the Japanese population. However, in view of the modest effect of this variant on disease risk, it is possible that our study was underpowered to detect some effects, if they exist. Further studies with larger sample sizes, involving at least several thousand patients and controls, are required to completely rule out the possible involvement of K121Q variant in the pathogenesis of insulin resistance or T2D in the Japanese

population. In addition, studies on genotype dependent changes, including in vivo assessment of insulin sensitivity (e.g., HOMA-IR and clamp studies), will help to understand the possible impact of ENPP1 on insulin resistance or T2D. Our data also suggest that ethnic variations in allele frequencies of disease-susceptibility variants for common diseases warrant detailed evaluation, and differences should be taken into account when interpreting their importance.

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