Brief Genetics Report

Common Variants in the *ENPP1* Gene Are Not Reproducibly Associated With Diabetes or Obesity

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The common missense single nucleotide polymorphism (SNP) K121Q in the ectoenzyme nucleotide pyrophosphate phosphodiesterase (*ENPP1*) gene has recently been associated with type 2 diabetes in Italian, U.S., and South-Asian populations. A three-SNP haplotype, including K121Q, has also been associated with obesity and type 2 diabetes in French and Austrian populations. We set out to confirm these findings in several large samples. We genotyped the haplotype K121Q (rs1044498), rs1799774, and rs7754561 in 8,676 individuals of European ancestry with and without type 2 diabetes, in 1,900 obese and 930 lean individuals of European ancestry from the U.S. and Poland, and in 1,101 African-American individuals. Neither the K121Q missense polymorphism nor the putative risk haplotype were significantly associated with type 2 diabetes or BMI. Two SNPs showed suggestive evidence of association in a meta-analysis of our European ancestry samples. These SNPs were rs7754561 with type 2 diabetes (odds ratio for the G-allele, 0.85 [95% CI 0.78–0.92], $P = 0.00003$) and rs1799774 with BMI (homozygotes of the delT-allele, 0.6 [0.42–0.88], $P = 0.007$). However, these findings are not supported by other studies. We did not observe a reproducible association between these three *ENPP1* variants and BMI or type 2 diabetes. *Diabetes* 55:3180–3184, 2006

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K.G.A. is employed by Genomics Collaborative, which owns a sample repository of samples that were used in this study.

Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.

SNP, single nucleotide polymorphism.

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Ectoenzyme nucleotide pyrophosphate phosphodiesterase (*ENPP1*), also known as plasma cell membrane glycoprotein 1 (PC-1), downregulates insulin signaling by inhibiting the insulin receptor's tyrosine kinase activity. This inhibition is proposed to confer insulin resistance in mammals. The *ENPP1* gene is located on 6q22-23, a locus linked to obesity and diabetes in several studies (1–4). Recent studies of variation in the *ENPP1* gene have found an association of the common missense single nucleotide polymorphism (SNP) K121Q (rs1044498) and of a three-marker haplotype (which includes K121Q) with the risk of diabetes and obesity. Abate et al. (5) reported that the Q-allele was associated with diabetes in South-Asian and Caucasian populations. Meyre et al. (6) described a three-SNP risk haplotype in the *ENPP1* gene (formed by the three minor alleles of rs1044498 K-allele, rs1799774 delT-allele, and rs7754561 G-allele) that was associated with increased risk of diabetes and obesity in adults and obese children. Bacci et al. (7) also reported an association of the minor allele in K121Q with insulin resistance and atherogenesis in diabetic individuals. Their meta-analysis of 4,425 control subjects and 2,834 patients with type 2 diabetes showed an odds ratio (OR) of 1.29 ([95% CI 1.09–1.53], $P = 0.003$) under a dominant model. In contrast, Matsuoka et al. (8) found that the Q-allele was not associated with diabetes and that the K-allele rather than the Q-allele was associated with obesity in both Caucasians and African Americans. Given this cumulative yet conflicting evidence for association with diabetes and obesity, we tested whether the K121Q variant or the risk haplotype (Q-delT-G) are associated with diabetes and/or obesity in several large case-control and family-based cohorts ascertained for both phenotypes.

RESEARCH DESIGN AND METHODS

Obese and lean individuals from the U.S. and Poland (Table 1) were selected from a collection of >60,000 subjects recruited by Genomics Collaborative for a diverse set of disease studies, including healthy people and groups with osteoarthritis, rheumatoid arthritis, asthma, hypertension, coronary artery disease, myocardial infarction, hyperlipidemia, stroke, type 2 diabetes, or

TABLE 1
Characteristics of obesity samples with allele frequencies for all three SNPs

Population	*n* (male/female)	Age (years)	BMI (kg/m^2)	Minor allele frequency rs1044498 (Q)	rs1799774 (delT)	rs7754561 (G)
U.S. and Poland	2,873	56.6 ± 9.1		0.14	0.22	0.27
Obese	886/1,032	56.5 ± 8.9	35.0 ± 3.4	0.14	0.21	0.27
Lean	439/516	56.6 ± 9.4	21.5 ± 0.8	0.15	0.24	0.27
African American	93/95	39.3 ± 8.7		0.79	0.79	0.81
Obese	46/50	37.7 ± 8.7	43.2 ± 5.9	0.81	0.80	0.79
Lean	47/45	40.8 ± 8.5	20.8 ± 0.6	0.77	0.78	0.98
African American (family based)	866	38.4 ± 11.0	30.0 ± 8.3	0.76	0.78	0.79
Men	382	38.0 ± 11.0	27.7 ± 7.2	0.78	0.78	0.80
Women	484	38.7 ± 11.0	31.8 ± 8.7	0.75	0.78	0.78

Data are means ± SD unless otherwise indicated.

osteoporosis. DNA samples were selected by determination of the BMI distribution in healthy control subjects for each decade of life, sex, and country of origin (U.S. or Poland), selecting subjects with a BMI between the 90th and 97th percentiles as obese case subjects and subjects with a BMI between the 5th and 12th percentiles as lean control subjects. These criteria were used to generate a case-control sample of self-described "whites" or "Caucasians" from Poland (700 obese and 331 lean) and the U.S. (1,218 obese and 624 lean) matched for age, sex, and grandparental country of origin.

African-American DNA samples (Table 1) were obtained from a larger cohort of families enrolled in studies of blood pressure at Loyola University in Maywood, Illinois. The survey enrolled a representative random sample of the population between 18 and 74 years of age, regardless of obesity phenotype. The two panels of DNA samples chosen from this cohort are *1*) 93 obese and 93 lean unrelated individuals (chosen from the top and bottom quartiles of the BMI distribution and matched by sex) and *2*) 824 individuals from 324 families for family-based association studies.

The diabetic individuals are presented in Table 2 and have been described elsewhere (9,10). Briefly, they comprise 321 Scandinavian parent-offspring trios; 1,189 Scandinavian siblings discordant for type 2 diabetes; a Scandinavian sample of 471 case-control pairs individually matched for age, BMI, and geographic region; a Swedish sample of 514 case-control pairs who were individually matched for sex, age and BMI; and an individually matched case-control sample totaling 127 pairs from the Saguenay Lac-St. Jean region in Quebec (Canada). In the Scandinavian samples, cases included subjects with type 2 diabetes or severely impaired glucose tolerance, defined as a 2-h blood glucose ≥8.5 but <10.0 mmol/l during a 75-g oral glucose tolerance test. In addition, two case-control European diabetes cohorts were also obtained from Genomics Collaborative: one comprised of 1,226 case and 1,226 control subjects from the U.S. and one comprised of 1,009 case and 1,009 control subjects from Poland, both matched for age, sex, and grandparental country of origin.

Genotyping: three SNPs in *ENPP1*. rs1044498 (C-allele corresponds to the 121Q-allele), rs1799774 delT, and rs7754561 A/G in the 3′ untranslated region were genotyped by an allele-specific primer extension of amplified products with detection by matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (11) using a Sequenom platform (12). Genotyping completion rates were 95.9% for the diabetes samples and 98.6% for the obesity samples. An overlap of 312 subjects in the diabetes and obesity panels

TABLE 2
Characteristics of diabetes samples with allele frequencies for all three SNPs

Population	*n* (male/female)	Age (years)	BMI (kg/m^2)	Fasting plasma glucose (mmol/l)	HbA_{1c} (%)* or plasma glucose at 2-h OGTT (mmol/l)†	Minor allele frequency rs1044498 (Q)	rs1799774 (delT)	rs7754561 (G)
U.S. case/control								
Diabetes	644/582	63 ± 11	33 ± 7	9.8 ± 3.0	8.0 ± 3.1*	0.14	0.22	0.25
NGT	644/582	61 ± 10	27 ± 5	5.1 ± 0.9	ND	0.16	0.23	0.28
Poland case/control								
Diabetes	422/587	62 ± 10	30 ± 5	8.9 ± 4.0	7.9 ± 1.3*	0.13	0.19	0.22
NGT	422/587	59 ± 7	26 ± 4	4.8 ± 1.2	ND	0.15	0.22	0.27
Scandinavia trios								
Probands	168/153	39 ± 9	27 ± 5	7.2 ± 2.6	8.5 ± 2.9†	0.13	0.20	0.18
Parents	236/236							
Sibships								
Diabetes/severe IGT sib	280/329	65 ± 10	29 ± 5	9.3 ± 3.3	14.3 ± 5.6†	0.16	0.22	0.21
NGT sib	275/305	62 ± 10	26 ± 3	5.4 ± 0.4	6.0 ± 1.1†	0.12	0.19	0.20
Scandinavia case/control								
Diabetes/severe IGT	252/219	60 ± 10	28 ± 5	9.8 ± 3.4	15.0 ± 5.3†	0.14	0.19	0.19
NGT	254/217	60 ± 10	27 ± 4	6.2 ± 1.8	6.8 ± 2.8†	0.13	0.21	0.20
Sweden case/control								
Diabetes/severe IGT	267/247	66 ± 12	28 ± 4	9.6 ± 2.9	6.5 ± 1.5*	0.15	0.16	0.18
NGT	267/247	66 ± 12	28 ± 4	5.5 ± 0.7	ND	0.16	0.17	0.19
Canada case/control								
Diabetes	70/57	53 ± 8	29 ± 5	6.4 ± 1.8	12.8 ± 2.1†	0.13	0.17	0.24
NGT	70/57	52 ± 8	29 ± 4	5.1 ± 0.6	6.1 ± 1.1†	0.12	0.22	0.30

Data are means ± SD. Plasma glucose was measured at baseline (fasting) and 2 h after an oral glucose tolerance test (OGTT). "Severe IGT" was defined as an oral glucose tolerance test 2-h blood glucose ≥8.5 but <10.0 mmol/l. IGT, impaired glucose tolerance; ND, not determined; NGT, normal glucose tolerance.

TABLE 3
Association of selected SNPs and haplotype with BMI for European and African-American populations

Population	*n*	rs1044498 (Q) OR (95% CI)	*P*	rs1799774 (delT) OR (95% CI)	*P*	rs7754561 (G) OR (95% CI)	*P*	Haplotype (Q-delT-G) OR (95% CI)	*P*
U.S. and Poland Obese/lean	1,918/955	0.98 (0.84–1.15)	0.84	0.85 (0.74–0.97)	0.02	1.01 (0.87–1.12)	0.83	0.96 (0.79–1.18)	0.37
African American Obese/lean	96/92	1.26 (0.76–2.07)	0.37	0.87 (0.53–1.46)	0.61	1.20 (0.72–2.02)	0.48		
		Heritability	*P*	Heritability	*P*	Heritability	*P*	Heritability	*P*
African American Families	846	0.009	0.72	−0.001	0.72	−0.002	0.79	−0.00009	0.49

The association tests were done under multiplicative, dominant, and recessive models (multiplicative shown). SNP rs1799774 was also associated with BMI in the Caucasian population in a recessive model. Association testing for the African-American families was computed in PBAT (pedigree-based association testing software package), listing the heritability or effect estimate and the *P* value (residual of BMI adjusted for age and sex). To be consistent with the literature, ORs of individual SNPs are reported as minor vs. major allele.

provided 933 duplicate genotypes, showing 99.7% consensus corresponding to an estimated error rate of 0.2%. For the two family-based studies, there were no apparent Mendelian inheritance errors in the Maywood population genotypes, and there were 3, 3, and 2 for rs1044498, rs1799774, and rs7754561, respectively, in the 321 Scandinavian families. This corresponds to an estimated error rate of ~1–2%. Removing the trios that generated Mendelian inheritance violations did not change the results.

Analysis methods. Phasing of chromosomes for haplotype reconstruction was performed by the weighted expectation-maximization algorithm incorporated into the software Haploview (13) (http://www.broad.mit.edu/mpg/haploview/) and as previously described (14) for the discordant sib pairs. In Haploview, haplotypes are assigned probabilistically in the tests of disease association. The probability estimate of the putative risk haplotype in each chromosome was compared against all other possible haplotypes at the same locus.

For case-control populations, χ^2 tests (1 df) of allele counts in case and control subjects were used to test for association with obesity or diabetes. The European-derived case-control panels have 99.8% power to detect an association conferring a 1.3-fold increased risk of diabetes and 93% power to detect a similar association for obesity using a χ^2 power calculation, assuming a two-tailed *P* value of 0.05 (15) (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html). Comparison of lean and obese individuals from the near extremes of the BMI distribution (case and hypernormal control subjects) is likely more powerful than comparison of individuals above and below a BMI of 30 kg/m^2. We also tested specific genetic models (dominant and recessive for the minor allele) and analyses stratified by sex as secondary analyses. For family-based samples, FBAT(family-based association testing method), as implemented in PBAT (pedigree-based association testing software package) (16), was used to test for association with obesity, either treating BMI as a quantitative trait adjusted for age and sex or dichotomizing at a BMI of 30 kg/m^2. Association to BMI for the control subjects in the diabetes cohort (labeled as NGT [normal glucose tolerance] in Table 2) was tested using log-transformed BMI as a quantitative trait.

For the diabetic trios and sib pairs, we performed the transmission disequilibrium test (17) and the discordant allele test (18), respectively, and the results were incorporated in the Mantel-Haenszel meta-analysis of the ORs as previously described (19); all *P* values are nominal and two tailed. Homogeneity of results was tested using a Breslow-Day statistic as previously described (19).

RESULTS

The allele frequencies of the three variants differed greatly between the European-derived and African-American populations (Tables 1 and 2). For obesity, there was no significant association of the K121Q variant or the three-marker risk haplotype with obesity represented by BMI in the European-derived and African-American populations (Table 3). The European-derived panels from the U.S. and Poland showed homogeneous results for each SNP, allowing pooling of association results using the Mantel-Haenszel method. No nominally significant association was observed with the K121Q variant or the three-marker haplotype when the U.S. or Poland populations were analyzed separately (data not shown). There was suggestive evidence of a novel association of rs1799774 with obesity best seen in a recessive model (OR 0.6 for homozygotes of the delT-allele [95% CI 0.42–0.88], $P = 0.007$); see Table 3 for the multiplicative model. However, the African-American samples did not show the same association. As an additional analysis, we tested the 3,663 control subjects from the Scandinavian, U.S., and Polish samples ascertained for diabetes and again saw no association with BMI in either group with K121Q, the three-marker haplotype, or rs1799774 under a recessive model (online appendix Table 1 [available at http://diabetes.diabetesjournals.org]). Finally, no other haplotype or SNP that we tested showed consistent association with obesity in our samples.

We did not reproduce an association with type 2 diabetes according to the previously proposed genetic models (Table 4). Despite having an estimated power of 99.8% to detect an association conferring a 1.3 increased odds of disease, neither the K121Q missense polymorphism nor the putative three-marker risk haplotype were associated

TABLE 4
Association of selected SNPs and the putative risk haplotype with type 2 diabetes

Population	rs1044498 (Q) OR (95% CI)	*P*	rs1799774 (delT) OR (95% CI)	*P*	rs7754561 (G) OR (95% CI)	*P*	Haplotype (Q-delT-G) OR (95% CI)	*P*
Scandinavia	1.11 (0.96–1.29)	0.16	0.95 (0.83–1.09)	0.48	0.92 (0.81–1.05)	0.21	1.06 (0.85–1.32)	0.61
U.S.	0.83 (0.71–0.97)	0.02	0.92 (0.80–1.06)	0.24	0.87 (0.77–0.99)	0.03	0.85 (0.70–1.03)	0.10
Poland	0.87 (0.73–1.03)	0.11	0.84 (0.72–0.98)	0.02	0.74 (0.64–0.86)	0.00006	0.89 (0.71–1.12)	0.32
Meta-analysis	0.94 (0.86–1.03)	0.20	0.91 (0.84–0.99)	0.02	0.85 (0.78–0.92)	0.00003	0.92 (0.81–1.04)	0.20

Scandinavia (plus Canada), $n = 4{,}206$; U.S. Caucasians, $n = 2{,}452$; Poland Caucasians, $n = 2{,}018$. The three SNPs and the haplotype formed by the minor alleles of each were tested for association with type 2 diabetes in our samples under a multiplicative, dominant, and recessive genetic model (multiplicative shown). Results from the various samples were combined by Mantel-Haenszel meta-analysis of the ORs. All *P* values are two tailed. To be consistent with the literature, ORs of individual SNPs are reported as minor vs. major allele.

with type 2 diabetes in our populations. Our result for the K121Q-allele (OR 0.94 [95% CI 0.86–1.03], $P = 0.2$) indicates that it is likely that the true OR in our population falls within this 95% CI (and is therefore <1.03). Given our data, it is unlikely that the actual OR for our populations lie within the range reported by Bacci et al. (7) in a meta-analysis of eight studies (1.29 [1.09–1.53]). A similar concurrent study (Weedon et al. [20]) also failed to detect any evidence of association with type 2 diabetes (K121Q 1.02 [0.93–1.12], $P = 0.61$).

Although neither the K121Q-allele nor the three-marker haplotype were associated with type 2 diabetes, in our populations the rs7754561 G-allele was nominally protective against type 2 diabetes (OR 0.85 [95% CI 0.78–0.92], $P = 0.00003$) (Table 4). A less robust nominal protection from type 2 diabetes was seen for the minor allele in rs1799774 (0.87 [0.79–0.97], $P = 0.009$ under a dominant model). However, these apparent associations are not consistent with results from other studies (see DISCUSSION). In addition, we tested for the reported association of these variants with fasting plasma glucose level (6) and did not detect a consistent association across our three populations (online appendix Table 2).

DISCUSSION

In summary, we were not able to detect a consistent association with obesity or diabetes phenotypes for either the K121Q minor allele or the risk haplotype Q-delT-G in large cohorts. Our results, those in the accompanying article by Weedon et al. (20), a recent Japanese study (21), and the conflicting data in the previous literature (5–8) (with some evidence for both the Q-allele and the K-allele being risk alleles) suggest that the previously reported associations to *ENPP1* may represent false positives or associations that are not easily generalizable. Our investigations are not an exact replication of the previously reported studies due to different ascertainment designs. However, we have successfully reproduced other associations with diabetes (*KCNJ11* [22,23], *PPARG* [10], and *TCF7L2* [24]) and obesity (*INSIG2* [25]) using these samples. In contrast to Meyre et al. (6), we did not test for association in children, possibly missing an effect specific to severe or early-onset obesity.

Our failure to reproduce associations of three selected variants in the *ENPP1* gene could be due to insufficient power to detect a modest effect. However, based on our 95% CIs, even quite modest risk effects consistent with previously published reports are unlikely (Tables 2 and 4). In light of the widely differing allele frequencies between populations of European and African ancestry, it is possible that population stratification could influence the results, either creating a false association or countering modest evidence (26). Family-based tests in our African-American cohort and in a subset of our diabetes samples are immune to population stratification. In addition, all subjects in the diabetes samples were of self-described northern European ancestry. In theory, strong interactions between *ENPP1* and unidentified population-specific genetic or environmental modifiers could also lead to heterogeneous results. Finally, the *ENPP1* gene has not been exhaustively surveyed; thus, a variant in weak linkage disequilibrium with the three markers tested could be contributing to weak but variable signals of association.

The significant association between the 3′ untranslated region SNP (rs7754561) and type 2 diabetes that we observed here was not found by Meyre et al. (6) (no effect seen in French subjects with diabetes and opposite effect in their Austrian samples) (genotype data kindly provided by D. Meyre and P. Froguel). Furthermore, it has not been detected by our colleagues, as described in the accompanying article by Weedon et al. (20). Thus, we do not feel that this represents a robust association between *ENPP1* and type 2 diabetes.

Only a few of the reported associations with diabetes and obesity have been consistently reproducible (27,28). While there may be a role for common genetic variation in *ENPP1* in obesity, diabetes, and related conditions, we believe that the current evidence does not convincingly support such a role. A meta-analysis of all published and unpublished data should have greater power to detect a modest effect of variation in this gene on diabetes and obesity. In addition, a more comprehensive assessment of genetic variation in and around *ENPP1* would be necessary to fully assess the role of this gene in obesity, insulin resistance, and type 2 diabetes.

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NOTE ADDED IN PROOF

After this manuscript was reviewed and revised, Grarup et al. (*Diabetologia* 49:2097−2104, 2006) reported a large study of Danish individuals in which they also failed to find an association of the K121Q-allele with type 2 diabetes; a modest association was seen between the Q-allele and BMI >25 kg/m^2. The meta-analysis presented by Grarup et al. does not include the data in this article or in that by Weedon et al. (20).

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