

**HAPLOTYPE STRUCTURE OF THE *ENPP1* GENE  
AND NOMINAL ASSOCIATION OF THE K121Q POLYMORPHISM  
WITH GLYCEMIC TRAITS IN THE FRAMINGHAM HEART STUDY**

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*Objective:*

A recent meta-analysis demonstrated a nominal association of the ectonucleotide pyrophosphate phosphodiesterase 1 (*ENPPI*) K121Q polymorphism with type 2 diabetes. We set out to confirm the association of *ENPPI* K121Q with hyperglycemia, expand this association to insulin resistance traits, and determine whether the association stems from K121Q or another variant in linkage disequilibrium with it.

*Research Design and Methods:*

We characterized the haplotype structure of *ENPPI* and selected 39 tag SNPs that captured 96% of common variation in the region (minor allele frequency  $\geq 5\%$ ) with an  $r^2 \geq 0.80$ . We genotyped the SNPs in 2,511 Framingham Heart Study participants and used age-sex-adjusted linear mixed effects models to test for association with quantitative metabolic traits. We also examined whether interaction between K121Q and BMI affected glycemic trait levels.

*Results:*

The Q allele of K121Q (rs1044498) was associated with increased fasting plasma glucose (FPG), HbA<sub>1c</sub>, fasting insulin, and insulin resistance by homeostasis model assessment (HOMA-IR; all  $P=0.01-0.006$ ). Two non-coding SNPs (rs7775386, rs7773477) demonstrated similar associations, but linear mixed effect models indicated that their effects were not independent from K121Q. We found no association of K121Q with obesity, but interaction models suggested that the effect of the Q allele on FPG and HOMA-IR was stronger in those with a higher BMI ( $P=0.008$  and  $0.01$  for interaction, respectively).

*Conclusions:*

The Q allele of *ENPPI* K121Q is associated with hyperglycemia and insulin resistance in whites. We found an adiposity-SNP interaction, with a stronger association of K121Q with diabetes-related quantitative traits in people with a higher BMI.

Enonucleotide pyrophosphatase phosphodiesterase 1 (*ENPPI*), also known as plasma cell membrane glycoprotein 1 (PC-1), is a transmembrane glycoprotein which down-regulates insulin signaling in cells by inhibiting the insulin receptor's tyrosine kinase activity, perhaps by interaction with its  $\alpha$  subunit (1). Within the coding region of *ENPPI* a K $\rightarrow$ Q missense single nucleotide polymorphism (SNP) at position 121 (K121Q; rs1044498) has been previously associated with insulin resistance and related abnormalities in some studies (2-7). The molecular mechanism thought to be responsible for the role of the Q121 variant is through a "gain of function" of the *ENPPI* protein inhibitory activity on the insulin receptor (8). It has also been reported that insulin receptor autophosphorylation in fibroblasts is decreased in Q allele carriers as compared to KK homozygotes (2). Thus, the *ENPPI* gene is considered to be a likely candidate gene for insulin resistance and type 2 diabetes (9).

Multiple studies have shown both positive and negative evidence of association between variants in *ENPPI* and obesity, type 2 diabetes and related traits. Most recently, Meyre *et al.* found that a haplotype formed by three SNPs in *ENPPI* (one of which was K121Q) were associated with childhood and adult obesity and type 2 diabetes (5). Three subsequent large association studies of variants in *ENPPI* detected no association of *ENPPI* K121Q with type 2 diabetes or obesity: Grarup *et al.* found no association of K121Q with type 2 diabetes in a Danish population (10); Lyon *et al.* found no significant association between three *ENPPI* SNPs (K121Q, rs1799774, and rs7754561) and body mass index (BMI) or type 2 diabetes (11); and Weedon *et al.* found no association with variants in *ENPPI* and type 2 diabetes or obesity in a study involving 8,089 subjects in the U.K. (12). In a comprehensive meta-analysis, we have recently shown that the

*ENPPI* K121Q variant confers a modestly increased risk of type 2 diabetes under a recessive genetic model in whites ( $P=0.005$ ), an effect which appears to be modulated by BMI (13). Although these studies were informative, only a handful of SNPs were genotyped, and common variation in the *ENPPI* locus has not been examined comprehensively. Given the conflicting body of evidence in the literature and incomplete evaluation of the *ENPPI* gene, we set out to confirm the association of *ENPPI* K121Q with hyperglycemia, expand the characterization of this association to quantitative insulin-related traits, assess the effect of adiposity on these associations, and determine whether the association stems from K121Q or another variant in linkage disequilibrium (LD) with it.

## METHODS

### Population samples

We used data from the Framingham Heart Study (FHS) to study associations between *ENPPI* variants and quantitative glycemic traits. The FHS is a community-based, multigenerational, longitudinal study of cardiovascular disease and its risk factors, including diabetes. The FHS is comprised of the Original Cohort, Offspring, and Generation 3 Studies. Subjects described in this present analysis include 2,511 individuals from the FHS Offspring cohort. In this analysis our principal diabetes-related quantitative traits come from Offspring examination 5 (1991-94) where data from a 75-gram oral glucose tolerance test (OGTT) is available for all Offspring without diagnosed diabetes. The study was approved by the Boston University's Institutional Review Board and written informed consent, including consent for genetic analyses, was obtained for all study participants. The demographic characteristics of the FHS study population are presented in Table 1.

An extensive array of diabetes-related quantitative traits has been collected in the FHS. Diabetes-related quantitative traits measured in this study include: glycated hemoglobin (HbA<sub>1c</sub>), fasting plasma glucose (FPG), fasting insulin, insulin resistance by homeostasis model assessment (HOMA-IR), B-cell insulin secretion by homeostasis model assessment (HOMA-B) (14), Gutt's 0-120 min insulin sensitivity index (ISI<sub>0-120</sub>) (15) and the time-averaged mean fasting plasma glucose level over exams 3-7 comprising 16 years (mean FPG). Laboratory methods for all quantitative traits have been described previously (16).

We used 2003 ADA clinical criteria to define diabetes, where a case was defined as use of oral hypoglycemic or insulin therapy, or a FPG  $\geq 7.0$  mmol/L at the index exam and FPG  $\geq 7.0$  mmol/L on at least one prior exam.

### SNP selection

We targeted a genomic region from ~20 kb upstream to ~10 kb downstream of *ENPPI*. We first downloaded Phase 2 HapMap ([www.hapmap.org](http://www.hapmap.org)) genotypes for the CEU population (accessed January 2006). We genotyped the previously reported non-HapMap SNP rs1799774 in the HapMap CEU samples so as to include it in its haplotype structure. We examined these 167 HapMap SNPs in Haploview (<http://www.broad.mit.edu/edu/mpg/haploview/>), and found that 95 passed quality control filters including genotyping call rate  $>75\%$ , Hardy-Weinberg equilibrium ( $P > 0.001$ ) and absence of Mendelian errors. We attempted to capture all remaining working variants with minor allele frequency (MAF)  $\geq 5\%$  by using *Tagger* (<http://www.broad.mit.edu/mpg/tagger/>), using a pair-wise approach, setting an  $r^2 \geq 0.8$  and forcing in the three previously reported SNPs (K121Q, rs1799774 and rs7754561). This procedure yielded 39 SNPs tagging the region, including rs1799774. We genotyped

these 39 SNPs (and an additional SNP, rs7773477, whose MAF is 4.5% in Framingham and is located at the 3' intron-exon junction of exon 2) in the FHS samples. Because 7 SNPs failed genotyping in these initial FHS samples, we repeated the tagging procedure forcing in all successful 32 SNPs as tags: this yielded 7 new SNPs, which were genotyped in the FHS samples for a total of 40 SNPs (39 tags and rs7773477).

### Genotyping

Genotyping was performed by allele-specific primer extension of multiplex amplified products with detection by matrix-assisted laser desorption ionization-time of flight mass spectroscopy on an iPLEX Sequenom platform. Average genotyping call rates were 97.7%, the minimum call rate was 94.7%, and the average consensus rate based on 254 duplicate samples was 99.5%.

### Statistical analysis

The quantitative traits were regressed against covariates in order to produce Studentized residuals. Two models were used: the first with sex, age and age<sup>2</sup> covariate adjustment and the second added BMI (kg/m<sup>2</sup>) to the other covariates to examine the strength of the subsequent SNP associations when adjusted for obesity. We adjusted for both age and age<sup>2</sup> to allow for a non-linear trend over time. The covariates from Offspring exam 5 were used for all the traits, except for mean fasting plasma glucose, in which case we used the 16-year average of each covariate.

The association between each residual and each SNP was assessed using a linear mixed effects (LME) model implemented in SOLAR (17) to account for the within-family correlation. Each SNP was included in a model as a fixed effect with additive coding, although additional dominant and recessive coding were evaluated to examine the association of rs1044498 (K121Q) with the quantitative traits. The models included

random effects to account for the covariance between family members; the covariance structure was determined by the degree of relatedness between each relative pair (17).

The role of BMI in the association between rs1044498 (K121Q) and the quantitative traits was further examined in LME models of the age-age<sup>2</sup>-sex adjusted residual with K121Q, BMI and an interaction term between K121Q and BMI as covariates.

To assess the association of each SNP with the type 2 diabetes phenotype, we used Cox proportional hazards survival analysis with diabetes as the outcome and the survival time as the age at the exam at which diabetes was determined. The survival time of individuals without diabetes was the age at their last exam. The model was implemented with the survival package in R (18), with the same adjustments as in the linear mixed effects models with covariates taken at the first exam. Trait correlation among siblings was modeled with a frailty term in the survival model (19).

To assess whether positive association signals were due to LD with K121Q or were independent we added the SNPs to LME models already containing K121Q. If the signals were independent of K121Q, we expected that they would remain significant in these models. Alternatively, if both K121Q and the other SNPs became non-significant, we would conclude that the signal in the other SNPs was not independent from K121Q.

Our study was formulated around a single primary hypothesis: we intended to replicate the association of K121Q with hyperglycemia as captured by diabetes-related traits and, if such association was confirmed, perform further covariate adjustment for improved characterization, and additional fine-mapping to determine the true source of the association signal. We have thus tested a unique SNP for association with several components of one composite trait (hyperglycemia) which is reflected in multiple correlated measures. We

believe this primary hypothesis should not be seen as making multiple unrelated comparisons, and therefore chose a nominal *P*-value of 0.05 to indicate statistical significance.

## RESULTS

An LD plot showing the completed haplotype structure of the *ENPPI* locus is presented in the online Supplemental Figure. The *ENPPI* gene region contains a high degree of LD. From the initial set of 167 SNPs, 96% of the 95 common variants with a MAF  $\geq 5\%$  were captured with an  $r^2 \geq 0.8$  (and 100% with an  $r^2 \geq 0.7$ ) by a set of 39 tag SNPs using single-marker (pairwise) tests. The list of the 39 successful tag SNPs used in this analysis, with chromosomal position, major allele, and MAF in both the HapMap and Framingham population is presented in Table 2. The list of 95 captured SNPs with their tags is listed in the online supplement (Electronic Supplementary Material [ESM] Table 1).

We examined whether individual SNPs in *ENPPI* were associated with hyperglycemic and insulin resistance traits in the FHS population. We first focused our attention on our principal polymorphism of interest, rs1044498 (K121Q), using three different genetic models: additive, dominant and recessive. Table 3 presents mean trait levels for each genotypic group, with *P* values for association with K121Q before and after adjustment for BMI. Several associations with insulin resistance traits reached nominal levels of significance: specifically, under the additive model the Q allele was associated with higher FPG ( $P=0.01$ ), HbA<sub>1c</sub> ( $P=0.006$ ), fasting insulin ( $P=0.006$ ), and HOMA-IR ( $P=0.006$ ); all of these associations remained significant after adjusting for BMI. Similar *P* values were obtained under the dominant genetic model. In this population sample, there were no differences in mean trait value across genotypic groups at *ENPPI* K121Q for

BMI ( $P=0.32$ ) or waist circumference ( $P=0.64$ ), both tested as continuous traits. When we compared the distribution of genotypes at this locus across individuals who were of normal weight (BMI  $<25$  kg/m<sup>2</sup>), overweight (BMI 25-30 kg/m<sup>2</sup>) or obese (BMI  $>30$  kg/m<sup>2</sup>), we found no association of K121Q with obesity as a categorical trait ( $P=0.66$ ). We also found no significant deviation from the null hypothesis of no association when our cohort was divided by BMI cutoffs at 25, 30 and 35 kg/m<sup>2</sup> (data not shown).

We then explored whether these consistent associations might be driven by other polymorphisms in the region. Table 4 displays the *ENPPI* SNPs for which we detected nominally significant associations with any of the glycemic traits under study. Figures 1A (glucose-related traits) and Figure 1B (insulin-related traits) display the  $P$  values for rs1044498 (K121Q) in comparison with those obtained for other SNPs across the genomic segment. Although K121Q was the SNP that showed the strongest association with fasting insulin and HOMA-IR (Figure 1B), two other SNPs (rs7775386 and rs7773477, located in intron 1 and at the exon 2 - intron 2 junction, respectively) achieved similar  $P$  values for associations with FPG and HbA<sub>1c</sub>; these two SNPs were also nominally associated with HOMA-IR (Table 4). In order to determine whether the effects of rs7775386 and rs7773477 were independent from K121Q or they produced an association signal simply because they are in tight LD with K121Q, we calculated the  $r^2$  among those SNPs in a subset of unrelated FHS participants. The  $r^2$  between K121Q and rs7775386 was 0.664, and between K121Q and rs7773477 was 0.285 (Table 4); however, given the high degree of relatedness within FHS pedigrees, these LD measures obtained from unrelated participants may be an underestimate of the true correlation between SNPs within the analytic dataset, where,

among related people, large chromosomal regions are expected to be identical by descent. We therefore also used LME models to examine simultaneously the effects of the three strongest association signals (K121Q, rs7773477 and rs7775386). While the association of rs7773477 with FPG remained nominally significant after addition of rs7775386 to the model, statistical significance disappeared after inclusion of K121Q to models that contained either rs7773477 or rs7775386. This indicates that the associations of these SNPs with diabetes-related quantitative traits are likely accounted for by their LD with K121Q, and that the latter is giving the strongest common variant association signal in the region.

Online supplementary table ESM Table 2 lists all the data for each polymorphism and quantitative traits examined. There was no significant association between any variant in *ENPPI* and BMI or waist circumference. There was no significant relationship between the non-HapMap SNP rs1799774 (which codes for a T/del change) and any insulin resistance trait. Other than a nominal  $P$  value of 0.02 for rs7775386, there was no significant association between K121Q or any other variant and risk for incident diabetes.

Finally, we also examined the interaction between K121Q and BMI, because of preliminary evidence suggesting that the effect of the Q allele may be modified by an increase in adiposity. There was a nominally significant interaction between genotype at *ENPPI* K121Q and BMI for the associations of the SNP with FPG (interaction  $P$  value=0.008,  $\beta$  estimate=0.017) and HOMA-IR (interaction  $P$  value=0.014,  $\beta$  estimate=0.016). This indicates a stronger genetic association of the Q allele with insulin resistance traits among people who have a higher BMI.

## DISCUSSION

The association of the K121Q polymorphism in *ENPPI* with insulin resistance and type 2 diabetes has been controversial. As far as insulin resistance, Pizzuti *et al.* found that non-obese, non-diabetic Q allele carriers were more insulin resistant than KK homozygotes, as defined both by OGTT and the euglycemic clamp (2). Subsequent studies reported both positive (20) and negative evidence (10) of association with insulin resistance. For type 2 diabetes, an initial positive result of association by Pizzuti *et al.* (2) was followed by replication in several independent studies (3-5) as well as confirmation in partial meta-analyses (10; 12; 21); however, three very large association studies (including one by members of our group) that comprised several thousand samples in other populations of European descent failed to reproduce the association, despite apparently adequate power to do so (10-12). In addition, five high-density genome-wide association studies (in samples that partially overlap those studied previously) have not reported a robust association at this locus (22-26). Nevertheless, a recent comprehensive meta-analysis by members of our group (13) documented a nominally significant ( $P=0.005$ ) association of the K121Q QQ genotype with type 2 diabetes in populations of European ancestry under a recessive model. The association appeared to be modified by BMI. The very modest effect of a single Q allele on the diabetes phenotype (summary odds ratio  $\sim 1.08$ ), the initial overestimate of this risk due to the phenomenon of the “winner’s curse”, the lack of power of subsequent studies to explore alternative genetic models, the confounder introduced by the widely divergent allele frequencies of the K121Q polymorphism in European and African populations, and the non-inclusion of a relevant covariate (BMI) in the assessment of its contribution to diabetes

risk may explain, in part, the conflicting results thus far reported in the literature.

Given the new evidence suggesting a real association of this polymorphism with type 2 diabetes, as well as functional reports implicating *ENPPI* and its polymorphism K121Q in mechanisms of insulin resistance (1; 8; 27-29), we decided to examine its association with insulin resistance traits in a homogeneous population cohort previously unexamined for this variant. In addition, we aimed to determine whether any association, if present, stemmed from *ENPPI* K121Q or another polymorphism in the region, and we aimed to characterize the putative modifying effect of BMI. The Framingham Offspring cohort is particularly advantageous for such a study: 1) as a population cohort, it is free of ascertainment biases which may restrict the range of variation around a quantitative glycemic or obesity trait; 2) it is an ethnically homogeneous sample; 3) it has undergone extensive phenotypic characterization in a longitudinal fashion; and 4) its family component reduces the risk of population stratification.

In this study, we captured most of the common genetic variation in *ENPPI* and studied its putative association with glycemic traits in a comprehensive manner. Using the FHS population to characterize the common variation across the *ENPPI* locus, we confirmed the association of the Q allele in *ENPPI* K121Q with hyperglycemia, as demonstrated by elevated FPG and HbA<sub>1c</sub>, under both the additive and dominant models. This supports the hypothesis that only one copy of the Q allele is necessary to be present to cause an effect on quantitative phenotypes. We also demonstrated that the effect of K121Q on hyperglycemia is likely mediated via insulin resistance, as the Q allele is also associated with elevated fasting insulin and HOMA-IR. The lack of an association with the Gutt insulin sensitivity index may reflect differences in insulin resistance at the tissue

level (basal hepatic insulin resistance versus peripheral glucose disposal after an oral load), or indicate an imperfect correlation of these surrogate measures with true insulin resistance. While other polymorphisms in the region showed similar associations with glycemic traits, our regression analysis demonstrated that the effect of two other significant SNPs was removed when incorporating K121Q in the models, suggesting that K121Q is the variant with the actual effect on glycemic and insulin resistance traits, as might be predicted by its impact on amino acid sequence. Because these associations represent confirmation of previous findings and other variants in the region were genotyped as a fine-mapping exercise, we do not believe statistical correction for the multiple variants analyzed is warranted.

Having established the association between *ENPPI* K121Q and hyperglycemia, we explored the interaction between K121Q and BMI, because of preliminary evidence that the effect of the Q allele on glycemic traits is mediated by an increase in adiposity (3; 5; 7; 13; 30), and suggestions that this variant may also contribute to obesity traits (5; 6; 31-33). While we observed no association of *ENPPI* K121Q with BMI or waist circumference, our interaction analysis supports the observation that a higher BMI strengthens this particular polymorphism's association with elevated insulin resistance and glucose levels. This finding is consistent with the hypothesis that the net effect of the *ENPPI* Q121 variant in modulating the risk of insulin resistance and related clinical outcomes is barely detectable in lean individuals, while becoming more evident in the context of an 'obesogenic' background, where the deleterious effect of the Q121 variant on the glucose disposal of skeletal muscle may be superimposed on that exerted by high BMI itself (30). This model is consistent with our previous meta-analysis in

which we noted that the Q121 variant confers a modest risk of type 2 diabetes in whites with a greater effect as BMI increases. Such BMI x genotype interactions may be particularly evident with regard to genes which cause hyperglycemia by augmenting insulin resistance, rather than in those which contribute to diabetes risk by diminishing insulin secretion. Due to the relationship between obesity and insulin resistance, there is more likely to be a correlation between increased adiposity and the effects of genes that modify insulin action.

In summary, our study adds further evidence in support of a potential causative role of the *ENPPI* gene in the inheritance and pathophysiology of type 2 diabetes. We found that the Q allele of K121Q in *ENPPI* appears to be the common variant most strongly associated with diabetes-related traits in whites, confirmed that K121Q is associated with hyperglycemia and a greater degree of insulin resistance, and found an adiposity-SNP interaction, with a greater strength of association of K121Q with diabetes-related quantitative traits in people with obesity.

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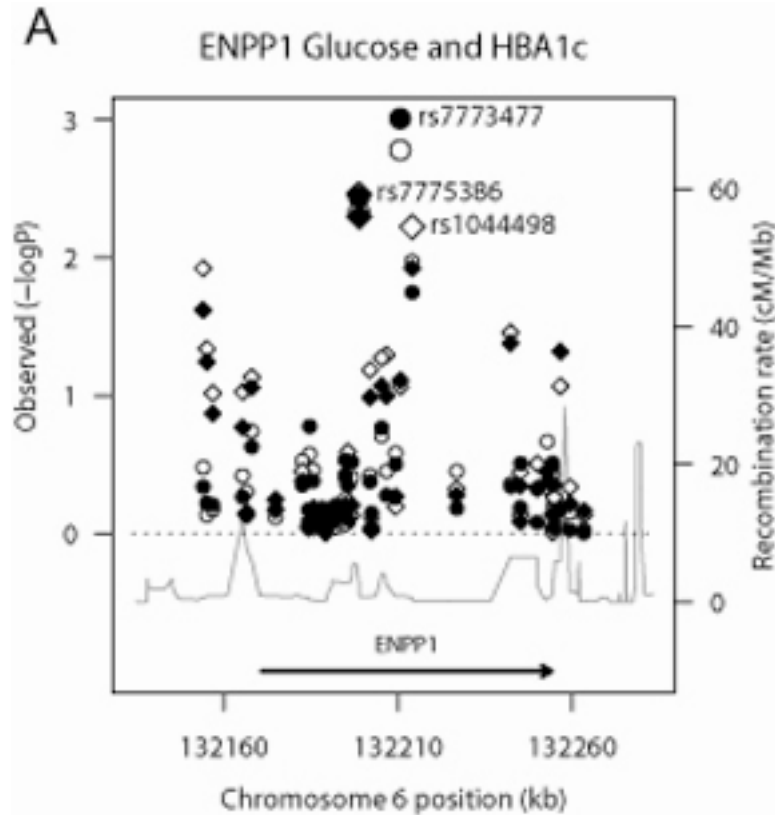


Figure 1A: Negative log base 10 of the  $P$  value for genetic associations with fasting plasma glucose (circles) and glycated hemoglobin (diamonds) under the additive model (left Y axis), graphed versus SNPs in the *ENPP1* region arranged by chromosomal position (X axis). The continuous line marked by the right Y axis indicates the recombination rate. The *ENPP1* gene is shown by the horizontal arrow at the bottom of the plot. Open symbols indicate traits adjusted for sex and age; closed symbols indicate additional adjustment for BMI.

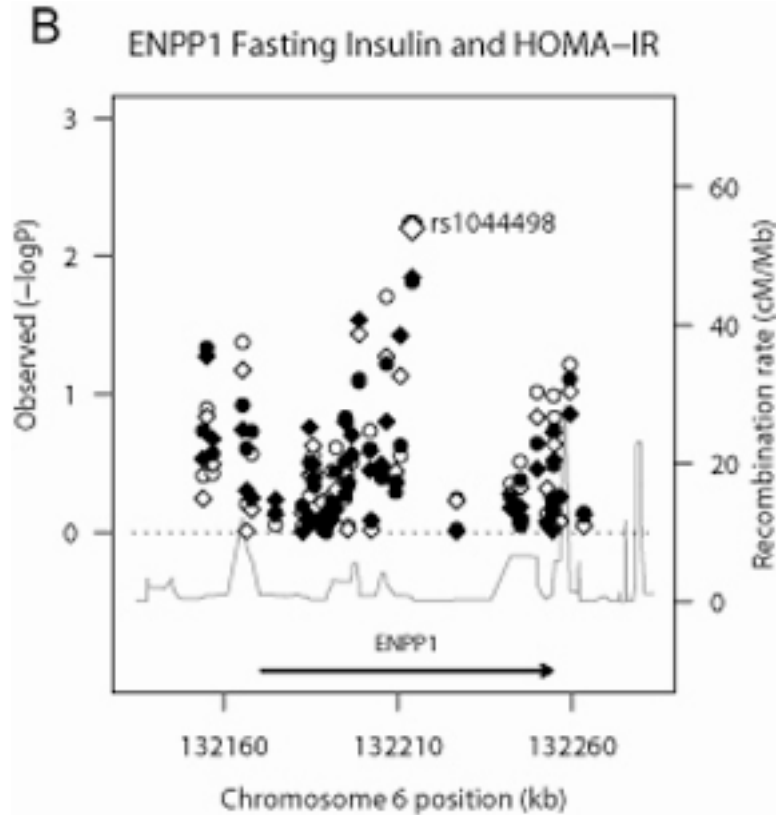


Figure 1B: Negative log base 10 of the  $P$  value for genetic associations with fasting insulin (circles) and insulin resistance by homeostasis model assessment (diamonds) under the additive model (left Y axis), graphed versus SNPs in the *ENPP1* region arranged by chromosomal position (X axis). The continuous line marked by the right Y axis indicates the recombination rate. The *ENPP1* gene is shown by the horizontal arrow at the bottom of the plot. Open symbols indicate traits adjusted for sex and age; closed symbols indicate additional adjustment for BMI.

## TABLES

TABLE 1: Demographic characteristics of the FHS participants

<b>Trait</b>	<b>N</b>	<b>Mean <math>\pm</math> SD or %</b>
Age at exam 5 (yrs)	2,397	54.1 $\pm$ 9.76
Gender (% female)	2,511	53.0%
Type 2 Diabetes	2,511	9.6%
Mean BMI (kg/m <sup>2</sup> )	2,387	27.4 $\pm$ 4.95
Exam 5 FPG (mg/dl)	2,365	100.2 $\pm$ 26.4
Exam 5 HbA <sub>1c</sub> (%)	1745	5.4 $\pm$ 0.97
Mean FPG (mg/dl)	2,510	99.4 $\pm$ 20.42
Exam 5 fasting insulin ( $\mu$ U/ml)	2,258	30.0 $\pm$ 12.27
Exam 5 HOMA insulin resistance	2,258	7.7 $\pm$ 4.91
Exam 5 Gutt insulin sensitivity index	2,146	25.7 $\pm$ 7.47
Exam 5 waist circumference (inches)	2,394	36.5 $\pm$ 5.65
Exam 5 HOMA-B $\beta$ -cell secretion	2,258	339.7 $\pm$ 269.96
Number of unrelated participants	1,436	
Number of pedigrees	282	
Number of sibpairs	1,004	
Number of avuncular pairs	114	
Number of cousin pairs	632	

*ENPPI POLYMORPHISMS AND GLYCEMIC TRAITS*

Table 2: Tag SNPs genotyped in the FHS sample

<b>Number</b>	<b>SNP</b>	<b>Position (NCBI 35)</b>	<b>M/m CEU</b>	<b>MAF CEU</b>	<b>M/m FHS</b>	<b>MAF FHS</b>
1	rs7752279	132154028	G/A	0.42	G/A	0.44
2	rs9493099	132155096	T/C	0.27	T/C	0.29
3	rs9493100	132156774	C/T	0.09	C/T	0.10
4	rs13211931	132165417	G/T	0.05	G/T	0.05
5	rs11154643	132166495	C/G	0.07	C/G	0.06
6	rs6935458	132168013	A/G	0.11	A/G	0.11
7	rs6569759	132174809	A/G	0.50	G/A	0.48
8	rs943004	132182569	G/A	0.06	G/A	0.07
9	rs7756163	132184356	T/C	0.39	T/C	0.38
10	rs12201710	132184591	G/A	0.22	G/A	0.25
11	rs1409182	132185636	G/A	0.16	G/A	0.12
12	rs9402345	132185906	G/A	0.10	G/A	0.11
13	rs9375830	132188213	G/A	0.22	G/A	0.20
14	rs6917903	132189309	G/C	0.38	G/C	0.42
15	rs1409181	132190993	G/C	0.48	C/G	0.47
16	rs2021966	132192132	T/C	0.44	T/C	0.46
17	rs9372999	132194845	C/A	0.10	C/A	0.12
18	rs858338	132194900	G/T	0.21	G/T	0.18
19	rs858339	132195590	T/A	0.32	T/A	0.29
20	rs703184	132196688	C/G	0.15	C/G	0.13
21	rs7775386	132198842	C/T	0.15	C/T	0.16
22	rs6916495	132201958	C/T	0.11	C/T	0.12
23	rs858342	132202336	A/G	0.27	A/G	0.25
24	rs858345	132205310	A/G	0.43	A/G	0.42
25	rs4141767	132206700	A/G	0.07	A/G	0.11
26	rs9402348	132209369	T/G	0.28	T/G	0.24
27	rs1044498*	132214061	A/C	0.12	A/C	0.17
28	rs9402349	132226801	A/C	0.12	A/C	0.12
29	rs9493116	132242326	A/G	0.05	A/G	0.07
30	rs7768480	132245125	A/G	0.06	A/G	0.11
31	rs1799774	132245167	T/del	0.33	T/del	0.25
32	rs7767111	132249978	G/A	0.09	G/A	0.06
33	rs1974201	132252814	C/G	0.10	C/G	0.23
34	rs7754561	132254387	A/G	0.20	A/G	0.28
35	rs9493120	132254694	G/A	0.06	G/A	0.06
36	rs9493121	132254883	A/G	0.09	A/G	0.05
37	rs1510	132256615	G/C	0.15	G/C	0.12
38	rs7753048	132259380	C/T	0.10	C/T	0.12
39	rs9373000	132263399	A/G	0.19	A/G	0.28

Position, major (M) and minor (m) nucleotides, and minor allele frequencies (MAF) for all 39 tag SNPs for HapMap (CEU) and Framingham (FHS) populations. \*K121Q SNP.

Table 3: Association between rs1044498 (K121Q) and diabetes-related quantitative traits

Trait	KK (n=1,982) Mean ± SD	KQ (n=695) Mean ± SD	QQ (n=74) Mean ± SD	% variance explained	<i>P</i> Additive	<i>P</i> Recessive	<i>P</i> Dominant
FPG (mg/dL)	99 ± 23.9	101 ± 29.6	107 ± 43.0	0.17% (0.11%)	0.01 (0.02)	0.07 (0.16)	0.02 (0.03)
HbA <sub>1c</sub> (%)	5.39 ± 0.93	5.48 ± 1.02	5.72 ± 1.24	0.40% (0.30%)	0.006 (0.01)	0.03 (0.03)	0.02 (0.03)
Mean FPG (mg/dL)	99.1 ± 19.92	99.6 ± 21.23	101 ± 21.5	0.005% (*)	0.21 (0.26)	0.42 (0.63)	0.25 (0.27)
Fasting insulin (μU/ml)	29.5 ± 11.57	31.3 ± 13.87	31.2 ± 12.09	0.32% (0.22%)	0.006 (0.01)	0.58 (0.96)	0.003 (0.006)
HOMA-IR	7.5 ± 4.71	8.06 ± 5.43	8.05 ± 4.37	0.29% (0.19%)	0.006 (0.01)	0.56 (0.95)	0.004 (0.005)
HOMA-B	343.8 ± 179.4	329.1 ± 444.7	336.8 ± 136.6	0.04% (0.03%)	0.40 (0.49)	—	—
Gutt's ISI	25.8 ± 7.36	25.4 ± 7.76	26.3 ± 7.42	0.03% (0.01%)	0.31 (0.42)	0.29 (0.15)	0.12 (0.15)
Waist circumference (inches)	36.5 ± 5.55	36.4 ± 5.93	37.6 ± 6.01	0.02% (0.04%)	0.64 (0.35)	0.43 (0.93)	0.80 (0.30)
BMI (kg/m <sup>2</sup> )	27.4 ± 4.83	27.5 ± 5.19	28.4 ± 6.26	0.06%	0.32	0.42	0.40

Associations between rs1044498 (K121Q) and selected quantitative metabolic traits in the FHS, with *P* values for the additive, recessive and dominant genetic models. The major (A) and minor (C) alleles code for the amino acids lysine (K) and glutamine (Q) respectively. The minor allele frequency is 15% in Framingham. All quantitative trait values are unadjusted means ± standard deviation, with *P* values without parentheses adjusted for sex and age, and *P* values in parentheses additionally adjusted for body mass index (BMI). FPG, fasting plasma glucose; mean FPG, FPG averaged over exams 3-7 comprising 16 years; HOMA-IR, insulin resistance by homeostasis model assessment; HOMA-B, β-cell secretion by homeostasis model assessment; Gutt's ISI, Gutt's insulin sensitivity index. \* indicates that the variance due to K121Q was not estimable due to instability.

Table 4: Nominally significant associations ( $P < 0.05$ ) of selected *ENPP1* SNPs with diabetes-related quantitative traits

SNP (MM/Mm/mm)	Alleles M/m	MAF	$r^2$	Trait	MM Mean $\pm$ SD	Mm Mean $\pm$ SD	mm Mean $\pm$ SD	$P$
rs7752279 (926/1,343/528)	G/A	0.43	0.106	HbA <sub>1c</sub>	5.38 $\pm$ 0.89	5.42 $\pm$ 0.95	5.53 $\pm$ 1.14	0.01 (0.02)
rs9493099 (1,375/1,164/225)	T/C	0.29	0.026	HbA <sub>1c</sub>	5.4 $\pm$ 0.94	5.43 $\pm$ 0.98	5.55 $\pm$ 1.09	0.046
				Fasting insulin	30.3 $\pm$ 12.15	29.9 $\pm$ 12.46	29.4 $\pm$ 12.37	(0.046)
rs13211931 (2,540/224/6)	G/T	0.04	0.063	Fasting insulin	29.9 $\pm$ 11.96	31.8 $\pm$ 15.34	34.7 $\pm$ 11.1	0.04
rs7775386 (2,031/642/55)	C/T	0.14	0.664	FPG	99 $\pm$ 23.4	102 $\pm$ 33.1	103 $\pm$ 35.5	0.005 (0.004)
				HbA <sub>1c</sub>	5.39 $\pm$ 0.9	5.53 $\pm$ 1.16	5.64 $\pm$ 1.05	0.004 (0.005)
				Mean FPG	98.8 $\pm$ 19.56	100.9 $\pm$ 23.16	99.7 $\pm$ 20.67	0.03 (0.02)
				HOMA-IR	7.5 $\pm$ 4.78	8 $\pm$ 5.4	7.8 $\pm$ 4.12	0.04 (0.03)
rs4141767 (2,230/489/34)	A/G	0.10	0.591	Fasting insulin	29.7 $\pm$ 11.71	31.3 $\pm$ 14.23	32.6 $\pm$ 13.07	0.02
rs7773477 (2,509/235/7)	G/T	0.05	0.285	FPG	99 $\pm$ 24.8	106 $\pm$ 40.8	103 $\pm$ 13.6	0.002 (0.001)
				HOMA-IR	7.6 $\pm$ 4.94	8.1 $\pm$ 4.94	8.8 $\pm$ 4.17	(0.04)
rs9493116 (2,377/343/20)	A/G	0.07	0.410	HbA <sub>1c</sub>	5.41 $\pm$ 0.95	5.56 $\pm$ 1.15	5.58 $\pm$ 0.54	0.03 (0.04)
rs1510 (2,105/608/30)	G/C	0.12	0.026	HbA <sub>1c</sub>	5.45 $\pm$ 1	5.33 $\pm$ 0.85	5.75 $\pm$ 1.16	(0.048)

Association between SNPs (listed in order of increasing chromosomal position, with genotype counts in parentheses under the rs number) and phenotypic traits that had nominal  $P$  values less than 0.05 using an additive genetic model. The minor allele frequencies (MAF) are based on data from FHS unrelated participants. The extent of LD of each SNP in relation to rs1044498 (K121Q) is shown by presenting  $r^2$  values obtained from the unrelated subset of our FHS sample. All quantitative trait values are crude means  $\pm$  standard deviation, with  $P$  values without parentheses adjusted for sex and age, and  $P$  values in parentheses

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additionally adjusted for body mass index (BMI). M, major allele; m, minor allele; FPG, fasting plasma glucose; mean FPG, FPG averaged over exams 3-7 comprising 16 years; HOMA-IR, insulin resistance by homeostasis model assessment.

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