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Functional ENTPD1 Polymorphisms in African Americans With Diabetes and End-Stage Renal Disease

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OBJECTIVE—The vascular ectonucleotidase ENTPD1 protects against renal injury and modulates glucose homeostasis in mouse models. We sought to determine whether human variation in ENTPD1 influences predisposition to diabetes or diabetic nephropathy.

RESEARCH DESIGN AND METHODS—We analyzed ENTPD1 single nucleotide polymorphisms (SNPs) in 363 African American control subjects, 380 subjects with type 2 diabetes and end-stage renal disease (DM-ESRD), and 326 subjects with ESRD unrelated to diabetes (non–DM-ESRD). Using human cell lines, we correlated disease-associated ENTPD1 haplotypes with ENTPD1 gene expression. Finally, we studied consequences of ENTPD1 deletion in a mouse model of type 2 diabetes (db/db).

RESULTS—A common ENTPD1 two-SNP haplotype was associated with increased risk for DM-ESRD (P = 0.0027), and an uncommon four-SNP haplotype was associated with protection against DM-ESRD (P = 0.004). These haplotypes correlated with ENTPD1 gene expression levels in human cell lines in vitro. Subjects with high ENTPD1-expressing haplotypes were enriched in the DM-ESRD group. By crossing ENTPD1-null mice with db mice, we show that ENTPD1 deletion has profound effects on metabolic syndrome traits. Specifically, deletion of ENTPD1 lowered glucose levels in control (db/−) mice with one functional leptin receptor and dramatically lowered weights in db/db mice with no functional leptin receptors. Similar effects were seen in aged ENTPD1-null mice with normal leptin receptors.

CONCLUSIONS—ENTPD1 polymorphisms appear to influence susceptibility to type 2 diabetes and/or diabetic nephropathy in African Americans. Studies in human cell lines and in vivo mouse data support a potential role for ENTPD1 genetic variation in susceptibility to type 2 diabetes. Diabetes 58:999–1006, 2009

Diabetes and its complications are enormous sources of mortality, morbidity, and cost in the U.S. African Americans are especially prone to diabetes and diabetic kidney disease (1). Genetic variation plays a major role in susceptibility to both development of diabetes and onset of diabetic renal injury (2,3). Finding genes responsible for this predisposition to disease could help predict who may be at high risk and also identify important pathways involved in disease pathogenesis.

ENTPD1, also known as CD39, is an ectonucleotidase that hydrolyzes ATP and ADP to AMP, initiating an enzymatic cascade that leads to the generation of adenosine (4). By regulating nucleotide levels, ENTPD1 controls the activity of both purinergic receptors (P2X and P2Y) and downstream adenosine (P1) receptors. ENTPD1 is widely expressed, particularly in blood vessels and on cells of the immune system (5–7). ENTPD1 is present in glomeruli, afferent arterioles, and larger vessels of the kidney (8,9). Mouse null for ENTPD1 sustained markedly more severe renal injury than wild-type mice in models of type 1 diabetes (9) and ischemia reperfusion injury (10).

Previously, a locus for susceptibility to diabetic and nondiabetic renal disease was located on human chromosome 10q24 in African Americans (11). Subsequently, others have also found loci in this chromosomal region associated with renal dysfunction in Caucasians with type 2 diabetes (12). It appears likely that one or more genes with effects on nephropathy are located in this region. ENTPD1 is located close to the linkage peak in both of these studies. More recently, we have reported that ENTPD1-null mice also exhibit abnormalities of glucose homeostasis (13). The close functional relationship between ENTPD1 and another ectonucleotidase that influences metabolic syndrome traits in humans, ENPP1, (14) suggests that ENTPD1 could affect glucose homeostasis in humans as well.

To determine whether variation in ENTPD1 might influence the development of diabetes or diabetic renal disease, we tested the frequency of single nucleotide polymorphisms (SNPs) in ENTPD1 in three groups of African American patients: 363 healthy control subjects, 326 patients with nondiabetic end-stage renal disease (non–DM-ESRD), and 380 patients with ESRD caused by type 2 diabetes (DM-ESRD). We support these genetic association results with functional studies in vitro using human cell lines and in vivo using mouse models with nonfunctional leptin receptors (db/db). We show that ENTPD1 gene expression in human cell lines correlates with the disease-associated genotypes, and mice null for ENTPD1 share metabolic phenotypes with humans who have low ENTPD1-expressing genotypes.
RESEARCH DESIGN AND METHODS

Collection of control and case subjects has been described previously (15,16). Briefly, all subjects were recruited in North Carolina, South Carolina, Georgia, Virginia, or Tennessee, and all were born in one of these five southeastern states. A total of 363 unrelated African American control subjects were recruited through community screening events, all without a history of type 2 diabetes or renal disease. A total of 380 unrelated African American patients with type 2 diabetes and ESRD were recruited (DM-ESRD group) from dialysis clinics. A total of 326 unrelated African American patients with ESRD but without a history of diabetes (non–DM-ESRD) were also recruited from dialysis clinics. A single investigator (B.I.F.) classified all ESRD patients into DM-ESRD or non–DM-ESRD groups. Non–DM-ESRD patients included those with hypertension-associated ESRD or primary glomerular diseases. The DM-ESRD groups included patients with either 1) diabetes and histologic evidence of diabetic nephropathy or 2) with diabetes ≥5 years before the initiation of renal replacement therapy and either diabetic retinopathy or proteinuria >500 mg per 24 h. DM-ESRD patients had no other known cause of renal disease. Patients with renal cystic diseases, hereditary nephritis, or ESRD related to urologic disease were all excluded from analysis.

Genotyping. A total of 18 SNPs tagging 80% of the known SNPs in the Yoruba (YRI) Hapmap population were selected using the program Tagger (www.broad.mit.edu/mpg/tagger). Tag SNPs all had minor allele frequency of ≥5%. rs3768774, the only common nonsynonymous coding variant in ENTPD1, was forced into SNP selection. Tag SNPs spanned the ENTPD1 gene on chromosome 19 from 97,504 to 97,613 Mb. We used a Sequenom MassArray platform (17) for genotyping.

Genetic analysis. SNPs with call rates <96% were excluded from analysis. All SNPs were in conformance with Hardy-Weinberg equilibrium (P < 0.05) in control subjects. Determination of haploblock structures and associations were performed using Haploview (www.broad.mit.edu/mpg/haplovew). Haplotypes with frequency >1% were included in the analysis. Permutation testing for haplotype associations was performed using Haploview software at 10,000 permutations.

In vitro ENTPD1 expression studies. Genotyped, Epstein-Barr virus–transformed lymphoblasts from the YRI HapMap (www.hapmap.org) collection and Perlegen (www.perlegen.com) African American panel were used for analysis. Cells were grown in RPMI-1640 media with 2 mmol/l glucose, 15% fetal bovine serum, penicillin-streptomycin mix, and 10 mmol/l Hepes buffer. Cells were seeded at 200,000 per ml and harvested 24–48 h later in log-phase growth. Then, mRNA was isolated using NReasy (Qiagen) according to the manufacturer’s instructions. A total of 0.5 μg of mRNA was reverse transcribed to cDNA using the TaqMan Reverse Transcription Kit (Applied Biosystems). Probe-primer sets for ENTPD1 and the 18S ribosomal subunit (loading control) were obtained from Applied Biosystems. Real-time PCR was performed on an Applied Biosystems 7700 system. ENTPD1 mRNA expression was normalized using 18S expression.

For protein isolation, cells were washed twice with ice-cold PBS and then lysed in an NP-40–based buffer with protease inhibitors (Roche mini-tabs). A total of 40 μg of protein were separated on a gradient gel under nonreducing conditions and transferred to polyvinylidene fluoride paper (Millipore) using a semidry transfer apparatus. For ENTPD1, we used a mouse monoclonal antibody (BU-61; Ancell) and a goat anti-mouse secondary antibody (Pierce). We used an anti–glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (Ambion) as a loading control. Band density was quantified with a Kodak ImageStation 2000M, and protein level was expressed as a ratio of ENTPD1 to GAPDH.

In silico, expanded ENTPD1 expression studies. For YRI HapMap subjects, ENTPD1 gene expression levels were downloaded from http://www.sanger.ac.uk/humgen/genevar/). Methods for the generation of this data are described by Stranger et al. (18). Genotyped were obtained from www.hapmap.org. Statistics were performed using Student’s t test on log2-transformed data.

Enzyme activity. A total of 250,000 lymphocytes were harvested in log-phase growth. ATPase and ADPase activity were determined with a Malachite Green assay for phosphate production as described previously using 200 μmol/l nucleotide as the substrate during a 10-min incubation (19). Tetramisole was also used to inhibit alkaline phosphatases (19).

Animal studies. All mouse studies were done with institutional animal care and use committee approval at Beth Israel Deaconess Medical Center. Mice in all four db groups originated from crossing db/db heterozygote breeders on a C57/BL6 background from Jackson Labs with ENTPD1+/− breeders backcrossed more than six times on a C57/BL6 background. Diabetic mice were db/db offspring with or without ENTPD1, and db+/- offspring were used as controls. In the aging study, male ENTPD1+/− mice backcrossed more than six times onto a C57/BL6 background were compared with age- and strain-matched C57/BL6 wild-type controls.
Hardy-Weinberg equilibrium criteria as described in RE-
of 18 SNPs passed quality standards and conformed to SNPs in the Yoruba (YRI) Hapmap population. Seventeen ENTPD1 that were predicted to cover 80% of the known athy (confer increased risk of diabetes and/or diabetic nephrop-
comparing control subjects with non–DM-ESRD subjects. A broader, unbiased haplotype analysis using all success-
sfully genotyped SNPs showed that 16 of the SNPs fell into a single haplotype block defining 10 haplotypes with frequency >1%. One haplotype was associated with pro-
tection against diabetes and/or diabetic nephropathy with $P = 0.004$ (odds ratio 0.34). This statistical significance of this protective haplotype remained unchanged when re-
duced to four SNPs (rs4918972, rs10748648, rs1342790, and rs3793744; GAGA in Table 2). After permutation analysis, the P value remained significant at 0.017. The GAGA haplotype is referred to as the four-SNP protective haplotype.

Animal phenotyping. Albuminuria was quantified using an albumin-to-creatinine ratio. Albumin was measured using the Albuwell-M Kit from Exocell, and creatinine was measured using the Creatinine Companion from Exocell. Blood glucose was measured using a One Touch Ultra 2 glucometer (Lifescan). For the db-based studies, nonfasting glucose levels were measured at 3:00 p.m. For the non-db studies (aging), glucose levels were measured after an 8h daytime fast.

For histopathology, mouse kidney tissue was fixed in 10% buffered formalin and embedded in paraffin then cut into 3-micron sections and stained with PAS stain. Glomerular area was calculated using Spot Imaging software. The glomerular sclerosis index has been described previously (9).

Haplotypes

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Control subjects</th>
<th>Case subjects</th>
<th>$\chi^2$</th>
<th>$P$</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG</td>
<td>0.829</td>
<td>0.799</td>
<td>0.857</td>
<td>9.0</td>
<td>0.0027</td>
<td>1.51 (1.15–1.99)</td>
</tr>
<tr>
<td>AA</td>
<td>0.108</td>
<td>0.124</td>
<td>0.091</td>
<td>4.2</td>
<td>0.0404</td>
<td>0.70 (0.51–0.98)</td>
</tr>
<tr>
<td>TG</td>
<td>0.063</td>
<td>0.077</td>
<td>0.051</td>
<td>4.1</td>
<td>0.0431</td>
<td>0.64 (0.42–0.98)</td>
</tr>
<tr>
<td>GAGA</td>
<td>0.432</td>
<td>0.428</td>
<td>0.436</td>
<td>0.10</td>
<td>0.7495</td>
<td>1.03 (0.84–1.27)</td>
</tr>
<tr>
<td>GTAG</td>
<td>0.393</td>
<td>0.386</td>
<td>0.398</td>
<td>0.23</td>
<td>0.6323</td>
<td>1.05 (0.85–1.30)</td>
</tr>
<tr>
<td>ATAG</td>
<td>0.139</td>
<td>0.141</td>
<td>0.138</td>
<td>0.04</td>
<td>0.8336</td>
<td>0.97 (0.72–1.30)</td>
</tr>
<tr>
<td>GAGA</td>
<td>0.023</td>
<td>0.034</td>
<td>0.042</td>
<td>0.36</td>
<td>0.5547</td>
<td>0.93 (0.66–1.32)</td>
</tr>
<tr>
<td>GTGA</td>
<td>0.013</td>
<td>0.010</td>
<td>0.016</td>
<td>0.09</td>
<td>0.3259</td>
<td>1.67 (0.65–4.26)</td>
</tr>
</tbody>
</table>

Association of DM-ESRD with two-SNP haplotypes consisting of the two nominally significant SNPs rs12763743 and rs3807983. The AG haplotype is referred to as the two-SNP risk haplotype, and the AA and TG haplotypes are referred to as the two-SNP protective haplotypes.

A broader, unbiased haplotype analysis using all success-
results. For this haplotype when comparing control sub-
to non–DM-ESRD subjects.

RESULTS

Using a set of markers chosen with the program Tagger, we genotyped 18 SNPs (minor allele frequency >5%) in ENTPD1 that were predicted to cover 80% of the known SNPs in the Yoruba (YRI) Hapmap population. Seventeen of 18 SNPs passed quality standards and conformed to Hardy-Weinburg equilibrium criteria as described in RESEARCH DESIGN AND METHODS above. These 17 SNPs tagged 79% of YRI HapMap SNPs.

Location of SNPs in the ENTPD1 gene, minor allele frequency of each SNP, and uncorrected P values in the populations in this study are shown in Table 1. When control subjects and subjects with non–DM-ESRD were compared, one SNP (rs65584026) showed nominal signifi-
cance ($P = 0.027$). Comparing control subjects and sub-
jects with DM-ESRD revealed two SNPs (rs12763743 and rs3807983) that were nominally significant (0.041 and 0.037, respectively).

We considered the possibility that the two nominally
significant SNPs for DM-ESRD might have additive effects. We used the two significant SNPs discovered in the DM-ESRD population to form a two-SNP haplotype for further analysis. Three common combinations accounted for all subjects tested ($AG = 82.9\%, AA = 10.8\%, and TG = 6.3\%$). The most common haplotype (AG) appeared to confer increased risk of diabetes and/or diabetic nephropathy ($P = 0.0027$, $P = 0.009$ after permutation; odds ratio 1.51) compared with the other two haplotypes, which were both nominally associated with protection from diabetes and/or diabetic nephropathy (Table 2). We refer to the AG haplotype as the two-SNP risk haplotype, and the combined group of AA and TG haplotypes as the two-SNP protective haplotypes. No difference was seen with re-
to these two-SNP risk or protective haplotypes when comparing control subjects with non–DM-ESRD subjects.
were homozygous for the AA protective haplotype and only one was homozygous for the TG protective haplotype. Since the AA and TG haplotypes were almost identical in terms of risk ($P = 0.04$, nominally protective) and odds ratios (0.70 and 0.64, respectively), we pooled these into a single group and compared ENTPD1 expression from their lymphocytes with those from the risk (AG) haplotype group using real-time PCR (Fig. 1A). Lymphocytes homozygous for the AG risk haplotype had 39% higher ENTPD1 expression levels than homozygotes for the protective AA or TG haplotypes (protective $5.62 \pm 0.36$ vs. risk $7.81 \pm 0.36; P = 0.007$), with no overlap between groups (range $5.09$–$6.30$ for protective and $6.91$–$9.53$ for risk). Not enough cell lines homozygous for the four-SNP protective haplotype (one cell line) were available for comparison.

To provide additional support for a correlation between haplotype and expression level, we used gene expression data from Stranger et al. (18) generated from YRI HapMap cell lines, which allowed us to incorporate haplotype heterozygotes into the analysis. For consistency, we analyze the two- and four-SNP haplotypes in terms of number of protective alleles. We pooled subjects with one or two protective alleles for the two-SNP haplotype and compared them with subjects with no protective alleles (two risk alleles). Subjects with zero protective haplotypes ($n = 31$) expressed 16% more ENTPD1 than subjects with one or two protective haplotypes ($n = 29; P = 0.027$) (Fig. 1B). We then pooled subjects with one or two copies of the protective four-SNP allele ($n = 7$) and compared them with those with no copies ($n = 53$). While the difference was not significant ($P = 0.09$), those with no protective alleles expressed 16% more ENTPD1 than those with one or two protective alleles (Fig. 1C). These cell lines with at least one protective four-SNP allele had the lowest mRNA expression levels of any haplotype group, including the protective two-SNP haplotype. To account for the fact that we were comparing subjects with protective four-SNP alleles versus a pool that contained many more subjects with one or more protective two-SNP allele, we then compared subjects based on their total number of protective alleles (two-SNP protective alleles plus four-SNP protective alleles) to integrate the effects of two-SNP and four-SNP haplotypes on ENTPD1 expression. Those with no protective alleles ($n = 29$) expressed 18% more ENTPD1 than subjects with at least one protective two-SNP allele or one protective four-SNP allele ($n = 31; P = 0.01$). Both haplotypes appear to correlate with ENTPD1 expression and were especially predictive of mRNA expression levels when used in tandem. The small number of subjects with the protective four-SNP allele prevented us from determining whether the effects of the two-SNP and four-SNP haplotypes were independent.

The protective four-SNP haplotype (GAGA) included a SNP causing an amino acid change (rs3793744: V to I at position 293). A second, uncommon haplotype (GTGA) also included the minor allele (A) at rs3793744 but was not associated with DM-ESRD when compared with control subjects. We compared expression levels between cell lines with one or more copies of each of these haplotypes. Remarkably, the nonprotective (GTGA) four-SNP haplotype including the minor allele at rs3793744 expressed 45% more ENTPD1 mRNA than the protective (GAGA) four-SNP allele with the same SNP at rs3793744 ($n = 3$ vs. $n = 7; P = 0.0006$). This finding suggests that differences in mRNA expression underly the effect of the four-SNP haplotypes.
haplotype rather than the amino acid substitution. We did note, though, that protein expression in the homozygotes for the protective four-SNP haplotype were surprisingly low compared with other cell lines (supplementary Fig. 1A and B). This finding was demonstrated in single samples from two separate collections (HapMap and Perlegen), and activity assays showed that the effect was not due to differential antibody binding at the amino acid change at position 293 (rs3793744) (supplementary Fig. 1C and D).

To further investigate the effects of ENTPD1 on either diabetes or diabetic nephropathy, we crossed ENTPD1-null mice with db/db mice to create a type 2 model of diabetes and type 2 diabetic nephropathy. Assessment for renal injury at 8 months of age demonstrated that in the nondiabetic mice (db/-), ENTPD1 deletion caused mildly increased albuminuria (wild type = 37.2 ± 2.3 μg/mg vs. knockout = 54.7 ± 6.7 μg/mg; P = 0.028) and a trend toward increased glomerular sclerosis (Fig. 2A and B). In the diabetic groups, there were no differences in albuminuria, sclerosis index, or glomerular area between db/db mice with and without ENTPD1 (Fig. 2C–E).

Metabolic syndrome parameters revealed more dramatic differences. In nondiabetic mice, nonfasting (3:00 P.M.) glucose was lower in ENTPD1-null mice at both 8 weeks (wild type = 208.1 ± 4.8 mg/dl vs. knockout = 176.8 ± 7.8 mg/dl; P = 0.006) and 6 months (wild type = 179.2 ± 5.7 mg/dl vs. knockout = 161.9 ± 5.8 mg/dl; P = 0.039) (Fig. 3A and B). No differences in glucose levels were seen in the db/db groups at these time points with or without ENTPD1 (Fig. 3C and D).

While weights of nondiabetic mice were no different (Fig. 4A and B), large differences were seen in db/db mice, with deletion of ENTPD1 protecting against obesity in the db/db mice at both 8 weeks (wild type = 41.4 ± 1.0 g vs. knockout = 37.6 ± 1.4 g; P = 0.026) and 6 months (wild type = 62.4 ± 0.9 g vs. knockout = 52.0 ± 1.3 g; P < 0.000001) (Fig. 4C and D).

We also aged wild-type and ENTPD1-null mice (leptin receptor normal) to see if aging would cause similar

![Graph A](image1.png)

**FIG. 2.** A: Albumin-to-creatinine ratio for db/- nondiabetic mice. n = 7 for ENTPD1 wild type and n = 12 for ENTPD1 knockout. B: Sclerosis index for db/- nondiabetic mice. n = 7 for ENTPD1 wild type and n = 12 for ENTPD1 knockout. C: Albumin-to-creatinine ratio for db/db mice. n = 13 for ENTPD1 wild type and n = 9 for ENTPD1 knockout. D: Sclerosis index for db/db mice. n = 13 for ENTPD1 wild type and n = 10 for ENTPD1 knockout. E: Glomerular area for db/db mice. n = 10 for ENTPD1 wild type and n = 8 for ENTPD1 knockout.
metabolic differences as we saw in the db/db model. At 2 years of age, ENTPD1-null mice had markedly lower fasting glucoses (wild type = 141.6 ± 5.1 mg/dl vs. knockout = 112.8 ± 3.3 mg/dl; P = 0.0009) and lower weights (wild type = 46.4 ± 2.7 g vs. knockout = 39.2 ± 0.7; P = 0.042) than wild type controls (Fig. 5A and B). Consistent with the db experiments, we did also note increased albuminuria in aged ENTPD1-null mice despite lower weights and blood glucoses (D.J.F., unpublished data).

**DISCUSSION**

Our previous studies in ENTPD1-null mice suggested a role for this ectonucleotidase in both resistance to renal injury and in glucose homeostasis. We tested polymorphic markers in the ENTPD1 gene in humans for association with diabetic ESRD and nondiabetic ESRD in African Americans, a group of Americans with high rates of both diseases. We found two common polymorphisms (rs12763743 and rs3897983) that are nominally associated with diabetic but not nondiabetic ESRD. When used together, these two polymorphisms showed even stronger association (P = 0.0027) with subjects in the diabetic nephropathy group. These two SNPs may represent independent, additive effects or they may together tag a causal SNP better than either of the SNPs alone. More general haplotype analysis also identified an uncommon four-SNP haplotype that appears to protect against diabetes or diabetic nephropathy (P = 0.004). The effect size of this uncommon haplotype is strong, conferring a relative risk of 0.34. Additional groupwise comparisons using pooled groups further suggested that the association was due to diabetes rather than nephropathy in the DM-ESRD groups.

To support association the association of ENTPD1 with diabetes and/or diabetic nephropathy, we tested HapMap cell lines derived from African subjects for ENTPD1 expression. Cell lines homozygous for the two-SNP (AG) risk haplotype expressed 39% more ENTPD1 mRNA than those cell lines with two protective haplotypes (AA and TG). We performed additional bioinformatic analysis that showed similar results: zero alleles of the two-SNP protective haplotypes led to 16% greater ENTPD1 expression than one or two protective alleles. The protective four-SNP haplotype also showed a trend toward lower ENTPD1 expression levels, though this analysis was limited by fewer representative cell lines and was confounded by comparison with a group including many protective two-SNP haplotypes. In fact, the protective four-SNP haplotype showed the lowest ENTPD1 expression of any group. By using an index that took into account the total number of both the two- and four-SNP protective haplotypes, we identified a strong correlation between genotype and ENTPD1 mRNA expression level using both haplotypes derived from our genetic association study. Additional experiments measuring ENTPD1 protein levels confirmed that the protective four-SNP haplotype was associated with very low ENTPD1 levels and may suggest an effect beyond mRNA expression, though we did not have suffi-
cient samples available to us to prove this hypothesis definitively.

We also used genetic studies in mice to assess the role of ENTPD1 on diabetes and diabetic nephropathy. While ENTPD1-null control mice did have increased albuminuria compared with wild-type mice, perhaps the more striking findings related to metabolic syndrome traits. In particular, ENTPD1-null mice had lower glucose levels in the absence of the homozygous db/db mutation and dramatically lower weights in the presence of the db/db mutation. Notably, previous work demonstrated that ENTPD1-null mice have higher leptin levels than wild-type mice (13), potentially a compensatory response that is disabled in the absence of one or both copies of the normal leptin receptor. The exact reason for this differential effect in db/db and db/− mice on blood glucose and weight still remains to be elucidated. However, even in the presence of normal leptin signaling, using wild-type mice rather than db/− heterozygotes as controls, aging accentuated both phenotypes (i.e., difference in glucose homeostasis and weight gain). Our mouse findings again suggest that the effect we discovered in humans may relate to low ENTPD1 protecting against diabetes in control subjects, though increased albuminuria in the nondiabetic CD39-null mice may point toward an effect on renal disease as well.

Further support for association between ENTPD1 and diabetes and diabetic nephropathy is the finding that
polymorphisms in another ectonucleotidase with a remarkably similar enzymatic mechanism, ENPP1, have also been associated with both diabetes and diabetic nephropathy. Nephropathy has been associated with both diabetes and diabetic nephropathy. First, in Caucasian populations (20–22), and subsequently in an African American population that included our subjects (23). Polymorphisms in the same gene also have an important influence on metabolic syndrome traits (14), and there appears to be an important interaction between weight and glucose homeostasis (24,25), just as we see with ENTPD1 in mice. While ENPP1 hydrolyzes extracellular ATP to AMP and pyrophosphate, ENTPD1 hydrolyzes extracellular ATP to AMP and two phosphate molecules. Low functional expression of either of these ectoenzymes could increase extracellular levels of ATP and decrease levels of the nucleoside adenosine.

The strength of our study rests on mutually supportive data built from genetic association of human disease with genetic variants, gene expression levels that correlate with the disease-associated haplotypes, and a mouse model that shows phenotypic differences based on expression level of the gene. Ultimately, replication in larger cohorts will be needed to validate our findings. Our data, along with previous studies with ENPP1, suggest that extracellular nucleotide metabolism may be a critical determinant of diabetes and/or diabetic nephropathy.

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No potential conflicts of interest relevant to this article were reported.

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