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A common non-synonymous variant in *GLUT9* is a determinant of serum uric acid levels in Old Order Amish

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Abstract

Objective—Uric acid is the primary end product of purine metabolism. Increased serum uric acid has been associated with gouty arthritis as well as with a variety of cardiovascular related phenotypes.

Methods—A 500,000 SNP genome wide association study of serum uric acid levels was performed in a cohort of Old Order Amish from Lancaster County, Pennsylvania.

Results—The scan confirmed a previously identified region on chromosome 4 to be strongly associated with uric acid levels ($p = 4.2\times10^{-11}$ for rs10489070). Follow-up genotyping revealed a non-synonymous coding SNP (Val253Ile; rs16890979) in *GLUT9* that was most strongly associated with uric acid levels, with each copy of the minor allele associated with 0.47 mg/dl less uric acid (95% confidence interval: 0.31 − 0.63; $p = 1.43 \times 10^{-11}$). The effect of this variant tended to be stronger in women than in men (p = 0.16 for sex × genotype interaction). The genotypic effect was not modified by the inclusion of several cardiovascular risk factors suggesting that *GLUT9* is directly related to uric acid homeostasis. The same SNP (rs10489070) identified in the Amish genome wide scan was significantly associated with gout in the Framingham Heart Study (p = 0.004).

Conclusions—We conclude that *GLUT9*, which is expressed in the kidney may be a novel regulator of uric acid elimination and a common non-synonymous variant in this gene contributes to abnormalities in uric acid homeostasis and gout.

INTRODUCTION

Uric acid is the primary end product of purine metabolism by xanthine oxidase. An elevated serum uric acid level is associated with gouty arthritis and kidney stones due to deposition of uric acid crystals in the joints and collecting ducts of the kidney, respectively. Serum uric acid is also an independent predictor of several cardiovascular and metabolic syndrome

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AUTHOR CONTRIBUTIONS

P.F. McArdle performed the analysis, oversaw the management of the data, and wrote the paper. A. Parsa, Y.P.C. Chang and M.R. Weir provided interpretation of the data. J.R. O'Connell wrote the software to perform the analysis. B.D. Mitchell and A.R. Shuldiner conceived of and designed the study. All authors approved of the final version of the manuscript.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

phenotypes in both healthy and at risk populations (1-3). While the direct causal mechanisms linking uric acid metabolism to these endpoints have not been unequivocally determined, clinical and experimental evidence supporting such an effect is mounting (4;5), and it has been suggested that decreasing uric acid levels may attenuate cardiovascular disease risk (6). Identifying genetic factors influencing variation in serum uric acid levels may contribute to the understanding of uric acid homeostasis and facilitate the identification of new targets for intervention. To this end, we performed a genome wide association study of serum uric acid levels in a cohort of 868 Old Order Amish from Lancaster County, Pennsylvania. Our findings confirm previous associations identified on chromosome 4 in Caucasian cohorts (7;8). We extend these findings to the identification of a common non-synonymous variant, Val253Ile in *GLUT9* that is likely functional. Finally, we demonstrate that a SNP in high linkage disequilibrium with this variant is associated with uric acid levels and gout in the Framingham Heart Study, thus linking the gene with this common and often disabling disease.

METHODS

Research subjects

The Heredity and Phenotype Intervention (HAPI) Heart Study began in 2003 with the goal of identifying genes that interact with environmental exposure to alter risk of cardiovascular disease (9). Old Order Amish individuals aged 20 years and older who were relatively healthy were recruited into the study. Exclusion criteria included severe hypertension (BP > 180/105 mm Hg), malignancy, and kidney, liver or untreated thyroid disease. The study protocol was approved by the Institutional Review Board at the University of Maryland, School of Medicine and informed consent was obtained from each study participant.

Physical examinations were conducted at the Amish Research Clinic in Strasburg, PA in the early morning following an overnight fast and a blood sample was taken. Uric acid levels drawn non-fasting at the screening exam were assayed by Quest Diagnostics (Baltimore, MD) and measured to the nearest 0.1 mg/dL.

Genotype analysis

Participants of the HAPI Heart Study were genotyped with the use of the Affymetrix GeneChip® Human Mapping 500K Array set. The GeneChip Genotyping Analysis Software (GTYPE 4.0) was used for automated genotype calling as part of the GeneChip Operating Software (GCOS) platform. The GTYPE-generated chip files were re-analyzed using the BRLMM genotype calling algorithm which provided improved call accuracy compared with the DM algorithm. Only samples with call rates > 93% on both microassays (the *NspI* and *StlyI* digestions) were used for analysis. The resulting mean call rates of the 861 resulting samples was 97.5%. Marker call rate was then assessed across the acceptable samples and markers with call rates of > 90% across samples and had minor allele frequency > 5% were considered for analysis (n = 361,034).

Data analysis

Genome wide association was performed using the measured genotype approach that modeled variation in uric acid as a function of measured environmental covariates (age, age^2, and sex), measured genotype and a polygenic component to account for phenotypic correlation due to relatedness. The polygenic component was modeled using the relationship matrix derived from the complete pedigree structure since all subjects are related. Specifically, the covariance between each pair of individuals within the pedigree is estimated as a function of their degree of relationship, the trait heritability, and the phenotypic variance of the trait. The model is thus defined as:

$$Y=X\beta+g+e,$$

where Y is a vector of uric acid values, and X is a design matrix accommodating an intercept and a vector of covariates and individual genotype values coded as 0, 1 or 2. β is a vector containing the estimates of the fixed effects. The g term is the polygenic component that is distributed multivariate normally with a mean of zero and a covariance equal to two times the kinship matrix times the expected variance due to the additive effect of genes. The e term is a normally distributed error component with mean zero. Generalized least squares estimates of the parameters of interest are given by:

$$\beta=\left(X^{T}V^{-1}X\right)^{-1}X^{T}V^{-1}Y$$
$$\text{var}\,(\beta)=\left(X^{T}V^{-1}X\right)^{-1}$$

where V is the variance-covariance matrix and is a function of residual trait heritability and the relationships implied by the pedigree structure. A 1-degree of freedom likelihood ratio test is used to assess significance of the measured genotype under the additive model. The genome-wide analysis was carried out using software developed in our group.

RESULTS

A genome wide association scan of uric acid levels was performed in 868 Amish participants of the Heredity and Phenotype Intervention (HAPI) Heart Study. The study sample included slightly more men (n=460) than women (n=408). Uric acid levels were higher in men than in women (4.54 ± 1.0 vs. 3.71 ± 0.9 mg/dl, $p < 0.0001$), see Table 1.

A total of 361,034 SNPs passed quality control measures with a minor allele frequency greater than 5% and comprised the genome wide scan. The results of the association tests for those SNPs with very strong evidence for association (n = 246 SNPs with $p < 0.0001$) are given in Supplemental Table S.1. The strongest association signal was on chromosome 4, in the same region as reported previously (7;8) (Figure 1). The most strongly associated SNP was rs10489070 ($p = 4.2 \times 10^{-11}$) with a cluster of 20 SNPs in linkage disequilibrium with rs10489070 that all provided strong evidence ($p < 10^{-7}$) for association with uric acid levels. These SNPs encompass an approximately 367 KB region that include *GLUT9* and *WDR1*.

GLUT9 is a class II member of the facilitated hexose transporter family (SLC2A). Substrate specificity is varied with some able to translocate both glucose and fructose (10). *GLUT9*, which codes for a 540 amino acid protein, is expressed primarily in liver, kidney and placenta and to some extent in chondrocytes, brain, lung and leukocytes (11). *GLUT9* also has a demonstrated splice variant, *GLUT9ΔN* which codes for a 512 amino acids protein expressed only in kidney and placenta (11). *GLUT9ΔN* was shown to be located in the apical membrane of human kidney proximal tubule epithelial cells, the primary site for renal uric acid regulation (12). The *WDR1* gene appears to affect actin disassembly and help regulate cell morphologic changes during mitosis (13). No potential functional correlation between *WDR1* and uric acid are known, and we therefore choose *GLUT9* as our target for further study.

GLUT9 contains 12 exons spanning 195 Kb and is described to have four non-synonymous coding SNPs, Ala17Thr (rs6820230), Val253Ile (rs16890979), Arg265His (rs3733591) and Pro321Leu (rs2280205) (dbSNP, build 128). We genotyped all four non-synonymous coding SNPs in our HAPI Heart sample; Val253Ile *GLUT9*, was in linkage disequilibrium

with rs10489070 (D' = 0.92, r^2 = 0.71) and showed the strongest association with uric acid in an additive fashion, p = 1.43×10^{-11} (Table 2). The Val253Ile substitution is in exon 8 of *GLUT9* and is located in the region between transmembrane domains 6 and 7. Valine at this position is highly conserved among the orthologs of *GLUT9* and is found in all known primate, rodent, and even tetraodon GLUT9 proteins, Table 3. This variant was the only significant association among the 4 coding SNPs when all were included in a single model providing evidence that it is associated with uric acid levels independently of other coding variants in the gene and thus is the most likely functional variant.

Since women have significantly lower uric acid levels than men, we examined the effect of Val253Ile in sex-stratified analysis. After adjusting for age, each copy of the Ile allele was associated with 0.47 mg /dl lower uric acid (95% confidence interval 0.31 − 0.63) among women and 0.27 mg/dl lower uric acid (95% confidence interval 0.10 − 0.45) among men (sex by genotype interaction p-value = 0.16). Additional analyses were carried out in women. These results were consistent with a potential modifying effect of estrogen on genotype - uric acid association. Among the 153 women reporting that they had reached menopause, the effect of the Ile allele was more similar to that observed in men, 0.35 mg/dl lower uric acid (95% confidence interval 0.05 − 0.64), while the greatest effect was observed among the 227 women who reported not yet reaching menopause, 0.53 mg/dl less uric acid per Ile allele (95% confidence interval 0.35 − 0.72).

Serum uric acid has been shown to be associated with a number of cardiovascular inflammation, and metabolic traits (14;15). We similarly found strong associations between uric acid levels and a panel of cardiovascular risk factors, including percent body fat, triglycerides, HDL, LDL, glucose, insulin, and estimated glomerular filtration rate (eGFR) calculated by the MDRD equation (16) (Table 4). However, no consistent significant associations were identified between the Val253Ile *GLUT9* variant and these cardiovascular and metabolic traits (Table 5). Similarly, inclusion of each risk factor into the model did not affect the relationship between Val253Ile *GLUT9* and uric acid. This result suggests that Val253Ile may affect serum uric acid levels independent of eGFR and known cardiovascular risk factors.

Subjects of the HAPI Heart Study were relatively healthy and gout phenotypes were not available. We thus sought to examine association between this clinically significant consequence of elevated uric acid levels and *GLUT9* genotype in subjects from the Framingham Heart Study (FHS). The Val253Ile *GLUT9* variant was not genotyped in the 100K GWAS that is publicly available (17), however, rs10489070 was on both the 100K FHS GWAS and the 500K Amish GWAS. This SNP is in linkage disequilibrium with Val253Ile *GLUT9* in the HapMap CEU samples (D' = 0.68, r^2 = 0.42) and was associated with uric acid levels in FHS (ex1 GEE p = 0.0001, ex2 GEE p = 0.002). The allele associated with increased uric acid levels was also strongly associated with gout in the FHS (GEE ß (SE) = −0.03 (0.009); p = 0.004). This demonstrates that common variation in *GLUT9* in addition to being associated with serum levels of uric acid has direct clinical relevance.

DISCUSSION

We performed a genome wide association study of serum uric acid levels and found strong association of multiple SNPs on chromosome 4 that exceeded that of genome-wide significance and replicates a previously identified association in the region of *GLUT9*. We further narrowed the most likely causative variant to a non-synonymous coding SNP in exon 8, rs16890979, which codes for a highly conserved Val to Ile amino acid change at position 253. The effect size associated with this non-synonymous SNP is large and resulted in our

most significant association. The age adjusted difference between Ile homozygotes and the Val homozygotes in our sample was nearly 1 mg/dl. A model of age, sex, genotype and BMI revealed that the Val253Ile variant explains 33% of the variation of uric acid in our sample.

As in other studies (14;15), our study indicates that uric acid is associated with a number of metabolic, inflammatory and CVD risk factors in the Amish. Uric acid was strongly associated with triglycerides, HDL cholesterol, creatinine and whole body fat mass, among others CVD risk factor traits Although genotype at the Val253Ile locus was strongly associated with uric acid, it was not consistently associated with these CVD-related markers; nor did adjustment for these CVD-related markers or eGFR alter the association of uric acid with genotype. The latter observation suggests that genotype may be in the direct causal pathway for uric acid homeostasis and not secondary to other associated factors. The lack of association between genotype and metabolic/CVD markers could either be due to a lack of causality between uric acid and increased metabolic/CVD risk or due to insufficient power since our sample is healthy with a low prevalence of elevated uric acid levels and clinically significant CVD. However, association of *GLUT9* variation with gout in FHS strongly supports the role of this gene in uric acid homeostasis that is clinically significant.

Serum urate level reflects the balance between production and excretion. The production is dependent on dietary protein intake and endogenous production and breakdown of purine by xanthine oxidase. The excretion is dependent primarily on renal elimination, which accounts for about 70% of urate excretion, the remaining being dependent on intestinal excretion (12). Interestingly, women have lower serum uric acid levels than their male counterparts, which has been shown to relate, at least in part, to increase renal excretion of uric acid in response to estrogen (18). Of potential relevance, in the Amish population as well as the Sardinia and Chianti populations, *GLUT9* genotype effect tends to be more pronounced in pre-menopausal women compared to post-menopausal women and males. This raises the possibility that *GLUT9* activity could be modulated by estrogen. Evidence for a gender interaction with *GLUT9* genotype was not identified in a study of British hypertensive subjects (8), but their sample had a mean age at time of phenotyping of 64 years (19) and it is possible that a sufficient number of pre-menopausal women were not available to detect an interaction. Sufficiently powered studies will be needed to formally test the hypothesis of a gene – sex interaction.

The mechanism by which *GLUT9* may affect uric acid levels is not known. However, there are at least two plausible mechanisms. The first relates to GLUT9's potential role in fructose homeostasis in kidney and liver. Increased fructose is known to increase uric acid levels secondary to increase production (20-23), and has been implicated as a potential cause of gout (24), kidney stones (25) and the metabolic syndrome (26;27). In accordance, hereditary fructosemia, which is caused be aldolase deficiency in the liver, is associated with hypoglycemia, jaundice, and hyperuricemia (28). *GLUT9* has also shown to be significantly up regulated in liver and kidney of diabetic rats (29), creating a potential link between the metabolic syndrome and hyperuricemia. A second plausible mechanism relates to uric acid excretion. The *GLUT9ΔN* splice variant is not only exclusively expressed in kidney and placenta but is located in kidney proximal tubules epithelial cells, the primary site of renal uric acid regulation and clearance. Future studies of GLUT9's role in uric acid homeostasis will be required to effectively test the proposed hypotheses.

Both valine and isoleucine are hydrophobic amino acids and thus the Val253Ile substitution may be regarded as conservative. However, in some proteins such substitutions at key positions leads to altered structure and function (30;31). Clinically relevant phenotypes involving valine to isoleucine substitutions have been implicated in disorders such as

rheumatoid arthritis and Alzheimer's disease (32-34). The mechanism by which the substitution alters function of the GLUT9 protein will require further investigation.

In summary, we identified *GLUT9* as an important genetic determinant of serum uric acid levels. This highly significant replication of the previous reports, including very similar effect size estimates, indicates that the association represents a true signal. The robustness of the association to adjustment of uric acid related covariates, association with gout, and identification of a highly conserved non-synonymous SNP, provide context for future mechanistic studies related to *GLUT9*, uric acid homeostasis and gout.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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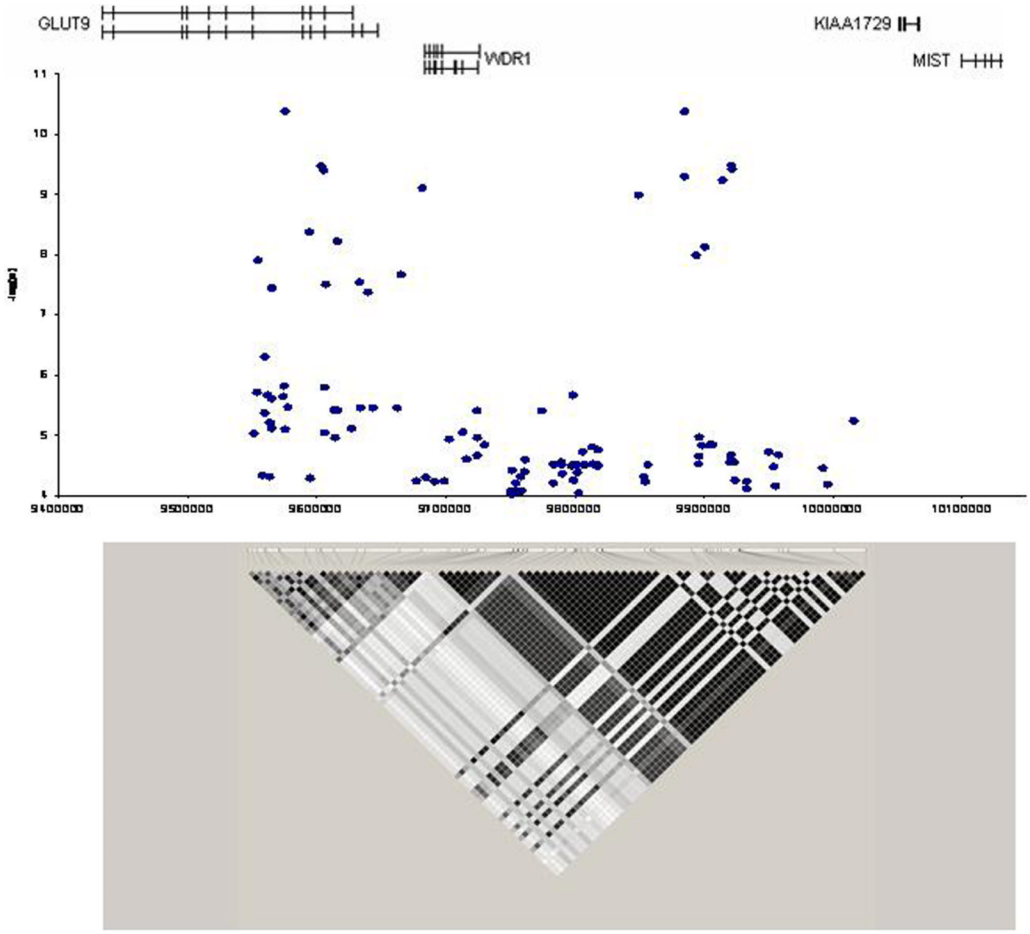

Figure 1.
Region on chromosome 4 strongly associated with serum uric acid levels. Two clusters of SNPs approximately 300 kb apart are in linkage disequilibrium and showed strong association with uric acid level. The region contains *GLUT9* and *WDR1*.

Table 1

Characteristics [mean (standard deviation)] by sex of HAPI Heart Study participants.

Trait	Women (n=408)	Men (n=460)	p
Age (yrs)	45.4 (14.2)	42.2 (13.6)	0.0007
Body Mass Index (kg/m^2)	27.8 (5.5)	25.6 (3.2)	<0.0001
Percent Body Fat (%)	34.4 (6.4)	18.4 (6.5)	<0.0001
Uric Acid (mg/dl)	3.71 (0.9)	4.54 (1.0)	<0.0001
SBP (mmHg)	121.4 (16.9)	121.5 (12.6)	0.9775
DBP (mmHg)	75.8 (8.4)	77.6 (8.8)	0.0019
Triglycerides (mg/dl)	73.8 (45.4)	63.9 (37.3)	0.0005

Table 2

Non-synonymous coding SNPs in *GLUT9*, linkage disequilibrium with strongest signal in genome wide scan and each other, effect size controlling for age, sex and family structure (simple model) and age, sex, family structure and other SNPs (full model).

		Allele	Linkage Disequilibrium (r^2)				Simple Model		Full Model	
SNP	**rsNumber**	**Frequency**	**Rs10489070**	**Val253Ile**	**Arg265His**	**Pro321Leu**	**Effect Size (SE); mg/dl**	**p**	**Effect Size (SE); mg/dl**	**p**
Ala17Thr	rs6820230	0.16	0.01	0.00	0.02	0.14	0.01 (0.07)	0.92	0.01 (0.08)	0.87
Val253Ile	rs16890979	0.17	0.71		0.02	0.09	0.44 (0.06)	1.43×10^{-11}	0.43 (0.07)	7.89×10^{-9}
Arg265His	rs3733591	0.12	0.02			0.00	0.10 (0.07)	0.16	0.03 (0.07)	0.65
Pro321Leu	rs2280205	0.46	0.18				0.12 (0.05)	0.01	0.00 (0.06)	0.94

Table 3

Amino acid sequences flanking the uric acid-associated non-synonymous SNP Val253Ile (rs16890979) in several species.

Species	Ref. Protein No.	Amino Acid Sequence (Val253Ile in bold)
Human	NP_064425	LLLEKHNEARAVKAFQTFLGKAD**V**SQEVEEVLAESRVQRSIRLVSVLELL
Chimpanzee	XP_520688.2	LLLEKHNEARAVKAFQTFLGKAD**V**SQEVEEVLAESRVQRSIRLVSVLELL
Orangutan	Q5RB09	LLLEKRNEARAVKAFQTFLGKAD**V**SREVEEV-AESRVQRSIRLVSVLELL
Mouse	NP_001012363.2	LLFEKHDEAGAMKAFQTFLGKAD**V**SQELEEALAESRVQRNLRLVSVLELL
Dog	XP_536240.2	LLFEKHDQAGAEKAFQTFLGKED**V**SREVEEVLAESRVQRNIQLVSVLELL
Rat	XP_577349.2	LLFEKHDEAGATKAFQTFLGKAD**V**SQELEEALAESRVQRNLRLVSVFELL
Chicken	XP_420789.2	LLLEKHNTSKAEKAFQTFLGKDD**V**SQEVEEVLAESRVQRNTKLVSVLQLL
Platypus	XP_001512025.1	LLFEKHDEAAATKAFQTFLGKDD**V**SQEIEDILAESRAQRNLRLESVPQLL
Opossum	XP_001371233.1	LLFEKHDEDGAEKAFQTFLGKMD**V**SQEMEEALEESRVQRNIRLVSVWELL
Pufferfish	CAG02006.1	LLMERRDEEGAKRAFQKFLGKDD**V**SEELEEVHAEARAQETLQTASVLQLM
Conserved		**LL-E**---------**AFQ**-FL**GK-DVS-E**-----A**E**-R-**Q**------**SV**--**L**-

Table 4

Association between uric acid and other quantitative traits in the HAPI Heart Study. Point estimates are effect on trait with each increase of 1 mg/dl of uric acid.

	Men		Women	
Trait	**Point Estimate (95% CI)**	**p**	**Point Estimate (95% CI)**	**p**
Triglycerides (mg/dl)	10.12 (13.36, 6.88)	<0.0001	19.42 (23.76, 15.09)	<0.0001
Fasting HDL Cholesterol (mg/dl)	−4.14 (−2.99, −5.28)	<0.0001	−5.97 (−4.35, −7.59)	<0.0001
Cholesterol HDL Ratio	0.28 (0.38, 0.17)	<0.0001	0.43 (0.57, 0.3)	<0.0001
eGFR (mL/min/1.73m2)	−5.03 (−3.5, −6.55)	<0.0001	−4.27 (−2.56, −5.97)	0.0001
Creatinine (mg/dL)	0.04 (0.05, 0.03)	<0.0001	0.03 (0.05, 0.02)	<0.0001
Glucose ln(mg/dl)	0.01 (0.03, 0)	0.01	0.04 (0.05, 0.02)	0.0002
Insulin ln(mU/ml)	0.11 (0.15, 0.07)	<0.0001	0.16 (0.22, 0.1)	<0.0001
Adiponectin ln(mg/ml)	−0.09 (−0.05, −0.12)	0.0007	−0.15 (−0.1, −0.2)	<0.0001
Leptin ln(pg/ml)	0.61 (0.86, 0.36)	0.0003	0.42 (0.57, 0.27)	<0.0001
Percent Body Fat (%)	2.01 (2.83, 1.19)	0.0003	2.1 (2.91, 1.3)	<0.0001
Body Mass Index (kg/m2)	1.23 (1.5, 0.95)	<0.0001	2.27 (2.77, 1.76)	<0.0001
Whole Body Fat Mass ln(g)	0.16 (0.23, 0.1)	<0.0001	0.14 (0.18, 0.09)	<0.0001
Whole Body Lean Mass ln(g)	0.02 (0.04, 0.01)	0.0008	0.04 (0.06, 0.02)	<0.0001
Hemoglobin (g/dL)	0.19 (0.27, 0.11)	0.0002	0.22 (0.31, 0.12)	<0.0001
C Reactive Protein ln(mg/L)	0.2 (0.31, 0.1)	0.0002	0.28 (0.38, 0.18)	<0.0001
Hematocrit (%)	0.49 (0.71, 0.26)	<0.0001	0.65 (0.92, 0.38)	0.0003
ALT (U/L)	1.28 (1.98, 0.57)	0.0004	1.95 (2.68, 1.22)	<0.0001
Red Blood Cell Count (mill/mcl)	0.06 (0.08, 0.03)	<0.0001	0.08 (0.12, 0.05)	<0.0001
White Blood Cell Count (thous/mcl)	0.1 (0.22, −0.02)	0.09	0.27 (0.39, 0.15)	<0.0001
DBP (mmHg)	1.72 (2.52, 0.92)	<0.0001	0.67 (1.54, −0.21)	0.13
SBP (mmHg)	2.03 (3.12, 0.94)	0.0003	0.86 (2.42, −0.7)	0.28

Table 5

Association between Val253Ile (rs16890979) and other quantitative traits in the HAPI Heart Study.

	Men		Women	
Trait	**Point Estimate (95% CI)**	**p**	**Point Estimate (95% CI)**	**p**
Triglycerides (mg/dl)	−2.43 (−9.11, 4.24)	0.48	−2.45 (−10.56, 5.67)	0.55
Fasting HDL Cholesterol (mg/dl)	1.15 (−1.17, 3.46)	0.33	3.04 (0.1, 5.98)	0.04
Cholesterol HDL Ratio	0.08 (−0.13, 0.3)	0.44	−0.24 (−0.48, −0.01)	0.05
eGFR (mL/min/1.73m2)	−0.74 (−3.88, 2.4)	0.65	−0.13 (−3.15, 2.9)	0.93
Creatinine (mg/dL)	0.01 (−0.02, 0.03)	0.57	0 (−0.03, 0.02)	0.75
Glucose ln(mg/dl)	0 (−0.02, 0.02)	0.92	−0.01 (−0.04, 0.01)	0.30
Insulin ln(mU/ml)	0.04 (−0.03, 0.12)	0.27	0.03 (−0.07, 0.13)	0.58
Adiponectin ln(mg/ml)	−0.04 (−0.12, 0.03)	0.24	0.04 (−0.04, 0.13)	0.32
Leptin ln(pg/ml)	0.02 (−0.46, 0.5)	0.94	−0.08 (−0.35, 0.19)	0.56
Percent Body Fat (%)	0.8 (−0.91, 2.51)	0.36	0.3 (−1.26, 1.86)	0.71
Body Mass Index (kg/m2)	0.04 (−0.55, 0.62)	0.90	−0.39 (−1.35, 0.57)	0.42
Whole Body Fat Mass ln(g)	0.08 (−0.05, 0.21)	0.25	0.02 (−0.08, 0.11)	0.74
Whole Body Lean Mass ln(g)	0.02 (−0.01, 0.04)	0.28	0 (−0.03, 0.03)	0.91
Hemoglobin (g/dL)	0.01 (−0.14, 0.16)	0.89	−0.02 (−0.19, 0.15)	0.80
C Reactive Protein ln(mg/L)	0.02 (−0.19, 0.23)	0.85	0.01 (−0.17, 0.19)	0.94
Hematocrit (%)	0.25 (−0.19, 0.69)	0.27	0.12 (−0.37, 0.6)	0.64
ALT (U/L)	−0.66 (−2.07, 0.75)	0.36	−1.48 (−2.78, −0.18)	0.03
Red Blood Cell Count (mill/mcl)	0.02 (−0.03, 0.07)	0.42	0.01 (−0.04, 0.07)	0.65
White Blood Cell Count (thous/mcl)	0.16 (−0.07, 0.39)	0.17	−0.16 (−0.38, 0.06)	0.15
DBP (mmHg)	0.28 (−1.33, 1.88)	0.74	−1.42 (−2.89, 0.04)	0.06
SBP (mmHg)	−0.36 (−2.5, 1.79)	0.75	−1.02 (−3.68, 1.65)	0.46