

Association of a Common Nonsynonymous Variant in *GLUT9* With Serum Uric Acid Levels in Old Order Amish

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Objective. Uric acid is the primary end product of purine metabolism. Increased serum uric acid levels have been associated with gouty arthritis as well as with a variety of cardiovascular-related phenotypes. This study was undertaken to investigate associations between uric acid levels and single-nucleotide polymorphisms (SNPs).

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 ($P = 4.2 \times 10^{-11}$ for rs10489070). Followup genotyping revealed that a nonsynonymous coding SNP (Val253Ile; rs16890979) in *GLUT9* was most strongly associated with uric acid levels, with each copy of the minor allele associated with a decrease of 0.47 mg/dl in the uric acid level (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 genotype effect was not modified by the inclusion of several cardiovascular risk factors, suggesting that *GLUT9* is directly related to uric acid homeostasis. The SNP identified in the genome-wide scan in the Amish population (rs10489070) was also significantly associated with gout in the Framingham Heart Study ($P = 0.004$).

Conclusion. Our findings indicate that *GLUT9*, which is expressed in the kidney, may be a novel regulator of uric acid elimination and that a common nonsynonymous variant in this gene contributes to abnormalities in uric acid homeostasis and gout.

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 in the collecting ducts of the kidney, respectively. The serum uric acid level is also an independent predictor of several cardiovascular and metabolic syndrome phenotypes in both healthy and at-risk populations (1–3). While the direct causal mechanisms linking uric acid metabolism to these end points 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 (CVD) risk (6).

Identifying genetic factors that influence 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 nonsynonymous variant, Val253Ile in *GLUT9*, that is likely func-

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tional. Finally, we demonstrate that a single-nucleotide polymorphism (SNP) in high linkage disequilibrium with this variant is associated with uric acid levels and gout in subjects in the Framingham Heart Study (9), thus linking the gene with this common and often disabling disease.

SUBJECTS AND METHODS

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 the risk of CVD (10). Old Order Amish individuals ages 20 years and older who were relatively healthy were recruited into the study. Exclusion criteria included severe hypertension (blood pressure >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 samples (nonfasting state) drawn at the screening examination were assayed by Quest Diagnostics (Baltimore, MD) and measured to the nearest 0.1 mg/dl.

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

Data analysis. A genome-wide association analysis was performed using the measured genotype approach, which models variation in the uric acid level as a function of measured environmental covariates (age, age squared, and sex), measured genotype, and a polygenic component to account for phenotype correlation due to relatedness. The polygenic component was modeled using the relationship matrix derived from the complete pedigree structure since all subjects were related. Specifically, the covariance between each pair of individuals within the pedigree was 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 0 and a covariance equal to 2 times the kinship

Table 1. Characteristics of the 868 subjects in the Heredity and Phenotype Intervention Heart Study*

Characteristic	Women (n = 408)	Men (n = 460)	P
Age, years	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
% body fat	34.4 ± 6.4	18.4 ± 6.5	<0.0001
Uric acid level, mg/dl	3.71 ± 0.9	4.54 ± 1.0	<0.0001
Systolic BP, mm Hg	121.4 ± 16.9	121.5 ± 12.6	0.9775
Diastolic BP, mm Hg	75.8 ± 8.4	77.6 ± 8.8	0.0019
Triglycerides, mg/dl	73.8 ± 45.4	63.9 ± 37.3	0.0005

* Values are the mean ± SD. BP = blood pressure.

matrix times the expected variance due to the additive effect of genes. The e term is a normally distributed error component with a mean of 0. Generalized least squares estimates of the parameters of interest are given by the formulas $\beta = (X^T V^{-1} X)^{-1} X^T V^{-1} Y$ and $\text{var}(\beta) = (X^T V^{-1} X)^{-1}$, 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-df 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 by our group.

RESULTS

A genome-wide association scan of uric acid levels was performed in 868 Amish participants in the HAPI Heart Study. The study sample included slightly more men (n = 460) than women (n = 408). Mean ± SD uric acid levels were higher in men than in women (4.54 ± 1.0 versus 3.71 ± 0.9 mg/dl; $P < 0.0001$) (Table 1).

A total of 361,034 SNPs, which passed quality control measures and had a minor allele frequency >5%, comprised the genome-wide scan. The results of association tests for SNPs with very strong evidence of association with uric acid levels ($P < 0.0001$; n = 246) are given in Supplemental Table 1 (available on the *Arthritis & Rheumatism* Web site at <http://www.mrw.interscience.wiley.com/suppmat/0004-3591/suppmat/>). The strongest association signal was on chromosome 4, in the same region as reported previously (7,8) (Figure 1). The SNP with the strongest association with uric acid levels was rs10489070 ($P = 4.2 \times 10^{-11}$). A cluster of 20 SNPs in linkage disequilibrium with rs10489070 all showed strong evidence of association with uric acid levels ($P < 10^{-7}$). These SNPs encompass a region of ~367-kb, which includes *GLUT9* and *WDR1*.

GLUT9 is a class II member of the facilitated glucose transporter family (solute carrier family 2).

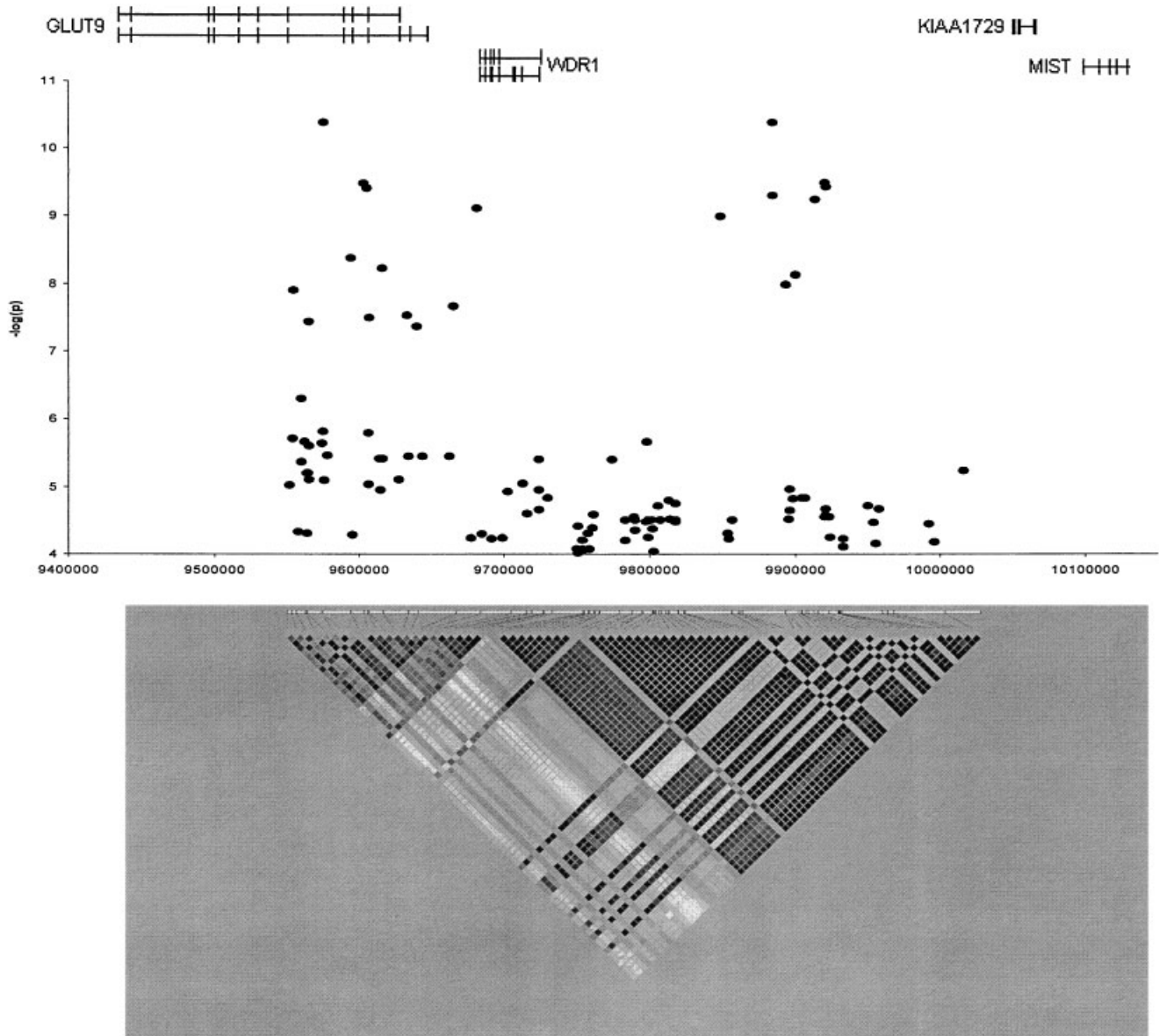


Figure 1. Region on chromosome 4 that was strongly associated with serum uric acid levels. Circles represent the negative log of the P value for association between uric acid levels and single-nucleotide polymorphisms (SNPs) located on chromosome 4. The x-axis shows the physical position, measured in basepairs. Two clusters of SNPs ~ 300 kb apart were in linkage disequilibrium and showed strong association with the uric acid level. The region contains *GLUT9* and *WDR1*. The graph shows linkage disequilibrium, as measured by r^2 , between each of the SNPs in the top portion of the figure.

Substrate specificity is varied, with some members able to translocate both glucose and fructose (11). *GLUT9*, which codes for a 540–amino acid protein, is expressed primarily in the liver, kidney, and placenta, and to some extent in chondrocytes, the brain, the lungs, and leukocytes (12). *GLUT9* also has a demonstrated splice variant, *GLUT9 Δ N*, which codes for a 512–amino acid

protein expressed only in the kidney and placenta (12). *GLUT9 Δ N* has been shown to be located in the apical membrane of human kidney proximal tubule epithelial cells, the primary site for renal uric acid regulation (13). The *WDR1* gene appears to affect actin disassembly and to help regulate cell morphologic changes during mitosis (14). No potential functional correlation between *WDR1*

Table 2. Nonsynonymous coding SNPs in *GLUT9*, linkage disequilibrium with the strongest signal in the genome-wide scan and with each other, and effect on uric acid, controlling for age, sex, and family structure (simple model) and for age, sex, family structure, and other SNPs (full model)*

	Ala17Thr	Val253Ile	Arg265His	Pro321Leu
rs no.	rs6820230	rs16890979	rs3733591	rs2280205
Allele frequency	0.16	0.17	0.12	0.46
Linkage disequilibrium, r^2				
rs10489070	0.01	0.71	0.02	0.18
Val253Ile	0.00	–	–	–
Arg265His	0.02	0.02	–	–
Pro321Leu	0.14	0.09	0.00	–
Simple model				
Effect size \pm SE, mg/dl	0.01 \pm 0.07	0.44 \pm 0.06	0.10 \pm 0.07	0.12 \pm 0.05
<i>P</i>	0.92	1.43 \times 10 ⁻¹¹	0.16	0.01
Full model				
Effect size \pm SE, mg/dl	0.01 \pm 0.08	0.43 \pm 0.07	0.03 \pm 0.07	0.00 \pm 0.06
<i>P</i>	0.87	7.89 \times 10 ⁻⁹	0.65	0.94

* The r^2 values for single-nucleotide polymorphisms (SNPs) in linkage disequilibrium with rs10489070 and for SNPs in linkage disequilibrium with each other are shown.

and the uric acid level are known, and we therefore chose *GLUT9* for further study.

GLUT9 contains 12 exons spanning 195 kb and is described as having 4 nonsynonymous coding SNPs, Ala17Thr (rs6820230), Val253Ile (rs16890979), Arg265His (rs3733591), and Pro321Leu (rs2280205) (dbSNP, build 128). We genotyped all 4 nonsynonymous coding SNPs in our sample from the HAPI Heart Study. Val253Ile in *GLUT9* was in linkage disequilibrium with rs10489070 and showed the strongest association with the uric acid level in an additive manner ($D' = 0.92$, $r^2 = 0.71$, $P = 1.43 \times 10^{-11}$) (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* glucose transporter 9 (*GLUT-9*) proteins (Table 3). This variant was the only one to show

a significant association with the uric acid level when all 4 coding SNPs 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 analyzed the effect of Val253Ile in patients stratified by sex. After adjusting for age, each copy of the Ile allele was associated with a decrease of 0.47 mg/dl in uric acid level among women (95% confidence interval [95% CI] 0.31–0.63) and a decrease of 0.27 mg/dl in uric acid level among men (95% CI 0.10–0.45) ($P = 0.16$ for sex–genotype interaction). Additional analyses were carried out in women. These results were consistent with a potential modifying effect of estrogen on the association between genotype and uric acid level. Among the 153 women reporting that they had reached menopause, the effect of the Ile allele

Table 3. Amino acid sequences flanking the uric acid–associated nonsynonymous SNP Val253Ile (rs16890979) in several species

Species	Ref. protein no.	Amino acid sequence*
Human	NP_064425	LLLEKHNEARAVKAFQTFGLGKAD V SQEVVEVLAESRVQRSTIRLVSVLELL
Chimpanzee	XP_520688.2	LLLEKHNEARAVKAFQTFGLGKAD V SQEVVEVLAESRVQRSIRLVSVLELL
Orangutan	Q5RB09	LLLEKRNEARAVKAFQTFGLGKAD V SREVEEV–AESRVQRSTIRLVSVLELL
Mouse	NP_001012363.2	LLFEKHDEAGAMKAFQTFGLGKAD V SQEELEALAESRVQRNLRLVSVLELL
Dog	XP_536240.2	LLFEKHQDQAGAEKAFQTFGLGKED V SREVEEVLAESRVQRNIQLVSVLELL
Rat	XP_577349.2	LLFEKHDEAGATKAFQTFGLGKAD V SQEELEALAESRVQRNLRLVSVLELL
Chicken	XP_420789.2	LLLEKHNTSKAEKAFQTFGLGKDD V SQEVVEVLAESRVQRNTKLVSVLQLL
Platypus	XP_001512025.1	LLFEKHDEAAATKAFQTFGLGKDD V SQEIEDILAESRAQRNLRLSVLQLL
Opossum	XP_001371233.1	LLFEKHDEDGAEKAFQTFGLGKMD V SQEMEEALEESRVQRNIRLVSVWELL
Pufferfish	CAG02006.1	LLMERRDEEGAKRAFQKFLGKDD V SEELVEVHAEARAQETLQTASVLQLM
Conserved		LL–E– ----- AFQ–FLGK–DVS–E– ----- AE–R–Q– ----- SV–L–

* Val253Ile is shown in boldface. SNP = single-nucleotide polymorphism.

Table 4. Association between uric acid level and other quantitative traits in subjects in the Heredity and Phenotype Intervention Heart Study*

Trait	Men		Women	
	Point estimate (95% CI)	<i>P</i>	Point estimate (95% CI)	<i>P</i>
Triglyceride level, mg/dl	10.12 (13.36, 6.88)	<0.0001	19.42 (23.76, 15.09)	<0.0001
Fasting HDL cholesterol level, mg/dl	-4.14 (-2.99, -5.28)	<0.0001	-5.97 (-4.35, -7.59)	<0.0001
Ratio of cholesterol to HDL	0.28 (0.38, 0.17)	<0.0001	0.43 (0.57, 0.3)	<0.0001
Estimated GFR, ml/minute/1.73 m ²	-5.03 (-3.5, -6.55)	<0.0001	-4.27 (-2.56, -5.97)	0.0001
Creatinine level, mg/dl	0.04 (0.05, 0.03)	<0.0001	0.03 (0.05, 0.02)	<0.0001
Glucose level, mg/dl†	0.01 (0.03, 0)	0.01	0.04 (0.05, 0.02)	0.0002
Insulin level, mU/ml†	0.11 (0.15, 0.07)	<0.0001	0.16 (0.22, 0.1)	<0.0001
Adiponectin level, mg/ml†	-0.09 (-0.05, -0.12)	0.0007	-0.15 (-0.1, -0.2)	<0.0001
Leptin level, pg/ml†	0.61 (0.86, 0.36)	0.0003	0.42 (0.57, 0.27)	<0.0001
% body fat	2.01 (2.83, 1.19)	0.0003	2.1 (2.91, 1.3)	<0.0001
Body mass index, kg/m ²	1.23 (1.5, 0.95)	<0.0001	2.27 (2.77, 1.76)	<0.0001
Whole body fat mass, gm†	0.16 (0.23, 0.1)	<0.0001	0.14 (0.18, 0.09)	<0.0001
Whole body lean mass, gm†	0.02 (0.04, 0.01)	0.0008	0.04 (0.06, 0.02)	<0.0001
Hemoglobin value, gm/dl	0.19 (0.27, 0.11)	0.0002	0.22 (0.31, 0.12)	<0.0001
CRP level, mg/liter†	0.2 (0.31, 0.1)	0.0002	0.28 (0.38, 0.18)	<0.0001
Hematocrit value, %	0.49 (0.71, 0.26)	<0.0001	0.65 (0.92, 0.38)	0.0003
ALT level, units/liter	1.28 (1.98, 0.57)	0.0004	1.95 (2.68, 1.22)	<0.0001
Red blood cell count, millions/ μ l	0.06 (0.08, 0.03)	<0.0001	0.08 (0.12, 0.05)	<0.0001
White blood cell count, thousands/ μ l	0.1 (0.22, -0.02)	0.09	0.27 (0.39, 0.15)	<0.0001
Diastolic BP, mm Hg	1.72 (2.52, 0.92)	<0.0001	0.67 (1.54, -0.21)	0.13
Systolic BP, mm Hg	2.03 (3.12, 0.94)	0.0003	0.86 (2.42, -0.7)	0.28

* Point estimates are the effect on the trait with each increase of 1 mg/dl in uric acid level. 95% CI = 95% confidence interval; HDL = high-density lipoprotein; GFR = glomerular filtration rate; CRP = C-reactive protein; ALT = alanine aminotransferase; BP = blood pressure.

† Values are the natural log.

was similar to that observed in men (a 0.35 mg/dl decrease in uric acid level [95% CI 0.05–0.64]), while the greatest effect was observed among the 227 women who reported that they had not yet reached menopause (a 0.53 mg/dl decrease in uric acid level per Ile allele [95% CI 0.35–0.72]). Menopause status was unknown for 28 of the women.

The serum uric acid level has been shown to be associated with a number of cardiovascular, inflammation, and metabolic traits (15,16). We similarly found strong associations between uric acid levels and a panel of CVD risk factors, including percentage of body fat, levels of triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol, glucose, and insulin, and estimated glomerular filtration rate (GFR) calculated using the Modification of Diet in Renal Disease Study equation (17) (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 the uric acid level. This result suggests that Val253Ile may affect serum uric acid levels independently of the estimated GFR and known cardiovascular risk factors.

Subjects in the HAPI Heart Study were relatively healthy, and gout phenotypes were not available. We therefore examined the association between this clinically significant consequence of elevated uric acid levels and the *GLUT9* genotype in subjects from the Framingham Heart Study. The Val253Ile *GLUT9* variant was not genotyped in the 100K genome-wide association scan that is publicly available (9); however, rs10489070 was genotyped in both the 100K Framingham Heart Study genome-wide association scan and the 500K Amish genome-wide association scan. This SNP is in linkage disequilibrium with Val253Ile *GLUT9* in the HapMap CEU samples (the Centre d'Etude du Polymorphisme Humain collection of samples from Utah residents with ancestry from northern and western Europe) ($D' = 0.68$, $r^2 = 0.42$) and was associated with uric acid levels in the Framingham Heart Study subjects (P for ex1 generalized estimating equation [GEE] = 0.0001 and P for ex2 GEE = 0.002). The allele associated with increased uric acid levels was also strongly associated with gout in the Framingham Heart Study subjects (GEE $\beta \pm$ SE = -0.03 ± 0.009 ; $P = 0.004$). This demonstrates that a common variation in *GLUT9*, in addition to being associated with serum levels of uric acid, has direct clinical relevance.

Table 5. Association between Val253Ile (rs16890979) and other quantitative traits in subjects in the Heredity and Phenotype Intervention Heart Study*

Trait	Men		Women	
	Point estimate (95% CI)	<i>P</i>	Point estimate (95% CI)	<i>P</i>
Triglyceride level, mg/dl	-2.43 (-9.11, 4.24)	0.48	-2.45 (-10.56, 5.67)	0.55
Fasting HDL cholesterol level, mg/dl	1.15 (-1.17, 3.46)	0.33	3.04 (0.1, 5.98)	0.04
Ratio of cholesterol to HDL	0.08 (-0.13, 0.3)	0.44	-0.24 (-0.48, -0.01)	0.05
Estimated GFR, ml/minute/1.73 m ²	-0.74 (-3.88, 2.4)	0.65	-0.13 (-3.15, 2.9)	0.93
Creatinine level, mg/dl	0.01 (-0.02, 0.03)	0.57	0 (-0.03, 0.02)	0.75
Glucose level, mg/dl†	0 (-0.02, 0.02)	0.92	-0.01 (-0.04, 0.01)	0.30
Insulin level, mU/ml†	0.04 (-0.03, 0.12)	0.27	0.03 (-0.07, 0.13)	0.58
Adiponectin level, mg/ml†	-0.04 (-0.12, 0.03)	0.24	0.04 (-0.04, 0.13)	0.32
Leptin level, pg/ml†	0.02 (-0.46, 0.5)	0.94	-0.08 (-0.35, 0.19)	0.56
% body fat	0.8 (-0.91, 2.51)	0.36	0.3 (-1.26, 1.86)	0.71
Body mass index, kg/m ²	0.04 (-0.55, 0.62)	0.90	-0.39 (-1.35, 0.57)	0.42
Whole body fat mass, gm†	0.08 (-0.05, 0.21)	0.25	0.02 (-0.08, 0.11)	0.74
Whole body lean mass, gm†	0.02 (-0.01, 0.04)	0.28	0 (-0.03, 0.03)	0.91
Hemoglobin value, gm/dl	0.01 (-0.14, 0.16)	0.89	-0.02 (-0.19, 0.15)	0.80
CRP level, mg/liter†	0.02 (-0.19, 0.23)	0.85	0.01 (-0.17, 0.19)	0.94
Hematocrit value, %	0.25 (-0.19, 0.69)	0.27	0.12 (-0.37, 0.6)	0.64
ALT level, units/liter	-0.66 (-2.07, 0.75)	0.36	-1.48 (-2.78, -0.18)	0.03
Red blood cell count, millions/ μ l	0.02 (-0.03, 0.07)	0.42	0.01 (-0.04, 0.07)	0.65
White blood cell count, thousands/ μ l	0.16 (-0.07, 0.39)	0.17	-0.16 (-0.38, 0.06)	0.15
Diastolic BP, mmHg	0.28 (-1.33, 1.88)	0.74	-1.42 (-2.89, 0.04)	0.06
Systolic BP, mmHg	-0.36 (-2.5, 1.79)	0.75	-1.02 (-3.68, 1.65)	0.46

* Point estimates are the effect on the trait with each copy of Ile at the locus. See Table 4 for definitions.

† Values are the natural log.

DISCUSSION

We performed a genome-wide association study of serum uric acid levels and found strong associations between uric acid levels and multiple SNPs on chromosome 4 that exceeded that of genome-wide significance and replicated a previously identified association in the region of *GLUT9*. We identified the most likely causative variant as a nonsynonymous coding SNP in exon 8, rs16890979, which codes for a highly conserved Val to Ile amino acid substitution at position 253. The effect size associated with this nonsynonymous SNP is large and resulted in the most significant association found in the present study. The age-adjusted difference in uric acid level between Ile homozygotes and Val homozygotes in our sample was \sim 1 mg/dl. A model including age, sex, genotype, and body mass index revealed that the Val253Ile variant explained 33% of the variation in uric acid level in our sample.

Consistent with the results of previous studies (15,16), our findings indicated that the uric acid level was associated with a number of risk factors for metabolic syndromes, inflammation, and CVD in the Amish. The uric acid level was strongly associated with levels of triglycerides, HDL cholesterol and creatinine, as well as whole body fat mass, among other risk factors for CVD.

Although Val253Ile was strongly associated with the uric acid level, it was not consistently associated with these CVD-related markers, nor did adjustment for these CVD-related markers or the estimated GFR alter the association of uric acid level with genotype. The latter observation suggests that the 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 or CVD markers could be due either to a lack of causality between uric acid and increased metabolic or CVD risk or to insufficient power, since the population studied was healthy, with a low prevalence of elevated uric acid levels and clinically significant CVD. However, association of *GLUT9* variation with gout in the Framingham Heart Study subjects strongly supports the hypothesis that this gene plays a clinically significant role in uric acid homeostasis.

The serum urate level reflects the balance between production and excretion. Production is dependent on dietary protein intake and endogenous production and breakdown of purine by xanthine oxidase. Excretion is dependent primarily on renal elimination, which accounts for \sim 70% of urate excretion, with the remaining dependent on intestinal excretion (13). Inter-

estingly, women have lower serum uric acid levels than men, which has been shown to relate, at least in part, to increased renal excretion of uric acid in response to estrogen (18). Of potential relevance, in the Amish population as well as in the Sardinia and Chianti populations (7), the *GLUT9* genotype effect tends to be more pronounced in premenopausal women than in postmenopausal women and in men. This raises the possibility that *GLUT9* activity could be modulated by estrogen. Evidence of a sex interaction with *GLUT9* genotype was not identified in a study of British subjects with hypertension (8), but the mean age at the time of phenotyping in that study was 64 years (19), and it is possible that an interaction was not detected because the study population included an insufficient number of premenopausal women. 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 the potential role of GLUT-9 in fructose homeostasis in the kidney and liver. Increased fructose is known to increase uric acid levels secondary to increased production (20–23), and has been implicated as a potential cause of gout (24), kidney stones (25), and metabolic syndrome (26,27). Hereditary fructosemia, which is caused by aldolase deficiency in the liver, is associated with hypoglycemia, jaundice, and hyperuricemia (28). *GLUT9* has also been shown to be significantly up-regulated in the liver and kidney of diabetic rats (29), creating a potential link between metabolic syndrome and hyperuricemia. A second plausible mechanism relates to uric acid excretion. The *GLUT9* ΔN splice variant is not only exclusively expressed in the kidney and placenta, but is also located in kidney proximal tubule epithelial cells, the primary site of renal uric acid regulation and clearance. Future studies of the role of GLUT-9 in uric acid homeostasis are needed to effectively test these 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 lead 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). Further investigation is needed to determine the mechanism by which the substitution alters the function of the GLUT-9 protein.

In summary, we identified *GLUT9* as an important genetic determinant of serum uric acid levels. This

highly significant replication of previously reported findings, 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, the association with gout, and the identification of a highly conserved nonsynonymous SNP, provide context for future mechanistic studies related to *GLUT9*, uric acid homeostasis, and gout.

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AUTHOR CONTRIBUTIONS

Dr. McArdle had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. McArdle, Parsa, Mitchell, Shuldiner.

Acquisition of data. Mitchell, Shuldiner.

Analysis and interpretation of data. McArdle, Parsa, Chang, Weir, O'Connell, Mitchell, Shuldiner.

Manuscript preparation. McArdle, Parsa, Chang, Weir, O'Connell, Shuldiner.

Statistical analysis. McArdle, O'Connell.

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