Serum uric acid concentrations and *SLC2A9* genetic variation in Hispanic children: the Viva La Familia Study[1–4]

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ABSTRACT

Background: Elevated concentrations of serum uric acid are associated with increased risk of gout and renal and cardiovascular diseases. Genetic studies in adults have consistently identified associations of solute carrier family 2, member 9 (*SLC2A9*), polymorphisms with variation in serum uric acid. However, it is not known whether the association of serum uric acid with *SLC2A9* polymorphisms manifests in children.

Objective: The aim was to investigate whether variation in serum uric acid is under genetic influence and whether the association with *SLC2A9* polymorphisms generalizes to Hispanic children of the Viva La Familia Study.

Design: We conducted a genomewide association study with 1.1 million genetic markers in 815 children.

Results: We found serum uric acid to be significantly heritable [$h^2 \pm$ SD = 0.45 $\pm$ 0.08, $P = 5.8 \times 10^{-11}$] and associated with *SLC2A9* variants (P values between 10^{-16} and 10^{-7}). Several of the significantly associated polymorphisms were previously identified in studies in adults. We also found positive genetic correlations between serum uric acid and BMI z score ($\rho_G = 0.45$, $P = 0.002$), percentage of body fat ($\rho_G = 0.28$, $P = 0.04$), fat mass ($\rho_G = 0.34$, $P = 0.02$), waist circumference ($\rho_G = 0.42$, $P = 0.003$), and waist-to-height ratio ($\rho_G = 0.46$, $P = 0.001$).

Conclusions: Our results show that variation in serum uric acid in Hispanic children is under considerable genetic influence and is associated with obesity-related phenotypes. As in adults, genetic variation in *SLC2A9* is associated with serum uric acid concentrations, an important biomarker of renal and cardiovascular disease risk, in Hispanic children. *Am J Clin Nutr* 2015;101:725–32.

Keywords: hyperuricemia, obesity, metabolic syndrome, SNP association, urate transporter

INTRODUCTION

Uric acid is the end product of dietary and endogenous purine metabolism (1). Purines are nitrogen-containing bases that form the backbone of nucleic acids, DNA, and RNA. Hyperuricemia or elevated concentrations of serum uric acid (SUA)[5] in adults have been linked to greater risk of metabolic disorders such as hypertension, inflammation, type 2 diabetes, metabolic syndrome, and renal and cardiovascular disease (CVD) (2–5). Studies in adults have also shown SUA to be an independent risk factor for CVD (6, 7). In the past few decades, SUA concentrations, which are highly affected by lifestyle factors such as diet, physical activity, and medications, have increased in the United States (8).

SUA concentrations have been strongly associated with the metabolic syndrome and its individual components in children and adolescents from the United States (9, 10) and other countries (11, 12). SUA in children is dependent on age and puberty stages. Concentrations of SUA increase from early childhood and stabilize by ~15–17 y of age (13). However, it was also observed that SUA concentrations are higher in overweight and obese children and increase at rates on par with normo-uremic and normal-weight children (14). In adults, hyperuricemia predicts hypertension (15, 16). Likewise, childhood hyperuricemia is strongly predictive of adult blood pressure (17). Jones et al. (18) showed that SUA concentrations in childhood are associated with adult blood pressure, particularly systolic, and thus may be an important tool to assess CVD risk in adulthood.

Variation in SUA concentrations is affected by genetic as well as environmental factors. Population studies have shown that SUA concentrations are significantly heritable, with heritability estimates ranging between 15% and 73% (19–22). Genomewide or candidate gene association studies in these populations have

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[5] Abbreviations used: CVD, cardiovascular disease; *DOK6*, docking protein 6; *ENPEP*, glutamyl aminopeptidase; GWAS, genomewide association study; LD, linkage disequilibrium; *SLC2A9*, solute carrier family 2, member 9; SNP, single nucleotide polymorphism; SOLAR, Sequential Oligogenic Linkage Analysis Routine; SUA, serum uric acid; VFS, Viva La Familia Study.

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shown SUA concentrations to be associated with single nucleotide variants [single nucleotide polymorphisms (SNPs)] in various uric acid transporter encoding genes. All of these genetic studies were conducted in adult populations. Genetic studies in children conducted thus far mainly focused on familial hyperuricemic nephropathy or juvenile gout. Thus, to identify genes and SNPs associated with variation in SUA concentrations, we conducted a genomewide association analysis of SUA concentrations in Hispanic children of the Viva La Familia Study (VFS) (23, 24).

METHODS

Study design and participants

The VFS was designed to investigate genetic and environmental factors affecting obesity and its comorbidities in Hispanic children. Its enrollment was not limited by country of origin; however, the majority of families were of Mexican-American descent. To qualify, families had to have at least 1 obese proband aged 4–19 y. Thus, the VFS represents a family-based cohort highly enriched for obesity. A total of 319 Hispanic families enrolled in the VFS in 2000–2004. The majority of the parents were either overweight (34%) or obese (57%), and 51% of the children were classified as obese (>95th BMI percentile) with BMI *z* scores ranging from 2.3 to 4.5. Anthropometric and body composition measurements were conducted in parents and children. Fasting blood samples were drawn for biochemical profiling of the children and for genotyping of children and parents. Subjects and study procedures are described in detail in previous publications (23, 24). The demographic, genetic, and phenotypic details of the adult populations used for comparison in this article were previously published (21, 22, 25, 26).

Ethics

All of the children and their parents gave written informed consent or assent. The protocol was approved by the Institutional Review Boards for Human Subject Research of Baylor College of Medicine and Affiliated Hospitals and the Texas Biomedical Research Institute. The VFS began in 2001 and is not registered.

Measurement of SUA and other phenotypes

Uric acid was oxidized to allantoin by uricase with the production of hydrogen peroxide. The peroxide reacts with 4-aminoantipyrine and 2,4,6-tribromo-3-hydroxy benzoic acid (Fisher Diagnostics) in the presence of peroxidase to yield a quinoneimine dye. The resulting change in absorbance is proportional to uric acid concentration in the sample. Methods used to measure fasting concentrations of biomarkers in blood are described elsewhere (27, 28). In addition, blood pressure measurements were taken by using an automated monitor. Anthropometric measurements were performed by using standardized techniques according to Lohman et al. (29). Body composition was determined by dual-energy X-ray absorptiometry (30).

Genotyping in Hispanic children

Genotyping of the 815 children for 1.1 million SNPs was conducted by using marker assays included on the Illumina HumanOmni1-Quad v1.0 BeadChips (30). Genotype calls were obtained after scanning on the Illumina BeadStation 500GX and analyzed by using the GenomeStudio software. Our genotyping error rate (based on duplicates) was 2 per 100,000 genotypes. The average call rate per individual sample was 97%. Specific markers were removed from analysis if they had call rates <95% (~4000 SNPs) or deviated from Hardy-Weinberg equilibrium at a 5% false-discovery rate (12 SNPs). SNP genotypes were checked for Mendelian consistency by using the program SimWalk2 (31). The estimates of the allele frequencies and their SEs were obtained by using the software program Sequential Oligogenic Linkage Analysis Routines (SOLAR version 7.6.2) (32).

Statistical analyses

Quantitative genetic analysis (univariate and bivariate)

A variance components decomposition method was used to estimate heritability of SUA and CVD-related phenotypes. This method is implemented in the software program SOLAR. To estimate the genetic contribution to the variation in SUA, its heritability was estimated. Total phenotypic variance can be partitioned into its genetic and environmental components. The fraction of total phenotypic variance (σ^2_P) resulting from additive genetic effects (σ^2_G) is called the heritability and is denoted by $h^2 = \sigma^2_G/\sigma^2_P$ (33).

A variance components approach was also used to estimate phenotypic and genetic correlations between SUA and CVD-related phenotypes. The approach is described in detail elsewhere (33). In short, the phenotypic correlation between SUA and other phenotypes can be explained in terms of its genetic and environmental correlation components. A model in which all of the variables are estimated is compared with a model in which the genetic correlation is constrained to zero. If the result of this statistical test is significant, then we infer that the phenotypes share effects of a common set of genes. For this comparison, the likelihood ratio test is distributed asymptotically again as a 1/2:1/2 mixture of a chi-square variable with 1 df and a point mass at zero (34).

Genomewide association analysis using measured genotype analysis

Association analyses were performed by using the SOLAR program (version 7.6.2). Each marker genotype was converted to a covariate measure equal to 0, 1, or 2 copies of the minor allele (or, for missing genotypes, the weighted covariate based on imputation). These covariates were included in the variance components mixed models for measured genotype analyses (35) vs. null models that incorporated the random effect of kinship and fixed effects such as age, sex, their interaction and higher order terms. For the initial genomewide association screen, we tested each SNP covariate independently as a 1-df likelihood ratio test. Empirical thresholds for genomewide significant and suggestive evidence of association were based on the distribution of *P* values from 10,000 simulated null genomewide association studies (GWASs; i.e., simulations of a heritable trait with no modeled SNP covariate effects using the VFS pedigree and genotypes). The threshold for significance ($P < 1 \times 10^{-7}$) was defined as the cutoff for the lower 5% tails of the empirical distribution, and the threshold for suggestive evidence ($P < 1 \times 10^{-6}$) was the minimum *P* value obtained not more than once per genome scan. The linkage disequilibrium (LD) was computed in SOLAR by using information for all genotyped SNPs on all individuals. The effective number of SNPs given LD was calculated by the method of Moskvina and

Schmidt (36), as implemented in SOLAR. The average ratio of the SNP effective number to the actual number obtained from analysis of 1989 nonoverlapping bins of SNPs was used to calculate the genomewide effective number of tests and thus the significance threshold for genomewide association. We performed a quantitative transmission disequilibrium test (implemented in SOLAR) to test for population stratification (37). An initial genomewide association screen was conducted on a residual trait after accounting for the covariates age, sex, their interaction and higher order terms. Also, to determine the covariates that might have a significant effect on modulating SUA concentrations, we tested several CVD risk factors. Of these, BMI *z* score and systolic blood pressure were found to be significant covariates for SUA. Thus, we used age, sex, and their interaction and higher order terms; BMI *z* scores; and systolic blood pressure to regress SUA concentrations and used the residual trait in our GWAS.

RESULTS

SUA concentrations in VFS

The study included data from 815 children participating in the VFS. The mean (±SD) SUA concentration in participating children was 5.2 ± 1.7 mg/dL. Significant heritability was detected for SUA concentrations [h^2 = 0.45 (0.08), $P = 5.8 \times 10^{-11}$]. The prevalence of hyperuricemia as classified by SUA concentrations >2 SDs from the mean was 25%.

SUA concentrations and CVD risk factors

Overall, SUA concentrations were significantly correlated phenotypically with several CVD risk factors. We partitioned the phenotypic correlation into genetic and environmental correlations. Significant positive genetic and environmental correlations were seen between SUA and obesity-related measurements such as BMI *z* score, percentage of body fat, and waist circumference. The genetic correlations indicate the presence of common genetic effects acting on SUA and each of these traits. However, for other phenotypes (liver function, lipids, systolic blood pressure, and diabetes-related phenotypes), only environmental correlations with SUA were significant (**Table 1**).

Genomewide association analysis

Genomewide association analysis of SUA concentrations showed strong associations with SNPs in solute carrier protein

TABLE 1
Genetic and phenotypic correlations of serum uric acid with cardiovascular disease risk factors[1]

Phenotype	Mean ± SD	ρ_G (SE)	P	ρ_E (SE)	P	ρ_P (SE)	P
Serum uric acid, mg/dL	5.22 ± 1.7	—	—	—	—	—	—
Age, y	10.61 ± 3.9	—	—	—	—	—	—
Anthropometric							
Birth weight, kg	3.49 ± 0.6	−0.01 (0.12)	9.5×10^{-1}	0.21 (1.3)	8.0×10^{-1}	0.009 (0.03)	8.2×10^{-1}
BMI *z* score	1.51 ± 1.0	0.45 (0.12)	2.0×10^{-3}	0.45 (0.07)	3.6×10^{-6}	0.45 (0.03)	9.6×10^{-38}
Fat, %	0.33 ± 0.09	0.28 (0.12)	3.9×10^{-2}	0.41 (0.08)	3.0×10^{-5}	0.35 (0.03)	1.0×10^{-23}
Fat mass, g	19.28 ± 12.2	0.34 (0.12)	1.7×10^{-2}	0.50 (0.07)	3.7×10^{-7}	0.43 (0.03)	9.4×10^{-36}
Waist circumference, cm	76.01 ± 18.0	0.42 (0.11)	3.0×10^{-3}	0.51 (0.07)	7.2×10^{-7}	0.47 (0.03)	2.0×10^{-43}
Waist-to-height ratio	0.53 ± 0.09	0.46 (0.11)	1.0×10^{-3}	0.45 (0.08)	1.8×10^{-5}	0.45 (0.03)	1.1×10^{-39}
Serum lipids, mg/dL							
Triglycerides	105.32 ± 56.8	0.17 (0.12)	2.0×10^{-1}	0.55 (0.08)	2.4×10^{-7}	0.37 (0.03)	5.8×10^{-26}
HDL cholesterol	46.83 ± 11.1	−0.05 (0.11)	6.5×10^{-1}	−0.49 (0.10)	1.5×10^{-5}	−0.25 (0.03)	1.3×10^{-12}
LDL cholesterol	103.88 ± 29.1	−0.04 (0.12)	7.1×10^{-1}	0.21 (0.12)	8.6×10^{-2}	0.06 (0.03)	7.1×10^{-2}
Total cholesterol	171.76 ± 34.4	0.005 (0.11)	9.7×10^{-1}	0.26 (0.14)	8.0×10^{-2}	0.09 (0.03)	9.0×10^{-3}
Adipokines/inflammatory markers							
Leptin, ng/mL	17.96 ± 15.0	0.24 (0.13)	9.8×10^{-2}	0.42 (0.08)	9.2×10^{-6}	0.34 (0.03)	2.0×10^{-23}
TNF-α, pg/mL	8.32 ± 2.4	0.01 (0.09)	9.2×10^{-2}	0.02 (0.22)	9.1×10^{-1}	0.01 (0.03)	7.7×10^{-1}
ICAM-1, pg/mL	279.92 ± 113	−0.06 (0.11)	5.9×10^{-1}	0.35 (0.12)	4.0×10^{-3}	0.11 (0.03)	3.0×10^{-3}
IL-6, pg/mL	2.10 ± 2.1	0.23 (0.14)	1.1×10^{-1}	0.12 (0.09)	1.9×10^{-1}	0.17 (0.03)	7.2×10^{-7}
MCP-1, pg/mL	312.80 ± 90.2	0.22 (0.12)	5.0×10^{-2}	−0.14 (0.12)	2.2×10^{-1}	0.06 (0.03)	8.1×10^{-2}
CRP, ng/mL	1188.5 ± 1427	0.24 (0.15)	1.2×10^{-1}	0.28 (0.08)	1.0×10^{-3}	0.26 (0.03)	5.3×10^{-15}
Liver function markers, U/L							
ALT	24.34 ± 24.3	0.08 (0.15)	5.9×10^{-1}	0.41 (0.08)	9.6×10^{-6}	0.28 (0.03)	1.2×10^{-15}
AST	25.11 ± 15.1	−0.07 (0.13)	5.8×10^{-1}	0.36 (0.09)	3.0×10^{-4}	0.15 (0.03)	1.1×10^{-5}
Diabetes-related phenotypes							
Glucose, mg/dL	92.31 ± 12.1	0.03 (0.12)	8.2×10^{-1}	0.17 (0.11)	1.1×10^{-1}	0.10 (0.03)	5.0×10^{-3}
Insulin, μU/mL	22.43 ± 18.5	0.26 (0.14)	9.2×10^{-2}	0.45 (0.07)	1.8×10^{-6}	0.37 (0.03)	7.7×10^{-27}
C-peptide, ng/mL	2.69 ± 1.8	0.002 (0.12)	9.9×10^{-1}	0.80 (0.14)	2.1×10^{-8}	0.31 (0.03)	3.6×10^{-19}
HOMA	5.26 ± 4.9	0.23 (0.14)	1.3×10^{-1}	0.46 (0.07)	1.3×10^{-6}	0.36 (0.03)	7.2×10^{-26}
Blood pressure, mm Hg							
Systolic	108.13 ± 10.9	0.19 (0.14)	1.9×10^{-1}	0.23 (0.08)	1.0×10^{-2}	0.21 (0.03)	3.6×10^{-10}
Diastolic	51.01 ± 6.9	−0.03 (0.15)	8.5×10^{-1}	0.04 (0.09)	6.8×10^{-1}	0.01 (0.03)	7.6×10^{-1}

[1]Estimates of the allele frequencies and their SEs were obtained by using the software program Sequential Oligogenic Linkage Analysis Routines (SOLAR), version 7.6.2 (32). ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; ICAM-1, intercellular adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1; ρ_E, estimate of environmental correlations; ρ_G, estimate of genetic correlations; ρ_P, estimate of phenotypic correlations.

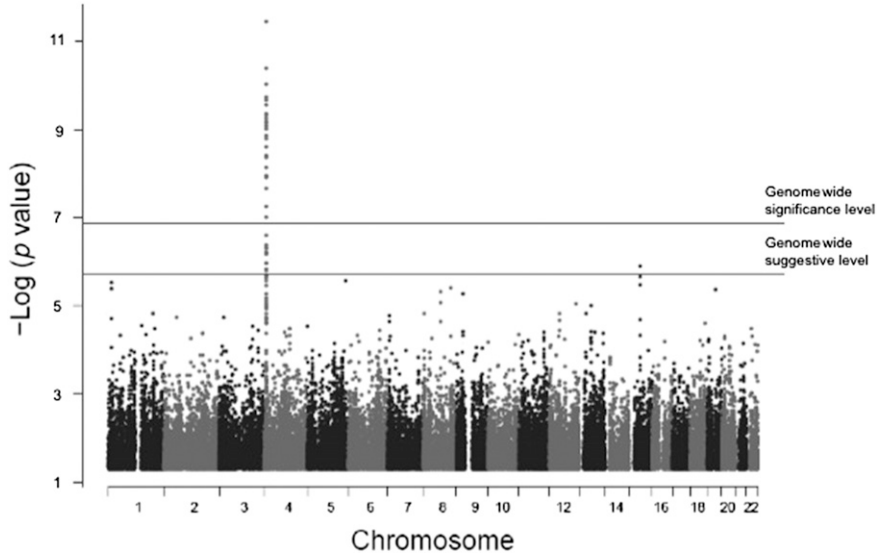

FIGURE 1 Genomewide association analysis of serum uric acid in Hispanic children.

2 family, member 9 (*SLC2A9*), on chromosome 4 (**Figure 1**). A total of 400 SNPs showed an association with SUA concentrations at a genomewide significance level of $P < 1 \times 10^{-7}$. All of the 400 SNPs were in the chromosome 4 region that spanned ~46 kb and covered both *SLC2A9* and the adjacent gene WD repeat-containing protein 1 (*WDR1*). Genotype distributions of all significantly associated SNPs conformed to Hardy-Weinberg equilibrium. Population stratification was not significant as per the quantitative transmission disequilibrium test and therefore did not confound our associations. The distribution of *P* values from GWASs of SUA showed no evidence of inflation due to population stratification (**Figure 2**). The relation pairs (n = 1242) that were used in the analysis are shown in **Table 2**. The top 20 significant associations are shown in **Table 3**. Frequencies of the minor alleles ranged between 0.24 and 0.49, whereas effect sizes (proportion of the residual phenotypic variance accounted by minor allele of the SNP) ranged between 8.5% and 10% (Table 3). Genotype-specific means of SUA concentrations showed that minor alleles of most of the SNPs are associated with lower concentrations of SUA, which is consistent with adult studies. Details of risk and minor alleles are given in Table 3. To uncover other signals and to offset the overwhelming

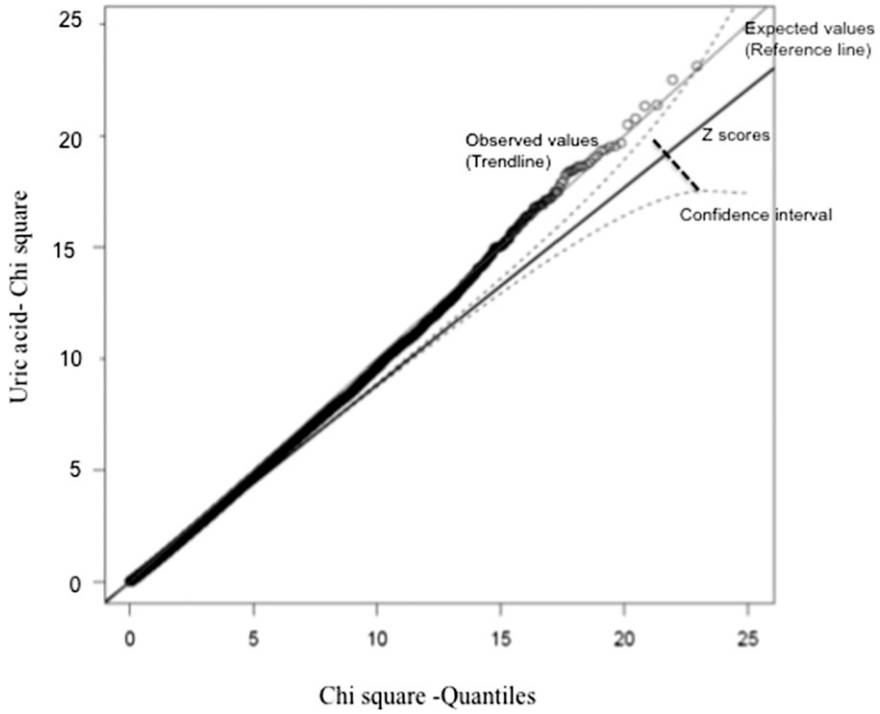

FIGURE 2 A Q-Q plot showing the absence of inflation due to population stratification and batch or clustering effects.

TABLE 2
Relation pairs used in this analysis

Relationships	Pairs, *n*
Identical sibling pair	2
Siblings	888
Half siblings and first cousins	2
Double first cousins	1
Half avuncular	5
First cousins	338
Half first cousins	6
Total	1242

association of *SLC2A9* and flanking SNPs, we conducted another GWAS with adjustment for the top 20 significant SNPs (from the chromosome 4 region) from the previous genomewide association analysis. Evidence of a suggestive association with SUA concentrations was found for 2 SNPs: rs12965305 from docking protein 6 (*DOK6*) and rs3796879 from glutamyl aminopeptidase (*ENPEP*). None of the SNPs were associated with SUA at a significance level of $P < 1 \times 10^{-7}$.

Extension of the *SLC2A9* association with SUA to children

For several of the significantly associated SNPs (rs6449213, rs10805346, rs1014290, and rs737267), an association with SUA was previously reported in Mexican Americans of the San Antonio Family Heart Study, American Indians of the Strong Heart Family Study, Western Alaska Natives of the Genetics of Coronary Artery Disease in Alaska Natives, Zuni Indians of the Genetics of Kidney Disease in Zuni Indians, and in European adult populations (**Table 4**). Interestingly, the effect sizes in Hispanic children were much higher than those in adults (3–5%) (38, 39).

DISCUSSION

To our knowledge, this is the first GWAS of SUA concentrations in children and the first to extend the strong association of variants in the *SLC2A9* genetic locus with SUA concentrations to children in a family-based study. GWASs have been conducted in several adult populations to identify loci associated with SUA concentrations. Most of these studies showed associations of *SLC2A9* SNPs with SUA concentrations. Vitart et al. (40) showed the association of *SLC2A9* SNPs with SUA concentrations in a Croatian sample and then replicated the result in a sample from the island of Orkney. Similar associations were reported in individuals from Germany (41), Sardinia (42), and the United Kingdom (43). Our group has replicated the association of SUA with *SLC2A9* SNPs in Mexican American (38), American Indian (39), Western Alaska Native, and Zuni Indian adult populations (44, 45). The main aim of all these family-based studies was to identify genetic determinants of complex diseases, mainly CVD and chronic kidney disease (21, 22, 25, 44, 45). All of these cohorts, except for Zuni Indians, were

TABLE 3
Significant associations of *SLC2A9* SNPs with serum uric acid concentrations[1]

						Genotype-specific means of serum uric acid concentrations[5]		
SNP	Base-pair position[2]	MGA *P* value	Minor allele/frequency	Risk allele[3]	Effect size,[4] %	11	12	22
rs11723388	9804900	3.3×10^{-16}	A/0.26	G	10	4.21 (2.3)	5.02 (2.1)	5.54 (2.5)
rs11721501	9798233	5.7×10^{-16}	A/0.26	G	9.9	4.17 (2.3)	5.02 (2.1)	5.55 (2.5)
rs6843466	9537003	1.2×10^{-15}	A/0.49	G	9.6	4.67 (1.7)	5.32 (1.6)	5.75 (1.9)
rs17251963	9751659	1.3×10^{-15}	G/0.25	A	9.7	4.12 (1.5)	5.04 (1.6)	5.53 (1.7)
rs13129697	9536065	1.4×10^{-15}	A/0.49	A	9.6	5.74 (1.9)	5.29 (1.6)	4.66 (1.7)
rs7696983	9604927	1.5×10^{-15}	A/0.26	G	9.3	4.03 (2.3)	5.06 (1.6)	5.51 (2.5)
rs13113918	9607591	1.5×10^{-15}	A/0.26	G	9.3	4.03 (2.3)	5.06 (1.6)	5.51 (2.5)
rs7683856	9610045	1.5×10^{-15}	A/0.26	G	9.3	4.03 (2.3)	5.06 (1.6)	5.51 (2.5)
rs9991278	9611763	1.5×10^{-15}	A/0.26	G	9.3	4.03 (2.3)	5.06 (2.1)	5.51 (2.5)
rs13111638	9605988	2.1×10^{-15}	A/0.25	G	9.1	3.97 (1.4)	5.07 (1.6)	5.49 (1.7)
rs4481233	9565177	2.4×10^{-15}	A/0.24	G	9.0	3.96 (2.3)	5.04 (1.6)	5.51 (1.8)
rs1978274	9854185	2.5×10^{-15}	C/0.29	A	9.5	4.28 (2.3)	5.06 (2.1)	5.56 (2.5)
rs7669607	9606899	2.4×10^{-15}	A/0.26	G	8.5	4.03 (2.3)	5.06 (1.6)	5.51 (2.5)
rs9991278	9611817	3.0×10^{-15}	A/0.26	G	9.1	4.03 (2.3)	5.07 (2.1)	5.51 (2.5)
rs11723439	9560917	3.5×10^{-15}	A/0.25	G	8.6	3.98 (1.3)	5.08 (1.7)	5.49 (1.7)
rs4697745	9914179	5.1×10^{-15}	A/0.27	G	9.1	4.24 (1.6)	5.04 (1.6)	5.55 (1.8)
rs7675964	994134	5.7×10^{-15}	G/0.49	G	9.2	5.73 (1.9)	5.28 (1.6)	4.69 (1.7)
rs938552	9925524	7.0×10^{-15}	A/0.41	G	8.9	4.53 (1.6)	5.23 (1.7)	5.60 (1.7)
rs6449213	9994215	1.3×10^{-14}	G/0.25	A	8.6	4.02 (1.4)	5.05 (1.6)	5.50 (1.7)
rs12510549	9885815	2.0×10^{-14}	G/0.26	A	8.5	4.32 (1.6)	5.04 (1.6)	5.53 (1.8)

[1]The top 20 associations are shown. Estimates of the allele frequencies and their SEs were obtained by using the software program Sequential Oligogenic Linkage Analysis Routines (SOLAR), version 7.6.2 (32). The final model included age, age-squared, sex, age × sex, age-squared × sex, BMI *z* score, and systolic blood pressure as covariates. MGA, measured genotype analysis; *SLC2A9*, solute carrier family 2, member 9; SNP, single nucleotide polymorphism.
[2]Position based on NCBI Genome Build 36.3.
[3]Risk allele = allele that is associated with increased serum uric acid concentrations (mg/dL).
[4]Effect size = proportion of residual phenotypic variance explained by the SNP.
[5]1 = minor allele; 2 = major allele.

TABLE 4
SLC2A9 SNP associations in VFS children that replicate results observed in adults[1]

	VFS	SAFHS	SHFS	GOCADAN	GKDZI	Studies in Europeans, *P*	Reference for European studies
Age, y	11.0 ± 4.0[2]	47.9 ± 14.8	39.5 ± 17.0	48.9 ± 14.8	36.8 ± 13.7	—	—
Serum uric acid, mg/dL	5.2 ± 1.7	5.4 ± 1.4	5.14 ± 1.5	5.25 ± 1.3	5.9 ± 1.7	—	—
Hyperuricemia,[3] %	25	22	17	14	31	—	—
Associated *SLC2A9* SNPs,[4] *P* value							
rs13129697	9.9×10^{-16}	—	—	—	1.8×10^{-5}	2.3×10^{-19}	(52)
rs13124563	2.0×10^{-12}	1.1×10^{-5}	—	—	—	—	—
rs7660895	1.2×10^{-14}	1.5×10^{-4}	—	—	1.6×10^{-6}	—	—
rs11723439	8.8×10^{-15}	—	—	—	3.1×10^{-7}	—	—
rs13111638	3.5×10^{-15}	—	—	—	1.5×10^{-7}	1.1×10^{-9}	(39)
rs4697701	2.8×10^{-15}	—	—	—	1.4×10^{-6}	—	—
rs6832439	3.8×10^{-14}	6.0×10^{-9}	7.7×10^{-31}	2.2×10^{-5}	8.5×10^{-8}	—	—
rs737267	7.7×10^{-14}	4.2×10^{-8}	2.9×10^{-29}	7.9×10^{-6}	4.5×10^{-7}	2.5×10^{-9}	(38)
rs6449213	2.1×10^{-14}	1.6×10^{-6}	1.5×10^{-29}	1.3×10^{-6}	4.3×10^{-8}	6.1×10^{-10}	(39)
rs10805346	8.4×10^{-13}	1.8×10^{-5}	5.4×10^{-28}	9.5×10^{-4}	1.2×10^{-4}	—	—
rs1014290	1.0×10^{-13}	1.9×10^{-4}	—	—	1.6×10^{-5}	5.6×10^{-8}	(38)
rs13125029	1.9×10^{-10}	4.3×10^{-5}	—	—	5.6×10^{-7}	—	—
rs4447863	7.4×10^{-13}	2.7×10^{-2}	—	—	1.4×10^{-6}	—	—
rs4697695	7.1×10^{-14}	4.0×10^{-2}	—	—	1.3×10^{-4}	—	—

[1]GKDZI, Genetics of Kidney Disease in Zuni Indians; GOCADAN, Genetics of Coronary Artery Disease in Alaska Natives; SAFHS, San Antonio Family Heart Study; SHFS, Strong Heart Family Study; *SLC2A9*, solute carrier family 2, member 9; VFS, Viva La Familia Study.

[2]Mean ± SD (all such values).

[3]Based on 2 SDs above the serum uric acid means in children and serum uric acid >7 and >6 mg/dL in men and women, respectively.

[4]SNPs for the VFS, SAFHS, and GKDZI were genotyped as part of genomewide association studies; SNPs for the SHFS and GOCADAN were generated for candidate gene studies.

recruited without regard to disease status. Nevertheless, the obesity rates were still high in these cohorts.

The concentrations of SUA in children are dependent on age, sex, and puberty stage. Hyperuricemia cutoffs in children are different from adults and are defined as SUA concentrations >2 SDs above the mean. In this study, ~25% children were hyperuricemic with the use of this criterion. The serum uric acid profile also varied according to age, with older children (≥13 y) having serum uric acid concentrations similar to adults. Hyperuricemia is caused by either increased production of uric acid or decreased renal excretion or a combination of both. In recent times, children with hyperuricemia increasingly present with obesity, as is the case in adults. Elevated SUA concentrations in children and adults are often associated with obesity and the metabolic syndrome (8, 11, 12, 14, 19, 46, 47). Several investigators have advocated for uric acid to be a component of the metabolic syndrome. In a cohort of obese children and adolescents, Denzer et al. (14) found positive correlations between SUA concentrations and BMI, serum triglycerides, and systolic blood pressure. They also pointed out that childhood hyperuricemia may be an indicator for pre–metabolic syndrome in obese youth and an unfavorable cardiovascular profile in obese adults. In Japanese junior high school students, hyperuricemia was strongly associated with cardiometabolic risk factors but only in boys (48). Consistent with other publications, we found positive correlations of SUA with all components of the metabolic syndrome, except for HDL-cholesterol concentrations. Likewise, SUA concentrations in childhood have been strongly associated with blood pressure (1, 15, 49–53) and are predictive of adult hypertension (51). We also found positive correlations of SUA with systolic and diastolic blood pressure. Soletsky and Feig (52) showed that uric acid–lowering therapy was effective in reducing blood pressure and systemic vascular resistance in obese adolescents. Our GWAS results, when additionally adjusted for BMI *z* score and systolic blood pressure, showed associations between SUA and *SLC2A9* SNPs with stronger effect sizes. This may be a reflection of the previous reports of a biological link between blood pressure, adiposity, and SUA, especially in children (8, 15, 17, 47, 51, 52). Our results of genetic correlations of SUA with obesity measurements, particularly waist circumference, are consistent with our results in Mexican-American adults (21).

The association of SUA concentrations with adiposity and inflammation is well recognized. Although the mechanism is not yet clear, obese adults and children have higher SUA concentrations as observed in this study and in other studies (14, 53, 54). Gillum (53) also found independent associations of SUA concentrations with systolic blood pressure and waist-to-hip ratio. Lyngdoh et al. (54) suggested that adiposity might be the link between hyperuricemia and hypertension. Wasilewska et al. (55) showed that children with hyperuricemia tend to have higher concentrations of circulating monocyte chemoattractant protein-1 and C-reactive protein, which is consistent with our results. In our study we found strong genetic correlations between SUA and adiposity measures, suggesting a common set of genes affecting both of these measures. We did not detect any significant genetic correlations of SUA with blood pressure, inflammation markers, or other cardiometabolic risk factors, although we did find strong phenotypic correlations between them.

This is the first GWAS of SUA in children. The heritability estimate for SUA concentrations in this cohort of Hispanic children was 45%, which is in the same range as reported in adults

(10, 19–22). The variation in several uric acid transporter genes have been associated with SUA concentrations in adults (European and non-European), with variants in *SLC2A9* being the most commonly associated across populations (39–43, 56). Interestingly, we replicated the *SLC2A9* findings; however, the associations and effect sizes were stronger in children. One of the SNPs, rs938552, is in strong LD with a missense polymorphism, rs16890979. Because strong genetic correlations were observed between obesity phenotypes and SUA concentrations, we conducted association analysis between them. None of the *SLC2A9* SNPs were associated with obesity phenotypes, which was in contrast to our results in Mexican-American adults in whom *SLC2A9* SNPs were significantly associated with BMI and waist circumference (38). In another study, Brandstätter et al. (57) showed that the association between *SLC2A9* and SUA concentrations was modified by BMI.

We conducted an additional GWAS for SUA, conditioned on significant *SLC2A9* SNPs, to uncover novel signals otherwise masked by *SLC2A9* SNPs. Although we did not find significant associations between SUA and other genes, we found suggestive evidence of an association of SUA concentrations with *DOK6* and *ENPEP*. None of these have been previously associated with SUA concentrations either in adults or in children. However, they have been shown to be associated with renal function (58) and blood pressure regulation (59), respectively. In a GWAS in individuals of African ancestry, urinary albumin-creatinine ratio was associated with *DOK6* (58). *DOK6* encodes a member of a family of intracellular adaptor proteins, expressed highly in human kidneys, that plays a role in rearranged during transfection signaling cascade (60). *ENPEP* encodes the glutamyl aminopeptidase that plays an important role in blood pressure regulation by facilitating conversion of angiotensin II to angiotensin III (59).

The majority of candidate biomarkers that are used in drug development are usually studied in adults. Given the association between SUA concentrations and increased risk of gout, hypertension, renal disease, and CVD in adults, and the ability to predict adult hypertension on the basis of pediatric SUA concentrations, the identification of such biomarkers will aid in the development of new treatment strategies that can be applied early in life. Although we were able to replicate results from studies in adults, lack of similar studies in children makes it difficult to replicate or validate our results. In conclusion, our genomewide association results of SUA concentrations in children replicate findings in adults and support *SLC2A9* genetic variation as an important pediatric biomarker of renal disease and CVD risk.

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