



Original Contribution

Association of Functional Polymorphism rs2231142 (Q141K) in the *ABCG2* Gene With Serum Uric Acid and Gout in 4 US Populations

The PAGE Study

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A loss-of-function mutation (Q141K, rs2231142) in the ATP-binding cassette, subfamily G, member 2 gene (*ABCG2*) has been shown to be associated with serum uric acid levels and gout in Asians, Europeans, and European and African Americans; however, less is known about these associations in other populations. Rs2231142 was genotyped in 22,734 European Americans, 9,720 African Americans, 3,849 Mexican Americans, and 3,550 American Indians in the Population Architecture using Genomics and Epidemiology (PAGE) Study (2008–2012). Rs2231142 was significantly associated with serum uric acid levels ($P=2.37 \times 10^{-67}$, $P=3.98 \times 10^{-5}$, $P=6.97 \times 10^{-9}$, and $P=5.33 \times 10^{-4}$ in European Americans, African Americans, Mexican Americans, and American Indians, respectively) and gout ($P=2.83 \times 10^{-10}$, $P=0.01$, and $P=0.01$ in European Americans, African Americans, and Mexican Americans, respectively). Overall, the T allele was associated with a 0.24-mg/dL increase in serum uric acid level ($P=1.37 \times 10^{-80}$) and a 1.75-fold increase in the odds of gout ($P=1.09 \times 10^{-12}$). The association between rs2231142 and serum uric acid was significantly stronger in men, postmenopausal women, and hormone therapy users compared with their counterparts. The association with gout was also significantly stronger in men than in women. These results highlight a possible role of sex hormones in the regulation of *ABCG2* urate transporter and its potential implications for the prevention, diagnosis, and treatment of hyperuricemia and gout.

ABCG2 protein, human; genetic association studies; gout; meta-analysis; polymorphism, single nucleotide; urate transporter; uric acid

Abbreviations: ARIC, Atherosclerosis Risk in Communities; BMI, body mass index; CARDIA, Coronary Artery Risk Development in Young Adults; CHS, Cardiovascular Health Study; CI, confidence interval; EAGLE, Epidemiologic Architecture for Genes Linked to Environment; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; PAGE, Population Architecture using Genomics and Epidemiology; SHFS, Strong Heart Family Study; SNP, single-nucleotide polymorphism.

Gout is one of the most common forms of inflammatory arthritis (1). The prevalence and incidence of gout have increased in recent decades (2, 3), and it now accounts for almost 4 million outpatient visits every year in the United

States (4). An elevated concentration of serum uric acid, or hyperuricemia, is a key risk factor for gout and may also be a risk factor for cardiovascular disease incidence and mortality, hypertension, and chronic kidney disease (5–7). Most

epidemiologic and genetic research on gout and hyperuricemia has been conducted in populations of European or Asian ancestry. In the United States, there have been 2 studies in African Americans (8, 9) but none in Mexican Americans or American Indians, despite epidemiologic evidence suggesting that the prevalence of gout in African Americans (830.4 per 100,000 persons) is higher than that in European Americans (752.9 per 100,000 persons) (3, 10).

Both environmental and genetic factors play important roles in the etiology of hyperuricemia and gout. Epidemiologic studies suggest that men are more likely to develop gout than women, and the prevalence increases with age in both sexes, especially among women after menopause (9). Other known environmental risk factors for hyperuricemia and gout include obesity, hypertension, type 2 diabetes, use of diuretics, and alcohol consumption (1). Genetic studies have demonstrated that serum uric acid levels are highly heritable (10). Several genomic loci influencing serum uric acid levels and the prevalence of gout have been identified in recent genome-wide association studies, mostly in populations of European descent (11–16).

A single-nucleotide polymorphism (SNP) consistently associated with both uric acid and gout is rs2231142, located on the ATP-binding cassette, subfamily G, member 2 gene (*ABCG2*; OMIM (Online Mendelian Inheritance in Man) number 603756). More specifically, the T allele at rs2231142 has been shown to be associated with increased serum uric acid levels and odds of gout in persons of European (14, 15, 17, 18), African (14), Japanese (19, 20), and New Zealand Pacific Island (21) ancestry. More importantly, the resultant missense polymorphism at codon 141 (glutamine to lysine, Q141K) has been shown to be a loss-of-function mutation in 2 independent functional studies (22, 23).

Previous studies have shown that the association between rs2231142 and uric acid and gout is stronger in men than in women, suggesting that sex modifies this association (14, 15); however, the evidence has been inconsistent (18). In addition, few studies have had sufficiently large sample sizes to systematically examine potential interactions with other major risk factors for gout, including age, diabetes, menopausal status, hormone therapy, obesity, alcohol consumption, hypertension, and antihypertensive treatment (14, 18). Lastly, the association between rs2231142 and serum uric acid and gout has not been established in Mexican-American or American Indian populations. Therefore, we conducted a study within the Population Architecture using Genomics and Epidemiology (PAGE) Study (24) to determine the association between rs2231142 and serum uric acid and gout in 4 general US populations and to specifically examine the potential heterogeneity of these associations across major gout risk factors.

MATERIALS AND METHODS

Study population

Five population-based studies were included as part of the PAGE Study, including 4 prospective cohort studies—the Atherosclerosis Risk in Communities (ARIC) Study (25), the Coronary Artery Risk Development in Young Adults

(CARDIA) Study (26), the Cardiovascular Health Study (CHS) (27), and the Strong Heart Family Study (SHFS) (28–30)—and 1 nationally representative, cross-sectional survey (the National Health and Nutrition Examination Survey (NHANES)) accessed by the Epidemiologic Architecture for Genes Linked to Environment (EAGLE) investigators. The current analysis included DNA and data from the Third National Health and Nutrition Examination Survey (NHANES III), conducted between 1988 and 1994, and yearly continuous NHANES data ascertained between 1999 and 2002 (NHANES 1999–2002) (31). The study designs and data collection methods of these 5 studies are summarized in Web Table 1, available at <http://aje.oxfordjournals.org/>. The participants of all 5 studies provided written informed consent. All 5 studies were approved by the institutional review boards of the participating institutions.

Participants were excluded if they did not identify themselves as belonging to one of the following 4 groups: European-American, African-American, Mexican-American, or American Indian; or if they did not consent to genetic research. Participants without rs2231142 genotype data or missing information on uric acid levels or gout were further excluded from the analysis. In all, the sample sizes for the analysis of serum uric acid in European Americans, African Americans, Mexican Americans, and American Indians were 22,734, 9,720, 3,849, and 3,550, respectively; and the sample sizes for the analysis of gout in European Americans, African Americans, and Mexican Americans were 13,783, 4,271, and 1,373, respectively.

SNP genotyping

In the ARIC Study, the CARDIA Study, the CHS, and the SHFS, genotyping was performed in the genetic laboratory at the University of Texas Health Science Center using TaqMan genotyping assays (Applied Biosystems by Life Technology, Carlsbad, California). In NHANES III and NHANES 1999–2002, rs2231142 was genotyped by the Vanderbilt University DNA Resources Core as part of EAGLE, using the iPLEX Gold assay (Sequenom, Inc., San Diego, California). *P* values for Hardy-Weinberg equilibrium were ≥ 0.01 in all study- and population-specific groups. The call rates for this marker in all 5 studies were greater than 95%, except for African Americans in ARIC (91.2%). In the NHANES III and NHANES 1999–2002 combined study, the genotype concordance rate of blind duplicated samples using the iPLEX Gold assay was 0.99.

Measurement of outcomes

The primary outcome was serum uric acid level, measured by means of a uricase method, at the baseline of each study. The secondary outcome was prevalence of gout. ARIC participants (at visit 4, 1996–1998) and NHANES III participants (1988–1994) were asked, “Has a doctor ever told you that you had gout?” Participants who answered “yes” were considered to have gout. Participants who did not know their gout status were excluded from the gout analyses. The sensitivity of self-reported gout in the ARIC Study was 82% by reference to a hospital discharge diagnosis of gout or use of

gout-specific medication as the gold standard (32). In the CHS (at visit 1, 1989–1993), a participant was considered to have gout only if he or she was receiving any of the following medications at the time of the study examination: allopurinol, probenecid, colchicine, sulfapyrazone, or colchicine/probenecid. In the SHFS, the CARDIA Study, and NHANES 1999–2002, gout status was not assessed.

Assessment of covariates

In all 5 studies, body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Obesity status was defined as normal (BMI <25), overweight (BMI of 25–29.99), or obese (BMI ≥30). Age groups were defined as young (age ≤50 years) or old (age >50 years). Alcohol consumption status was defined as self-reported current drinking or not. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, current use of antihypertensive medication, or self-reported hypertension. Hypertensive participants were further categorized into untreated or treated patients based on their self-reported antihypertensive medication use. Type 2 diabetes was defined as nonfasting blood glucose levels ≥200 mg/dL, fasting blood glucose levels ≥126 mg/dL, current use of antidiabetic medication, or self-reported diabetes.

Self-reported menopausal status (premenopausal vs. postmenopausal) was used. In the ARIC Study, a woman was defined as postmenopausal if she 1) had had at least 24 consecutive months of amenorrhea, 2) had undergone a bilateral oophorectomy, or 3) had undergone a hysterectomy and was aged 55 years or older. In the NHANES, postmenopausal status was defined as not having had a menstrual period for at least 24 months. In the SHFS, postmenopausal status was defined as having not had a menstrual period in the previous 12 months. Women who did not meet postmenopausal criteria were categorized as premenopausal. All women in the CARDIA Study (age <30 years) were premenopausal, and all women in the CHS (age ≥65 years) were postmenopausal. Among postmenopausal women, use of hormone therapy was defined as self-reported current use of estrogen or estrogen and progestin. The assessment of menopausal status and hormone therapy use is described in Web Table 1.

Statistical analysis

All study-specific results were stratified by self-reported race/ethnicity (European-American, African-American, Mexican-American, or American Indian). In the ARIC Study, the CARDIA Study, CHS, and NHANES, the association between rs2231142 and either serum uric acid or gout was examined using unadjusted and multivariable linear or logistic regression (adjusting for sex, age, BMI, hypertension, antihypertensive treatment, type 2 diabetes, and current alcohol drinking). Results from the unadjusted and multivariable-adjusted models were very similar, and therefore we report results from multivariable models. An additive genetic model was assumed. In the SHFS, a variance components-based biometrical model was used to account for the random effects of kinship (33).

To estimate the population-specific association between rs2231142 and serum uric acid or gout, population- and study-specific β coefficients or odds ratios were meta-analyzed across studies using a fixed-effects model. Since the associations between rs2144300 and serum uric acid and gout were consistent across 4 populations, we further meta-analyzed the data across populations using a random-effects model to obtain the average effect of the SNP on serum uric acid and gout.

To detect potential heterogeneity of the association between rs2144300 and serum uric acid across major gout risk factors (34), in each study, we first stratified European-American, African-American, Mexican-American, or American Indian populations into different covariate subgroups (sex, menopausal status, hormone therapy use, age group, obesity status, hypertension, type 2 diabetes, and drinking status) to examine covariate-specific associations with serum uric acid. The joint estimate of the β coefficient of rs2231142 for each covariate subgroup was obtained by pooling results across studies and populations successively using a fixed-effects model. We used Cochran's Q test (35, 36) and a random-effects model to assess heterogeneity across each stratified covariate. A Q statistic with $P_{\text{het}} < 0.05$ indicated significant heterogeneity. Parallel heterogeneity analyses were performed for gout, except that we only examined factors that had significant heterogeneity in the analysis of serum uric acid level, which included sex, menopausal status, and hormone therapy.

To examine the potentially confounding effect of population stratification, we performed a sensitivity analysis in the ARIC Study by adjusting for principal components that were derived from genome-wide association study data (37) and were associated with the outcomes. For American Indians, to test for population stratification, we compared the likelihood of a model in which the association parameters, β_b (between-family genotype score) and β_w (within-family genotype score), were estimated with the likelihood of a model in which they were constrained to be equal, as would be the case in the absence of population stratification (38, 39).

Analyses were performed with either Stata 11 (StataCorp LP, College Station, Texas) or SAS, version 9.2 (SAS Institute Inc., Cary, North Carolina) in the ARIC Study, the CHS, and the CARDIA Study. Analyses in EAGLE were conducted remotely in SAS, version 9.2, using the Analytic Data Research by Email (ANDRE) portal of the Centers for Disease Control and Prevention Research Data Center in Hyattsville, Maryland. Analyses in the SHFS were implemented in the SOLAR (Sequential Oligogenic Linkage Analysis Routines) software package (version 4.4; Southwest Foundation for Biomedical Research, San Antonio, Texas) (40, 41). Meta-analyses were conducted using Stata 11. Aggregate statistics related to this work will be available via the database of Genotypes and Phenotypes (dbGaP) as part of the PAGE Study.

RESULTS

Baseline characteristics of the 39,853 participants are given by population in Table 1. In the serum uric acid analyses, a total of 22,734 European-American, 9,720

Table 1. Characteristics of 39,853 Participants in an Analysis of the Relationship of the *ABCG2* rs2231142 Polymorphism With Uric Acid and Gout, by Race/Ethnicity, PAGE Study, 2008–2012

	Race/Ethnicity							
	European-American		African-American		Mexican-American		American Indian	
	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%
Uric acid analysis	(n = 22,734)		(n = 9,720)		(n = 3,849)		(n = 3,550)	
Serum uric acid level, mg/dL	5.7 (1.5)		5.7 (1.6)		5.2 (1.4)		5.1 (1.5)	
Body mass index ^a	26.7 (5.1)		28.2 (6.3)		27.7 (5.7)		32.3 (7.9)	
Age, years	53.5 (11.1)		44.2 (12.3)		41.2 (18.7)		39.96 (17.0)	
Female sex		54.5		58.9		51.1		52.0
Postmenopausal		58.9		40.6		37.0		30.0
Hormone therapy ^b		15.5		9.6		4.0		9.0
Current alcohol drinker		64.8		50.8		59.6		58.0
Hypertension		32.3		39.5		28.0		32.0
Antihypertensive treatment		53.3		41.1		12.2		20.0
Type 2 diabetes		8.6		12.5		11.9		23.0
Gout analysis	(n = 13,783)		(n = 4,271)		(n = 1,373)		(N/A)	
Gout prevalence		4.4		5.9		1.0		N/A
Body mass index	26.7 (4.8)		28.7 (6.1)		27.0 (5.6)		N/A	
Age, years	57.9 (7.2)		50.4 (10.1)		37.1 (18.7)		N/A	
Female sex		56.1		61.9		50.6		N/A
Postmenopausal		66.8		52.5		27.4		N/A
Hormone therapy		19.3		14.7		7.6		N/A
Current alcohol drinker		61.5		39.2		57.8		N/A
Hypertension		33.8		47.1		24.0		N/A
Antihypertensive treatment		61.2		54.9		7.9		N/A
Type 2 diabetes		8.9		13.4		10.6		N/A

Abbreviations: N/A, not applicable; PAGE, Population Architecture using Genetics and Epidemiology; SD, standard deviation.

^a Weight (kg)/height (m)².

^b Self-reported current use of estrogen or estrogen and progestin (among postmenopausal women).

African-American, 3,849 Mexican-American, and 3,550 American Indian participants were included. The average serum uric acid levels in European Americans, African Americans, Mexican Americans, and American Indians were 5.7 mg/dL, 5.7 mg/dL, 5.2 mg/dL, and 5.1 mg/dL, respectively. The mean age in years was 53.5 in European Americans, 44.2 in African Americans, 41.2 in Mexican Americans, and 40.0 in American Indians. Slightly over half the study population was female, and the proportion of postmenopausal women ranged from 30.0% to 58.9% across populations. Overall, the prevalence of gout was 4.4% in European Americans, 5.9% in African Americans, and 1.0% in Mexican Americans. Baseline characteristics of the population- and study-specific samples are shown in Web Table 2.

Association between rs2231142 and serum uric acid levels and gout

Frequencies of the T allele in rs2231142 were 0.11 in European Americans, 0.03 in African Americans, 0.19 in Mexican Americans, and 0.20 in American Indians

(Table 2). SNP rs2231142 was consistently associated with serum uric acid levels across studies ($P_{\text{het}} > 0.05$). The population- and study-specific results are shown in Web Table 3. In the meta-analysis, each copy of the T allele was significantly associated with an increase in serum uric acid concentration of 0.31 mg/dL ($P = 2.37 \times 10^{-67}$) in European Americans, 0.21 mg/dL ($P = 3.98 \times 10^{-5}$) in African Americans, 0.22 mg/dL ($P = 6.97 \times 10^{-9}$) in Mexican Americans, and 0.18 mg/dL ($P = 5.33 \times 10^{-4}$) in American Indians (Table 2). After pooling of data across populations, the joint estimate of the β coefficient of rs2231142 associated with serum uric acid level was 0.24 (95% confidence interval (CI): 0.17, 0.31; $P = 1.37 \times 10^{-80}$).

Similarly, a consistent association was observed with gout across studies ($P_{\text{het}} > 0.05$) (Web Table 3). In the meta-analysis, each copy of the T allele was significantly associated with an approximately 2-fold increase in the odds of gout: The odds ratios were 1.71 ($P = 2.83 \times 10^{-10}$) in European Americans, 1.80 ($P = 0.01$) in African Americans, and 2.97 ($P = 0.01$) in Mexican Americans (Table 2). After pooling across populations, the joint estimate of the odds

Table 2. Associations Between the *ABCG2* rs2231142 Polymorphism and Serum Uric Acid Level and Gout, by Race/Ethnicity, PAGE Study, 2008–2012

Race/Ethnicity	Frequency of the T allele (Proportion) ^a	Uric Acid Level			Gout		
		β^b	95% CI	P Value ^c	OR ^d	95% CI	P Value ^c
European-American	0.11	0.31	0.28, 0.50	2.37×10^{-67}	1.71	1.45, 2.02	2.83×10^{-10}
African-American	0.03	0.21	0.11, 0.32	3.98×10^{-5}	1.80	1.14, 2.84	0.01
Mexican-American	0.19	0.22	0.16, 0.29	6.97×10^{-9}	2.97	1.25, 11.04	0.01
American Indian	0.20	0.18	0.08, 0.29	5.33×10^{-4}	N/A	N/A	N/A
Overall effect size		0.24	0.17, 0.31	1.37×10^{-80}	1.75	1.50, 2.04	1.09×10^{-12}

Abbreviations: CI, confidence interval; N/A, not applicable; OR, odds ratio; PAGE, Population Architecture using Genetics and Epidemiology.

^a Minor allele on the forward strand of the human genome reference sequence of National Center for Biotechnology Information build 37.1. The minor allele was the nonreference allele in all analyses.

^b Increase in serum uric acid level (mg/dL) associated with each copy of the T allele in rs2231142.

^c P value for the multivariable linear (β) or logistic regression (OR) analysis adjusting for sex, age, body mass index, hypertension, antihypertensive treatment, type 2 diabetes, and current alcohol drinking.

^d Increase in the odds of gout associated with each copy of the T allele in rs2231142.

ratio for the association of rs2231142 with gout was 1.75 (95% CI: 1.50, 2.04; $P = 1.09 \times 10^{-12}$).

Association between rs2231142 and serum uric acid levels by subgroup

In each of the 4 populations studied, the association between rs2231142 and serum uric acid level was consistently stronger in men than in women (Figure 1). Probably because of smaller sample sizes in the non-European-American populations, the sex difference in the genetic effects was statistically significant only in the European Americans ($P_{het} = 0.001$). After pooling of data across populations, a significantly stronger association between rs2231142 and serum uric acid level was observed in men ($n = 18,223$; $\beta = 0.35$, $P = 3.9 \times 10^{-52}$) than in women ($n = 21,360$; $\beta = 0.20$, $P = 6.35 \times 10^{-12}$), with significant heterogeneity between these 2 estimates ($P_{het} = 1.64 \times 10^{-5}$; Table 3).

In addition, the association between rs2231142 and serum uric acid level was also stronger in postmenopausal women ($n = 11,083$; $\beta = 0.27$, $P = 6.06 \times 10^{-10}$) than in premenopausal women ($n = 8,040$; $\beta = 0.14$, $P = 3.10 \times 10^{-5}$), with significant heterogeneity ($P_{het} = 0.02$). Among postmenopausal women, hormone therapy users showed a stronger association ($n = 1,930$; $\beta = 0.41$, $P = 2.59 \times 10^{-10}$) than non-users ($n = 8,468$; $\beta = 0.25$, $P = 2.86 \times 10^{-13}$), with significant heterogeneity ($P_{het} = 0.03$). We also tested heterogeneity in the association between rs2231142 and serum uric levels across age groups, obesity status, hypertension, type 2 diabetes, and drinking status but did not observe any significant heterogeneity (Table 3). The population-, study-, and covariate-specific β coefficients for the association of rs2231142 with serum uric acid (for sex, menopausal status, and hormone therapy use) are shown in Web Tables 4–6.

Association between rs2231142 and gout by subgroup

Table 4 summarizes the association between rs2231142 and the prevalence of gout by sex, menopausal status, and

hormone therapy use. Across the 4 populations, the association between rs2231142 and prevalent gout was significantly stronger in men ($n = 6,620$; odds ratio (OR) = 2.03, $P = 1.53 \times 10^{-13}$) than in women ($n = 9,018$; OR = 1.37, $P = 0.03$; $P_{het} = 0.03$). Among women, this association was statistically significant only in postmenopausal women ($n = 6,985$; OR = 1.45, $P = 0.03$), not in premenopausal

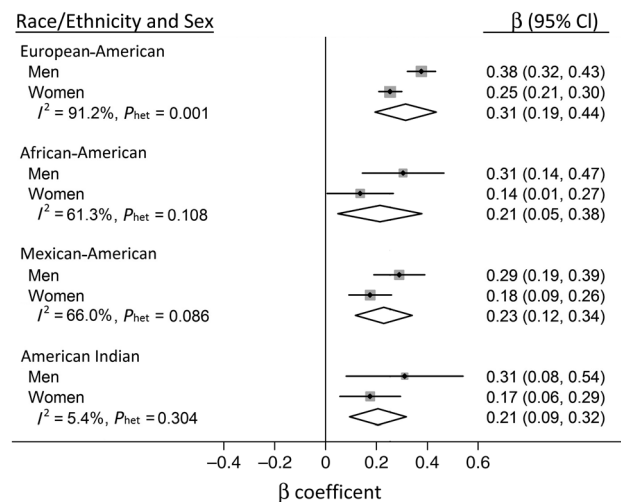


Figure 1. Sex-specific (squares) and pooled (diamonds) associations between rs2231142 and serum uric acid levels in the Population Architecture using Genetics and Epidemiology (PAGE) Study, by race/ethnicity and sex, 2008–2012. Beta coefficients (β) and 95% confidence intervals (CIs) were obtained from a multivariable linear regression model adjusting for sex, age, body mass index, hypertension, antihypertensive treatment, type 2 diabetes, and current alcohol drinking. *I*² and *P*_{het} values were obtained from Cochran’s Q test using a random-effects model. The size of each square is proportional to the percent weight that each race/ethnicity- and sex-specific group contributed in the overall association. The diamond represents the pooled β coefficient for each population. Bars, 95% CI.

Table 3. Associations Between the *ABCG2* rs2231142 Polymorphism and Serum Uric Acid Level According to Major Risk Factors for Gout, PAGE Study, 2008–2012

Gout Risk Factor	No. of Participants	β^a	95% CI	<i>P</i> Value ^b	<i>P</i> _{het} ^c
Sex					1.64×10^{-5}
Male	18,223	0.35	0.31, 0.40	3.9×10^{-52}	
Female	21,360	0.20	0.15, 0.26	6.35×10^{-12}	
Menopausal status					0.02
Premenopausal	8,040	0.14	0.07, 0.20	3.10×10^{-5}	
Postmenopausal	11,083	0.27	0.18, 0.35	6.06×10^{-10}	
Hormone therapy ^d					0.03
No	8,468	0.25	0.18, 0.31	2.86×10^{-13}	
Yes	1,930	0.41	0.28, 0.54	2.59×10^{-10}	
Age group, years					0.14
Young (≤ 50)	18,656	0.24	0.21, 0.28	5.32×10^{-39}	
Old (>50)	20,927	0.30	0.23, 0.37	5.03×10^{-18}	
Obesity status ^e					0.87
Normal	14,813	0.27	0.23, 0.31	1.10×10^{-36}	
Overweight	13,879	0.25	0.17, 0.33	1.92×10^{-9}	
Obese	11,161	0.29	0.17, 0.40	1.96×10^{-6}	
Hypertension					0.19
Normotensive	26,357	0.24	0.19, 0.29	7.69×10^{-22}	
Untreated hypertension	5,361	0.22	0.13, 0.31	1.96×10^{-6}	
Treated hypertension	7,906	0.35	0.23, 0.46	6.04×10^{-9}	
Type 2 diabetes					0.72
No	35,241	0.26	0.20, 0.31	8.02×10^{-21}	
Yes	4,365	0.28	0.17, 0.39	8.63×10^{-7}	
Current alcohol drinking					0.93
No	12,747	0.29	0.19, 0.38	1.49×10^{-9}	
Yes	18,880	0.28	0.24, 0.32	1.95×10^{-47}	

Abbreviations: BMI, body mass index; CI, confidence interval; PAGE, Population Architecture using Genetics and Epidemiology.

^a Increase in serum uric acid level (mg/dL) associated with each copy of the T allele in rs2231142.

^b *P* value for the multivariable linear regression analysis adjusting for sex, age, body mass index, hypertension, antihypertensive treatment, type 2 diabetes, and current alcohol drinking.

^c *P* value from Cochran's *Q* test using a random-effects model to assess heterogeneities across subgroups.

^d Self-reported current use of estrogen or estrogen and progestin (among postmenopausal women).

^e Normal, BMI (weight (kg)/height (m)²) <25; overweight, BMI 25–29.99; obese, BMI ≥ 30 .

women ($n = 2,033$; OR = 0.96, $P = 0.94$; $P_{\text{het}} = 0.43$). Lastly, among postmenopausal women, the association between rs2231142 and gout was significant only in non-hormone therapy users ($n = 5,014$; OR = 1.53, $P = 0.03$), not in hormone therapy users ($n = 1,320$; OR = 1.06, $P = 0.39$; $P_{\text{het}} = 0.08$). The population-, study-, and covariate-specific odds ratios for the association of rs2231142 with gout are shown in Web Tables 7–9.

In the sensitivity analysis conducted in the ARIC Study, the results obtained from models with and without adjustment for principal components did not differ, and the causal inference was not changed in European Americans and African Americans. For American Indians, as implemented in SOLAR, the tests for population stratification for rs2231142 from previous

quantitative-trait linkage disequilibrium tests were not significant ($P > 0.05$) (38, 39).

DISCUSSION

In a meta-analysis of 39,853 persons from 4 different population-based samples (European Americans, African Americans, Mexican Americans, and American Indians), we demonstrated that the functional variant rs2231142 (Q141K) in the *ABCG2* gene was significantly associated with both serum uric acid level ($P = 2.37 \times 10^{-67}$, $P = 3.98 \times 10^{-5}$, $P = 6.97 \times 10^{-9}$, and $P = 5.33 \times 10^{-4}$ in European Americans, African Americans, Mexican Americans, and American Indians, respectively) and the odds of

Table 4. Associations Between the *ABCG2* rs2231142 Polymorphism and Gout According to Major Risk Factors for Gout, PAGE Study, 2008–2012

Gout Risk Factor	No. of Participants	OR ^a	95% CI	P Value ^b	P _{het} ^c
Sex					0.03
Male	6,620	2.03	1.68, 2.45	1.53×10^{-13}	
Female	9,018	1.37	1.03, 1.82	0.03	
Menopausal status					0.43
Premenopausal	2,033	0.96	0.38, 2.47	0.94	
Postmenopausal	6,985	1.45	1.04, 2.02	0.03	
Hormone therapy ^d					0.08
No	5,014	1.53	1.03, 2.27	0.03	
Yes	1,320	1.06	0.93, 1.21	0.39	

Abbreviations: CI, confidence interval; OR, odds ratio; PAGE, Population Architecture using Genetics and Epidemiology.

^a Increase in the odds of gout associated with each copy of the T allele in rs2231142.

^b P value for the multivariable logistic regression analysis adjusting for sex, age, body mass index, hypertension, antihypertensive treatment, type 2 diabetes, and current alcohol drinking.

^c P value from Cochran's Q test using a random-effects model to assess heterogeneities across subgroups.

^d Self-reported current use of estrogen or estrogen and progestin (among postmenopausal women).

gout ($P = 2.83 \times 10^{-10}$, $P = 0.01$, and $P = 0.01$ in European Americans, African Americans, and Mexican Americans, respectively). After pooling of data across populations, each copy of the T allele in rs2231142 was associated with a 0.24-mg/dL increase in serum uric acid levels (95% CI: 0.17, 0.31; $P = 1.37 \times 10^{-80}$) and a 1.75-fold increase in the odds of gout (95% CI: 1.50, 2.04; $P = 1.09 \times 10^{-12}$). Moreover, there was a significantly stronger association between rs2231142 and both serum uric acid and gout in men compared with women. Lastly, we also observed heterogeneity in the association between rs2231142 and serum uric acid levels across menopausal and hormone therapy status categories, with a stronger association in postmenopausal women and hormone therapy users than in their counterparts.

Our study showed, for the first time, that rs2231142 is associated with serum uric acid levels and gout in Mexican Americans and American Indians, 2 admixed populations that had not been previously studied. Despite differences in minor allele frequencies at this variant across populations (minor allele frequencies ranging from 0.03 to 0.20), the associations between rs2231142 and the phenotypes were direction-consistent and robust across populations. Moreover, our study confirmed the previously observed heterogeneity between men and women in the association between rs2231142 and uric acid in our European-American samples and further demonstrated such heterogeneity in African Americans, Mexican Americans, and American Indians. The inclusion of a large proportion of postmenopausal women (>30.0%) and postmenopausal women receiving hormone therapy (ranging from 4.0% to 15.5%) in the serum uric acid analysis provided adequate statistical power to examine heterogeneity across categories of menopausal status and hormone therapy use, which had not been assessed previously. Lastly, similar to previous studies, the

association between rs2231142 and serum uric acid level did not differ significantly by age group, obesity status, diabetes, hypertension, or alcohol drinking status, which suggests either a lack of effect modification or very modest effect modification by these major gout risk factors, which the current study did not have adequate statistical power to discover. With a sample size of 39,853 in the present study and assuming that 50% of the population is exposed to the environmental factor, we had 80% power to detect an interaction effect as low as 0.10 (the difference in the β coefficients between men and women was 0.15 in our uric acid analysis) (42).

The ABCG2 protein, encoded by the *ABCG2* gene, has been shown to mediate renal urate secretion as a urate efflux transporter in the (luminal) brush-border membrane of kidney proximal tubule cells (23). The Q141K (rs2231142) polymorphism occurs in a highly conserved region of the gene and has been shown to be a loss-of-function mutation in 2 independent functional studies (22, 23). Moreover, several previous studies have reported regulation of the ABCG2 protein by estrogen. A series of studies showed that the ABCG2 protein has a putative estrogen response element (43) and that estrogens, along with their derivatives, antagonists and agonists, can regulate ABCG2 transporter activity in K562 human leukemia cells (44, 45), human placental BeWo cell lines (46), an ex vivo blood-brain barrier model from rats and mice (47), and mouse liver in vivo (48). The direction of regulation varies with the differences in sex hormones, model organisms, target organs, and substrates used to test ABCG2 protein function. One study found that higher expression of ABCG2 in kidney tissues from male rats appeared to be due to a suppressive effect of estradiol, because castration of the males had no effect on kidney *ABCG2* mRNA levels while ovariectomy of the female rats led to increased expression (49). If these results from model

systems are reflective of the human kidney, one might expect that the consequence of the Q141K loss-of-function polymorphism would be relatively less in persons with high estrogen levels (they had decreased *ABCG2* mRNA expression to begin with) compared with those with low estrogen levels. Our results were consistent in that the magnitudes of the associations for both serum uric acid and gout were smaller in women compared with men and in premenopausal women compared with postmenopausal women. However, a significantly stronger association with uric acid in hormone therapy users compared with nonusers was not expected based on previous animal data.

Several methodological issues may explain this apparent inconsistency. First, the hormone therapy measurement in our study was based on self-report. We did not have information on specific regimen, duration, or adherence. In addition, the heterogeneity between hormone therapy users and nonusers may be due not to their difference in sex hormone levels but to other factors which can influence the use of hormone therapy. Moreover, it is possible that endogenous and exogenous hormones may interact differently with the *ABCG2* protein. Lastly, it is also possible that the interaction between *ABCG2* protein and estrogen is different in various animal models, including humans. Thus, the statistical interaction between rs2231142 and hormone therapy use will need to be replicated in other populations and further investigated at the biological level.

The results of the current study should be interpreted with some limitations in mind. First, this was a cross-sectional study. While it is clear that genes can influence serum uric acid levels and gout and the reverse cannot be true, the same cannot be assumed for the relationship between the phenotypes and the gout risk factors examined, except for sex. Second, the statistical power of the gout analysis was limited because of the smaller sample sizes, the dichotomized nature of the trait, differences in study-specific definitions of gout, and the limitation of self-reported gout. Third, medications which may affect uric acid levels were not considered in the analyses; however, this may not have confounded our results, because the use of such medications is unlikely to be associated with genotype status at rs2231142. Lastly, we were not able to assess whether the observed association in Mexican Americans was due to confounding by population stratification; however, this is not likely to be the case, because rs2231142 is a functional polymorphism and the results of our sensitivity analyses in the other 3 populations showed that adjustment for population substructure did not alter the associations between rs2231142 and outcomes.

There are several aspects of the study that strengthened our findings. First, we used a large, multiethnic sample with data collected from 5 well-characterized population-based studies in the United States, providing unbiased estimates of allele frequencies and effect sizes across the general population. Second, the phenotypes and the covariates were carefully harmonized across the studies, which minimized the potential decrease in power due to study heterogeneities. Third, given the consistency of associations between rs2231142 and serum uric acid and gout across the 4 populations, we were able to meta-analyze across the populations to obtain the average effect and to increase statistical power.

Lastly, the Q141K variant is a well-studied functional polymorphism, thus providing a strong a priori hypothesis and biological plausibility. Moreover, our statistical interaction results are supported by some biological mechanisms, though more direct evidence is still needed.

In conclusion, the common polymorphism rs2231142, which leads to a change from glutamine to lysine in codon 141 (Q141K) of the *ABCG2* gene, is significantly associated with elevated serum uric acid levels and increased prevalence of gout in European Americans, African Americans, Mexican Americans, and American Indians. In addition, this SNP appears to have a differential effect on serum uric acid levels and gout prevalence by sex and heterogeneous associations across menopausal status and hormone therapy use with uric acid levels, which sheds light on sex and age differences in susceptibility to gout. More research is warranted on the possible role of sex hormones in the regulation of *ABCG2* urate transporter and the potential implications for the prevention, diagnosis, and treatment of hyperuricemia and gout.

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