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Genome-wide Association Study of 24-Hour Urinary Excretion of Calcium, Magnesium, and Uric Acid

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Abstract

Objectives: The urinary excretion of organic and inorganic substances and their concentrations have attracted extensive attention for their role in the pathogenesis of urinary stone disease. The urinary excretion of specific factors associates with sex and age and seems to have a hereditary component, but the precise genomic determinants remain ill-defined.

Methods: Genome-wide association studies previously conducted in 3 cohorts (Genetic Epidemiology Network of Arteriopathy study, January 1, 2006, through December 31, 2012; the combined Nurses' Health Study (NHS), NHS II, and Health Professionals Follow-up Study, January 1, 1994, through December 31, 2003; and the Prevention of Renal and Vascular End-stage Disease study, January 1, 1997, through December 31, 1998) were combined into meta-analyses to evaluate genetic associations with available urinary phenotypes relevant to stone pathogenesis (calcium, magnesium, and uric acid excretion; total urine volume).

Results: One region on chromosome 9q21.13 showed strong evidence of an association with urinary magnesium excretion. The strongest signal in this region was near *TRPM6*, whose protein product mediates magnesium transport in the colon and kidney, and *C9orf40*, *C9orf41*, *NMRK1*, and *OSTF1* (rs1176815; $P=1.70\times10^{-14}$, with each copy of the A allele corresponding to a daily 5.29-mg decrease in magnesium excretion). The single nucleotide polymorphism (SNP) that achieved genome-wide significance for calcium excretion (rs17216707 on chromosome 20; $P=1.12\times10^{-8}$) was previously associated with fibroblast growth factor 23 levels, which regulate phosphorus and vitamin D metabolism. Urine volume and uric acid excretion did not have any genome-wide significant SNPs.

Conclusion: Common variants near genes important for magnesium metabolism and bone health associate with urinary magnesium and calcium excretion.

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Kidney stones are common, affecting 10% of people during their lifetime, and are associated with a great health care burden and economic cost.[1] Most human kidney stones (~75%) are composed of predominantly calcium oxalate or calcium phosphate.[2] Risk factors for these common stones include the urinary concentration of solutes that contribute to the overall supersaturation for crystals that compose the stones. For example, greater excretion of urine calcium increases the risk of calcium stones,[3] excretion of uric acid alters the risk of uric acid and calcium stones,[4,5] and greater total urinary volume tends to decrease the risk of all stone types. Magnesium is a calcium oxalate crystallization inhibitor[6] that influences calculated calcium oxalate supersaturation,[7] although only limited data associate magnesuria with kidney stone risk.[5] Given the association of urinary supersaturation with kidney stone risk,[8] many groups have evaluated the effects of environment (including diet) and genetics on the urinary excretions relevant to stone disease.[9]

It has long been known that there is a strong heritable component to calcium kidney

stone risk (~50%) that approaches or even exceeds that of the common diseases diabetes and hypertension.[10] Recent studies have reported that many of the traits associated with kidney stone risk also have a strong heritable component, including urinary calcium excretion, magnesium excretion, and even urinary volume.[11–13] Previous genome-wide association studies (GWASs) implicated several genes in the pathogenesis of calcium stones, including claudins 14, 16, and 19.[14,15] However, relatively few studies have looked for genetic determinants of the specific urinary traits that associate with kidney stone risk.

Thus, in the present study we took advantage of several large cohorts with available genome-wide single nucleotide polymorphism (SNP) data as well as 24-hour urine data for several determinants of urinary supersaturation to perform a meta-analysis and determine whether any genetic loci were significantly associated with those urinary traits that were available across the cohorts (calcium, magnesium, uric acid, and urine volume).

METHODS

Participating studies include the Genetic Epidemiology Network of Arteriopathy (GENOA) study, the Nurses' Health Study (NHS), the NHS II, the Health Professionals Follow-up Study (HPFS),[5] and the Prevention of Renal and Vascular End-stage Disease (PREVEND) study.[16] All the participants were of European ancestry. The present analysis was approved by the institutional review boards at Mayo Clinic, Brigham and Women's Hospital, and the University of Michigan.

GENOA Study

As part of the Family Blood Pressure Program, the GENOA study recruited non-Hispanic white, hypertensive siblings from the Rochester, Minnesota, area to examine the genetic underpinnings of hypertension in phase 1 (January 1, 1996, through December 31, 2001).[17] Two ancillary studies conducted on the GENOA study samples include the phase 3 GENOA Genetics of Chronic Kidney Disease (CKD) study (January 1, 2006, through December 31, 2010) and the Genetic Determinants of Urinary Lithogenicity (GDUL) study (January 1, 2006, through December 31, 2012). These studies captured detailed kidney stone information on the participants who were invited to collect 24-hour urine samples once at the CKD and twice at the GDUL study visits.[18,19] Urinary determinants of supersaturation were measured in the Mayo Clinic Renal Testing Laboratory. Participants with end-stage renal failure (stage 5 CKD) were excluded from the study. Multiple urine samples were collected from most participants (1 measure, n=333; 2 measures, n=295; 3 measures, n=183). Where 2 or more urine samples were collected, values were averaged for analysis. Intraclass correlation coefficients for 24-hour urine excretions across time showed that most of the urine measures were relatively stable (calcium=0.82, magnesium=0.73, uric acid=0.66, urine volume=0.79). The mean time between the earliest CKD study and the latest GDUL study urine collections was 1.7 years (range, 0.9-3.6 years).

NHS, NHS II, and HPFS

The NHS was established in 1976 with more than 120,000 female registered nurses aged 30 to 55 years. The NHS II was established in 1989 with more than 116,000 female nurses aged 25 to 42 years. The HPFS was established in 1986 with more than 51,000 male health care professionals aged 40 to 75 years. All 3 cohorts have been followed via biennially mailed questionnaires that include questions about lifestyle practices, a Food Frequency Questionnaire, and newly diagnosed diseases such as nephrolithiasis.[5] Additional information was obtained from self-reported cases, including symptoms and kidney stone type. In validation studies, permission to obtain medical records was requested from newly diagnosed cases in all 3 cohorts. The diagnosis of stone disease was confirmed in more than 90% of these cases. Twenty-four–hour urine samples were collected from participants with a history of confirmed nephrolithiasis and from randomly selected control subjects from January 1, 1994, through December 31, 2003. Participants with a history of kidney stones performed the collections after the diagnosis. All 24-hour urine collections were performed using the Mission Pharmacal system.[5]

PREVEND Study

The PREVEND study was designed to investigate the relationship of urinary excretion with kidney and cardiovascular disease in a large cohort drawn from the general population of Groningen, the Netherlands.[16] The PREVEND study selected 7579 nondiabetic patients aged 28 to 75 years from a population-based cohort. These inhabitants were asked to send in a morning urine sample. A sample population of all individuals with an albumin concentration greater than 0.001 g/dL (to convert to g/L, multiply by 10) in the morning urine sample, compared with a randomly selected sample of the remainder of the population (morning urine albumin excretion <0.001 g/dL), made 2 visits to an outpatient clinic. Individuals using insulin and those who were pregnant were excluded. The visits, completed from January 1, 1997, through December 31, 1998, included anthropometric and blood pressure measurements, two 24-hour urine collections, an electrocardiogram, and a fasting blood sample. All the participants completed a questionnaire on demographic data and cardiovascular and renal history. Urinary calcium level, magnesium level, uric acid excretions, and total urinary volume were calculated as the mean of the two 24-hour urine collections.

Urine Measures Available for Analysis

Twenty-four–hour measures of 4 urinary excretions available across all the cohorts were assessed: calcium, volume, uric acid, and magnesium. Although urine concentration of key variables is the final factor that contributes to supersaturation, the final concentration for any analyte is the combined result of urinary excretion and urinary volume, and volume and excretions are under distinct regulatory influences. Participants could have been taking medications. These medications were not held for urine collections, were not itemized in detail for all participants in all cohorts, and, hence, were not adjusted for in the analysis.

All the urine measures were quantified as continuous variables. In each study, we assessed normality and removed outliers (±4 SD from the mean) for each urinary measure.

Covariates

All the models included age, sex, height, weight, and the top 10 study-specific principal components estimated from independent genome-wide genotypes to control for population structure. Models for calcium, magnesium, and uric acid also included sodium because sodium intake (and hence urine excretion) could influence their renal handling.

Genetic Measures

Internal quality control of genotype data was undertaken by each cohort. Quality checks per individual included exclusion of those with a poor genotype call rate (<95%) and a check for relatedness. The SNPs were excluded if they had a poor call rate (<95%), were out of Hardy-Weinberg equilibrium ($P<10^{-5}$), had high duplicate discordance rates, or were monomorphic. Genotyped data from each of the studies were imputed to the 1000G Phase I Integrated Release Version 3 (March 2012) using the cosmopolitan (worldwide) reference panel. Imputation dosages (range, 0-2) were used in the GWAS analyses.

GWAS Analyses

The GWAS analyses were performed by each of the studies, for each of the urine outcomes of interest, separately. Linear regression models were used for unrelated studies, and the GENOA study used linear mixed effects models with "family" as a random intercept. Each urine measure was regressed on study-specific covariates, including age, sex, height, weight, and top 10 study-specific principal components. Calcium, magnesium, and uric acid models also included sodium. Quantile-quantile (QQ) and Manhattan plots were created using the results from each study-outcome combination. Results of the GWAS analyses, including SNP, base pair position, beta estimate, standard error, and sample size, were retained for subsequent meta-analyses.

Meta-analysis

Before meta-analysis, GWASs from each cohort were corrected for genomic control. A fixed effects, inverse variance–weighted

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TABLE 1. Cohort-Specific Descriptive Statistics

Characteristic	GENOA study (n=811)	HPFS (n=553)	NHS (n=494)	NHS II (n=635)	PREVEND study (n=3969)
Age (y), mean ± SD	66±9	64±8	66±8	50±6	49±13
Height (cm), mean ± SD	168±10	179±7	164±6	165±7	174±9
Weight (kg), mean ± SD	88±19	84±12	71±15	74±19	79±14
BMI, mean ± SD	31±6	25±8	26±6	27±7	26±4
Sex, M/F	344/467	553/0	0/494	0/635	2027/1942
(No. [% male])	(42)	(100)			(51)
24-h urine measures (total daily excretion), mean ± SD					
Calcium (mg)	156±88	194±97	201±102	207±95	164±81
Magnesium (mg)	108±41	124±42	102±41	102±38	96±38
Uric acid (mg)	443±172	625±220	443±157	504±157	290±120
Volume (mL)	1958±700	1715±643	1846±680	1749±725	1579±530
Sodium (mEq)	139±58	182±71	139±57	150±64	144±51

BMI = body mass index; GENOA = Genetic Epidemiology Network of Arteriopathy; HPFS = Health Professionals Follow-up Study; NHS = Nurses' Health Study; PREVEND = Prevention of Renal and Vascular End-stage Disease.

meta-analysis was performed on summary statistics using METAL software.[20] The SNPs that were included in the meta-analysis had to be present in at least 2 participating studies, have a study-specific minor allele frequency of at least 1%, and have an imputation quality of at least 80%. The SNP associations were considered genome-wide significant at $\alpha=5\times10^{-8}$ and suggestive at $\alpha=1\times10^{-6}$. For genome-wide significant associations, regional plots were generated using LocusZoom software,[21] and evidence of association with gene expression in proximal genes was examined using the GTEx Portal (multiple tissue types; https://gtexportal.org/home) and the NephQTL browser (kidney tissue).[22]

RESULTS

Descriptive Statistics

Table 1 gives descriptive statistics for each of the individual studies. Descriptive statistics for the NHS, NHS II, and HPFS are presented separately, although they were combined into a single cohort for the purposes of analysis. On average, the GENOA study and the NHS had the oldest participants (mean age, 66 years), and the PREVEND study had the youngest (mean age, 49 years). The GENOA study participants weighed more (mean weight, 88 kg) and had a higher body mass index (calculated as the weight in kilograms divided by the height in meters squared) (mean body mass index, 31) than the other cohorts, and NHS participants weighed the least (71 kg for NHS and 74 kg for NHS II). The HPFS had the highest mean daily levels of magnesium (124 mg), uric acid (625 mg), and sodium excretion (182 mEq), and the NHS had the highest mean daily calcium excretion (201 mg for the NHS and 207 mg for the NHS II). The PREVEND study had the lowest mean daily levels of magnesium (96 mg) and uric acid excretion (290 mg), and the GENOA study had lower levels of calcium (156 mg). Mean daily sodium excretion was least in the NHS and GENOA study cohorts (139 mEq). The GENOA and PREVEND studies were approximately 50% female, the NHS and NHS II were 100% female, and the HPFS was 100% male.

Meta-analyses

The QQ and Manhattan plots for each urinary trait in each study are presented in the supplemental material (available online at http://mcpiqojournal.org). Results are presented in order of their generally accepted association with kidney stone risk. Figure 1 shows QQ plots for the meta-analyses of calcium excretion, urine volume, uric acid excretion, and magnesium excretion. Manhattan plots for each of these traits are shown in Figure 2, and SNPs with $P<1\times10^{-6}$ in each of 4 meta-analyses are shown in Table 2.

The QQ and Manhattan plots for these traits in each analyzed cohort separately (GENOA study, NHS/NHS II/HPFS, and PREVEND study) are shown in Supplemental Figures 1 through 4 (available online at http://mcpiqojournal.org).

Both the urine calcium and magnesium excretion trait meta-analyses resulted in genome-wide significant findings. One region on chromosome 9 (77.5-77.8 Mb) showed strong evidence of an association with urinary magnesium excretion. Figure 3 contains a regional plot of the urinary magnesium meta-analysis. The strongest signal in the region was rs1176815 ($P=1.70\times10^{-14}$). Each copy of the A allele of this SNP was associated with a daily decrease of 5.3 mg in magnesium excretion, and the frequency of the A allele in this study was 43%. The SNP was most strongly associated in the PREVEND study ($P=9.13\times10^{-10}$) and less strongly associated in the other cohorts (GENOA study, $P=5.33\times10^{-03}$; and NHS/NHS II/HPFS, $P=1.80\times10^{-4}$). However, daily effect estimates for rs1176815 were consistent across cohorts (−5.37, −5.13, and −5.07 mg in the PREVEND study, the GENOA study, and the NHS/NHS II/HPFS, respectively), and the test for heterogeneity confirmed that the effects were consistent across cohorts (heterogeneity $P=.98$).

Regional plots for this area of interest on chromosome 9 for urinary magnesium excretion separately for each study are provided in Supplemental Figure 5 (available online at http://mcpiqojournal.org). The SNPs in this region were genome-wide significant ($P<5\times10^{-8}$) or suggestive ($P<1\times10^{-6}$) for magnesium excretion in both the PREVEND study (smallest P value: rs1176816; $P=1.9\times10^{-10}$) and the NHS/NHS II/HPFS (rs2800249; $P=2.9\times10^{-6}$). The association signal spanned the gene region of *C9orf40*,

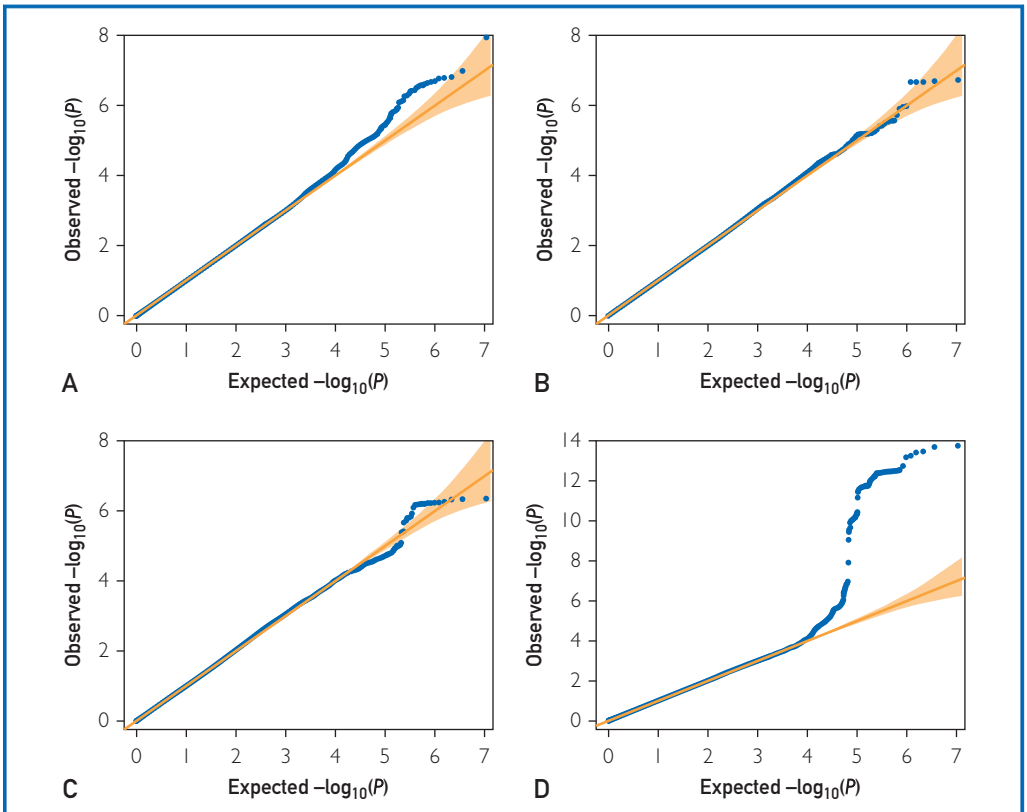

FIGURE 1. Quantile-quantile plots for meta-analysis of urine traits: calcium (A), urine volume (B), uric acid (C), and magnesium (D). Fixed effects, inverse variance–weighted meta-analyses were performed on summary statistics, corrected for study-specific genomic control lambda. Meta-analysis λ values were as follows: λ=0.9967 (A); λ=0.9977 (B); λ=0.9884 (C); λ=1.0019 (D).

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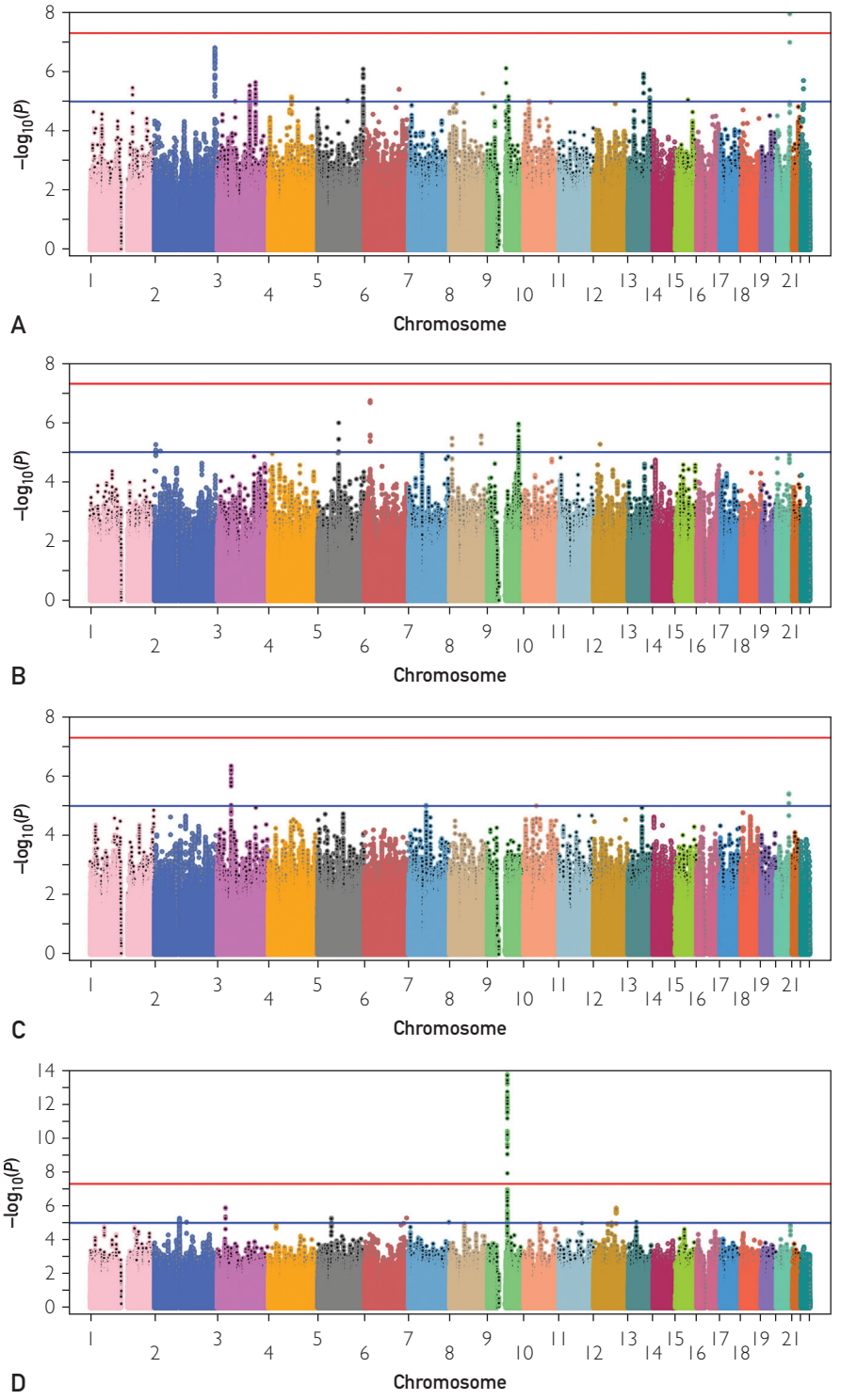

FIGURE 2. Manhattan plots for meta-analysis of urine excretion traits: calcium (A), urine volume (B), uric acid (C), and magnesium (D). Fixed effects, inverse variance–weighted meta-analyses performed on summary statistics, corrected for study-specific genomic control lambda. Top line (red) represents genome-wide significant finding ($P<5\times10^{-8}$), and bottom line (blue) represents genome-wide suggestive finding ($P<1\times10^{-6}$).

C9orf41, *NMRK1*, and *OSTF1*. It was upstream of *TRPM6* but was separated from this gene by a recombination hotspot. A query of the GTEx Portal showed that the lead SNP, rs1176815, was associated with *C9orf40* expression in transverse colon tissue ($P=7.8\times10^{-10}$) but not with other genes in the region. It was also marginally associated with *OSTF1* gene expression in glomerulus tissue in the NephQTL database.

The single genome-wide significant finding for calcium excretion was on chromosome 20 (rs17216707; $P=1.12\times10^{-8}$, with each copy of the *T* allele increasing daily calcium excretion by an estimated 10.9 mg). This SNP was not associated with gene expression in any tissue types in the GTEx or NephQTL databases. Supplemental Figure 6 (available online at http://www.mcpiqojournal.org) contains a regional plot of the urinary calcium meta-analysis, and separate regional plots by study are provided in Supplemental Figure 7 (available online at http://www.mcpiqojournal.org). Because the daily effect estimates across cohorts were heterogeneous for this SNP (10.33, 2.19, and 18.00 mg for the PREVEND study, the GENOA study, and the NHS/NHS II/HPFS, respectively; heterogeneity $P=5.85\times10^{-2}$), and because no other SNPs in this region demonstrated a significant or suggestive association with urine calcium excretion, we acknowledge that this finding may be a false-positive. Meta-analyses for urine volume and urinary uric acid excretion did not reveal any genome-wide significant SNPs.

DISCUSSION

There is strong evidence for heritability of the most common form of nephrolithiasis, idiopathic calcium stones. In addition, there is strong evidence for heritability of urinary traits associated with idiopathic calcium stone risk.[13] In the present study we performed a meta-analysis of available data from large cohorts in the United States and Europe to determine whether any genetic regions are associated with the 4 urinary traits that are important for kidney stone risk. A significant association was found for 24-hour urine magnesium excretion in a region on chromosome 9 near a gene known to mediate magnesium transport in the intestine and the kidney (*TRPM6*), as well as several other genes (*C9orf40*, *C9orf41*, *NMRK1*, and *OSTF1*). No significant associations were found for urinary calcium, uric acid, and urine volume, except for a single association for calcium.

Urinary calcium excretion is a key risk factor for idiopathic calcium stones.[5,18] Previous GWASs have identified genes that associate with having ever developed a kidney stone. Several of these implicated genes are important for calcium metabolism, including claudins 14, 16, and 19.[14,15] Vezzoli et al[23] suggested that the calcium-sensing receptor gene was a component of the complex genetic background that regulates calcium excretion. Specifically, the Arg990Gly (R990G) polymorphism could facilitate calcium-sensing receptor activation and increase calcium excretion, with carriers of a minor 990G allele susceptible to hypercalciuria.[24] However, we did not find significant evidence in these cohorts for any polymorphisms in this gene affecting calcium excretion, although the direction of effect for the Arg990Gly polymorphism in this meta-analysis was consistent with previous findings: carriers of the *G* (Gly) allele of rs1042636 (chromosome 3, 122003769) had 3.43-mg/24 h greater urinary calcium excretion than those with the Arg allele ($P=.21$).

An early GWAS[15] also implicated *SLC34A1* on chromosome 6 in calcium excretion (Table 2). The present meta-analysis revealed only 1 SNP (rs17216707) significantly associated with urine calcium excretion, which may be a false-positive result because no other SNPs in this region were strongly associated. However, rs17216707 is upstream of *CYP24A1*, a gene that encodes the primary catabolic enzyme for 1,25-dihydroxyvitamin D (calcitriol) and 25-hydroxyvitamin D. This SNP has recently been associated with fibroblast growth factor 23 (FGF23) concentration, which is a bone-derived hormone that plays a role in regulating phosphorus and vitamin D metabolism.[25] The *T* allele was associated with higher circulating FGF23 levels. In the present analysis, the *T* allele was associated with greater calcium excretion. In a previous study of first-time stone formers, the relationship between FGF23 and serum calcium differed compared with controls, suggesting lack of FGF23 suppression in stone formers,[26] consistent with the present observations. We did not identify significant signals in other chromosomal regions of genes previously implicated in calcium metabolism and kidney

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TABLE 2. Results for Each Urine Excretion Trait With Meta-analysis $P<1\times10^{-6}$[a,b]

SNP	Chr	Position	Coded|noncoded allele	Coded allele frequency	Beta	*P* value				Effect direction[c]	In gene
						GENOA study	NHS/NHS II/HPFS	PREVEND study	Combined cohort		
Calcium											
rs17216707[d]	20	52732362	T|C	0.80	10.93	1.93×10^{-1}	1.14×10^{-5}	1.08×10^{-5}	1.12×10^{-8}	+ + +	
rs3762592	2	234307549	T|C	0.62	7.85	2.58×10^{-1}	6.73×10^{-2}	7.60×10^{-7}	1.55×10^{-7}	+ + +	*DGKD*
rs11142400	9	73017959	A|G	0.34	−7.64	9.21×10^{-2}	4.50×10^{-4}	6.49×10^{-4}	7.58×10^{-7}	− − −	*KLF9*
rs9565451	13	79433941	A|G	0.42	7.43	8.00×10^{-1}	2.30×10^{-2}	3.92×10^{-6}	1.20×10^{-6}	+ + +	
rs7584554	2	234308782	T|G	0.46	6.97	2.95×10^{-1}	5.97×10^{-2}	9.98×10^{-6}	1.42×10^{-6}	+ + +	*DGKD*
rs6004867	22	26380058	T|C	0.07	−25.52	2.67×10^{-2}	1.20×10^{-5}	—	1.97×10^{-6}	− − ?	*MYO18B*
rs9814789	3	148701115	A|G	0.25	8.07	1.18×10^{-1}	1.14×10^{-1}	2.00×10^{-5}	2.27×10^{-6}	+ + +	
rs7539883	1	165118761	A|G	0.69	7.43	3.29×10^{-2}	1.95×10^{-2}	4.71×10^{-4}	3.41×10^{-6}	+ + +	
rs4131290	5	176780682	A|C	0.71	−7.57	1.85×10^{-1}	4.41×10^{-1}	2.97×10^{-6}	3.87×10^{-6}	− − −	*LMAN2*
rs9402632	6	135051813	A|G	0.61	−13.05	3.29×10^{-2}	2.12×10^{-5}	—	3.89×10^{-6}	− − ?	*LOC101928277*
rs4150258	13	103503791	T|C	0.39	6.97	4.31×10^{-1}	1.26×10^{-2}	7.85×10^{-5}	4.06×10^{-6}	+ + +	*ERCC5, BIVM-ERCC5*
rs58084056	8	128909031	A|G	0.21	15.23	8.48×10^{-1}E-02	8.42×10^{-6}	—	5.37×10^{-6}	+ + ?	*PVT1*
rs12684403	9	82423014	T|C	0.43	6.59	9.02×10^{-1}E-01	6.90×10^{-4}	2.39×10^{-4}	6.75×10^{-6}	− + +	
rs11721752	4	90270991	T|C	0.26	7.58	5.83×10^{-1}E-01	4.84×10^{-2}	2.49×10^{-5}	6.95×10^{-6}	+ + +	
rs28849216	15	69147146	A|G	0.57	13.44	6.66×10^{-1}E-04	3.15×10^{-3}	—	8.82×10^{-6}	+ + ?	
rs11012063	10	20761567	T|C	0.89	−10.23	5.67×10^{-1}E-01	4.49×10^{-2}	4.15×10^{-5}	9.62×10^{-6}	− − −	
rs153644	5	115840528	T|C	0.22	7.84	1.68×10^{-1}E-01	3.00×10^{-4}	5.19×10^{-3}	9.66×10^{-6}	+ + +	*SEMA6A*
rs11128156	3	70027374	T|C	0.67	−6.93	3.09×10^{-1}E-02	5.78×10^{-2}	5.38×10^{-4}	9.79×10^{-6}	− − −	
rs2102958	3	126419805	T|C	0.83	−8.74	5.63×10^{-1}E-01	1.10×10^{-4}	2.33×10^{-3}	9.81×10^{-6}	− − −	
Urine volume											
rs140619252	6	23235249	T|C	0.13	0.19	6.08×10^{-1}	1.70×10^{-7}	9.19×10^{-2}	1.87×10^{-7}	+ + +	
rs1877144	12	25771073	A|G	0.71	−0.13	3.83×10^{-1}	4.92×10^{-1}E-06	5.63×10^{-1}	5.45×10^{-6}	− − −	*LMNTD1*
rs62123774	2	23370512	T|C	0.93	−0.24	1.18×10^{-1}	8.31×10^{-6}	5.47×10^{-1}	9.20×10^{-6}	− − ?	*LOC101929251*
Uric acid											
rs6445664	3	54484100	A|C	0.16	−15.85	3.65×10^{-1}	1.16×10^{-1}	1.13×10^{-6}	4.41×10^{-7}	− − −	*CACNA2D3*
rs17728712	20	50064221	A|G	0.24	12.41	5.25×10^{-2}	1.45×10^{-3}	1.58×10^{-3}	3.82×10^{-6}	+ + +	*NFATC2*
rs55788337	7	68945417	T|G	0.67	10.77	1.04×10^{-1}	6.30×10^{-3}	1.09×10^{-3}	9.44×10^{-6}	+ + +	
rs10857574	10	49665226	A|G	0.49	−18.80	7.85×10^{-3}	3.40×10^{-4}	—	9.81×10^{-6}	− − ?	*ARHGAP22*

Continued on next page

TABLE 2. Continued

SNP	Chr	Position	Coded|noncoded allele	Coded allele frequency	Beta	*P* value				Effect direction[c]	In gene
						GENOA study	NHS/NHS II/HPFS	PREVEND study	Combined cohort		
Magnesium											
rs1176815	9	77552195	A|C	0.43	−5.29	5.33×10^{-3}	1.80×10^{-4}	9.13×10^{-10}	1.7×10^{-14}	− − −	
rs2278540	3	32408916	A|G	0.65	−3.65	2.94×10^{-1}	1.94×10^{-2}	2.46×10^{-5}	1.26×10^{-6}	− − −	*CMTM8*
rs112505569	12	87802066	T|C	0.84	−4.44	6.59×10^{-1}	1.80×10^{-4}	3.18×10^{-4}	1.32×10^{-6}	− − −	
rs9365555	6	163757127	A|G	0.69	5.49	1.01×10^{-1}	9.73×10^{-6}	—	5.07×10^{-6}	+ + ?	
rs35930	5	53581724	A|G	0.20	−3.84	3.25×10^{-1}	4.60×10^{-4}	1.84×10^{-3}	5.14×10^{-6}	− − −	*ARL15*
rs62156223	2	97412167	T|C	0.94	−6.63	2.11×10^{-1}	3.25×10^{-2}	1.04×10^{-4}	5.32×10^{-6}	− − −	
rs77054396[d]	7	156754975	A|G	0.94	−6.88	2.08×10^{-4}	3.59×10^{-2}	9.45×10^{-3}	8.87×10^{-6}	− − −	*NOM1*
rs114290302[d]	2	124739919	C|G	0.08	9.73	2.95×10^{-1}	1.67×10^{-6}	—	9.00×10^{-6}	+ + ?	
rs3116597	13	51086364	C|G	0.23	3.54	8.61×10^{-1}	9.75×10^{-2}	5.47×10^{-6}	9.12×10^{-6}	+ + +	*DLEU1*

[a]Chr = chromosome; GENOA = Genetic Epidemiology Network of Arteriopathy; HPFS = Health Professionals Follow-up Study; NHS = Nurses' Health Study; PREVEND = Prevention of Renal and Vascular End stage Disease; SNP = single nucleotide polymorphism.
[b]Pruned for linkage disequilibrium<0.8.
[c]Direction corresponds to the beta direction for AI for the GENOA study, NHS/NHS II/HPFS, and the PREVEND study in order. A question mark indicates that the SNP was not available for the indicated cohort.
[d]These SNPs do not pass the Cochran Q test for heterogeneity (α=0.10), resulting in observed effect sizes (or test statistics) that are heterogeneous across samples.

stone risk. Of note, the present cohorts were composed of a mixed European ancestry, and it is possible that genetic features associated with calcium excretion differ in this population vs the more homogeneous Icelandic population used in the previous study.[15] We studied 5 established large cohorts of European ancestry with a common set of available urinary traits and did not study kidney stones as a phenotype; thus, the study population contained a mixture of kidney stone formers and controls. It remains possible that the underlying mechanism of hypercalciuria contributes to kidney stone risk and that genetic factors relevant to urinary calcium excretion differ between stone formers and controls. Thus, further work is needed to define the genetic determinants of calcium excretion in the setting of kidney stone risk.

Magnesium is a known inhibitor of calcium oxalate and calcium phosphate crystallization. Furthermore, urinary magnesium concentration affects calculated calcium oxalate and calcium phosphate supersaturation.[7] Nevertheless, to date, limited clinical data specifically associate higher urinary magnesium excretion with reduced kidney stone risk[5] or suggest that supplemental magnesium reduces crystallization of calcium salts in the urine.[27] The extracellular magnesium concentration is tightly regulated by the balance between intestinal absorption and renal excretion.[28] The claudin 16-19 complex in the thick ascending limb of the nephron regulates paracellular magnesium and calcium transport, with claudin 14 functioning as a negative inhibitory protein.[14] Mutations in the transient receptor potential melastatin 6 gene (*TRPM6*), specifically expressed in the apical surface of the distal convoluted tubule and colon, are associated with profound hypomagnesemia.[29–31] Patients often present early in life with serum magnesium concentrations of 0.2 mM or lower and seizures.[32] Defects in *TRPM6* impair magnesium transport in the distal convoluted tubule and the colon, thereby reducing absorption from the colon and reabsorption in the kidney.[33] The hypocalcemia often observed is thought to result from hypomagnesemia-induced hypoparathyroidism.[28]

In the present study, we identified a consistent and strong association of a region

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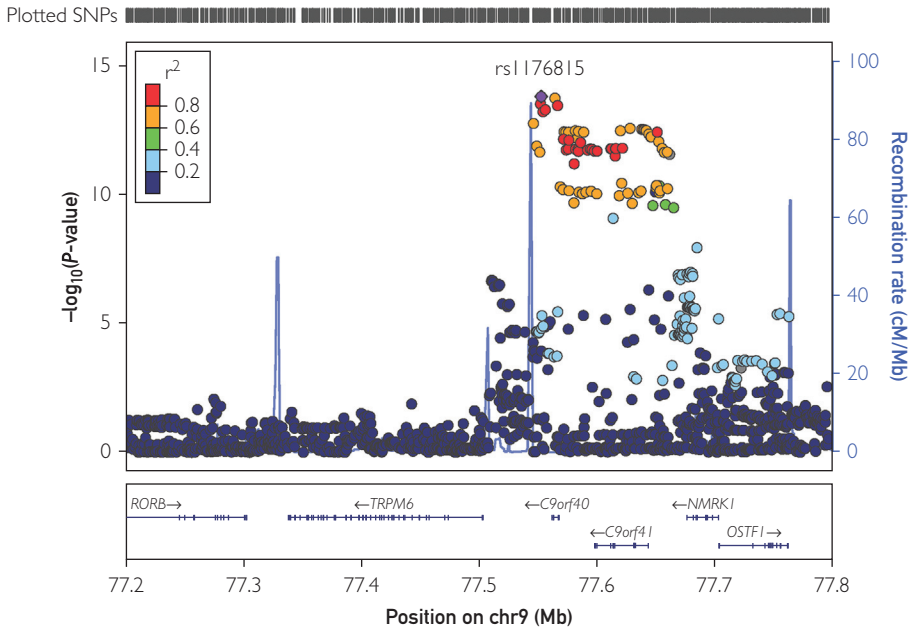

FIGURE 3. Regional plot for genome-wide significant results on chromosome 9 (chr9) for the magnesium excretion meta-analysis. The left y-axis shows −log10(*P*) from the association between single nucleotide polymorphisms (SNPs) and magnesium excretion; right y-axis, the SNP recombination rate based on the 1000 Genomes 2012 Europe reference panel (hg19); x-axis, chromosomal location and gene regions; and r^2 color code, degree of linkage disequilibrium (correlation) with the most strongly associated SNP (purple diamond).

on chromosome 9 immediately upstream of *TRPM6* that is associated with urinary magnesium excretion. Given the profound effect of *TRPM6* mutations on magnesium transport, it is possible that genetic variation influencing *TRPM6* expression or function affects magnesium metabolism. Although it is less clear whether genetic variability in *TRPM6* affects kidney stone risk, the present findings have clear significance for understanding human magnesium balance. Other genes in this region include the nicotinamide riboside kinase 1 (*NMRK1*), the osteoclast-stimulating factor 1 (*OSTF1*), and 2 predicted genes (*C9orf40* and *C9orf41*). These genes are less likely to be implicated in magnesium metabolism. The most significant SNP in this region, rs1176815, showed consistent effects across cohorts, with each copy of the *A* allele associated with a decrease of approximately 5 mg of magnesium excretion per day. The *A* allele is relatively common in the European ancestry population (approximately 43%). It is important to note, however, that there is little evidence that rs1176815 influences gene expression of *TRPM6* in any tissue type examined to date. Stronger evidence exists for regulation of other nearby genes by rs1176815, including *OSTF1* and *C9orf40*.

Gout and calcium urolithiasis often associate with each other.[34,35] Although genetic variability in the renal uric acid transporter *SLC2A9* is associated with serum uric acid concentration, it was not associated with urinary uric acid excretion.[36] This null association was expected because this gene does not influence uric acid generation, which would influence uric acid excretion. *ABCG2*, also known as *BCRP*, encodes a high-capacity urate exporter, the dysfunction of which raises gout/hyperuricemia risk due to reduced intestinal secretion and "overload" hyperuricemia.[37] An older literature describes an entity termed *hyperuricosuric calcium*

urolithiasis,[38] and a randomized controlled trial supports the utility of lowering uric acid excretion with allopurinol as a treatment for these patients.[39] However, a large study that adjusted for other urinary factors found that uric acid excretion is not an independent predictor of overall kidney stone risk.[5] Nonetheless, higher urine uric acid excretion is a known risk factor for uric acid nephrolithiasis. Although a previous study suggested a heritable component to urinary uric acid excretion,[13] in the present analysis we do not find any genetic regions that associate with uric acid excretion. It is not clear why, but lack of diet data across all cohorts to adjust for in this meta-analysis is one limitation that could have limited our power to detect a genetic component.

Twenty-four—hour urine volume is also a key determinant of urinary supersaturation. A recent meta-analysis confirms that higher urine volumes are associated with lower kidney stone risk.[9] In addition, there are recent studies suggesting, somewhat surprisingly, that urine volume has a heritable component.[13] One possibility is that the heritability of urine volume relates to thirst. However, we did not find any specific genetic regions that associated with urine volume in the present study. It is possible that more detailed phenotypes (eg, urine and serum osmolality) might be needed.

The initial reason for this study was to examine genetic contributions to urine phenotypes relevant to urinary stone disease. The present findings suggest that in a European population, common genetic variants might not contribute to a great enough extent that we were able to detect them in the sample size. Thus, diet and environment (including medications) may be at least equally as important and may have blurred our ability to detect associations. There is also a heritable component to diet,[13] and this might account for some of the heritability of stone disease. There may also be several genes in this European population contributing to any given urinary trait, and thus we may not have been powered to detect these. It is also possible that a population more enriched with stone formers may have had better power to detect the relevant genetic risks. Finally, although low magnesium excretion is not a clear kidney stone risk factor, the strong genetic association that was identified is of at least physiologic interest and might have relevance for other clinical scenarios (eg, risk of hypomagnesemia on diuretics).

The present study has certain weaknesses. Quantitative data for urinary risk factors was available only for a subset of solutes in all the cohorts. In addition, despite pooling data from multiple cohorts, the total number of participants with the common data set numbered just 6462. This might explain, in part, the lack of findings for genetic associations with important traits such as calcium excretion. Not all the cohorts had a quantitative dietary history, so this could not be accounted for in the analysis. Participants could have been taking medications that can influence urinary excretions of the measured analytes (eg, thiazides, allopurinol). The possible effects of variable bone turnover between subjects or mild CKD were not accounted for in the analysis. Nevertheless, this remains one of the larger studies to have quantitative 24-hour urinary data for important traits associated with kidney stone risk.

CONCLUSION

A meta-analysis of data from 5 relatively large cohorts identified potential candidate genes that influence urinary magnesium excretion (*TRPM6* on chromosome 9q21.13) and calcium excretion (*CYP24A1* on chromosome band 20q13.2). We did not identify genetic associations with urinary uric acid excretion or urine volume. Further studies are necessary to understand the genetics of urinary risk factors for kidney stones and, indeed, kidney stones themselves.

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Drs Ware and Smith contributed equally to this work.

SUPPLEMENTAL ONLINE MATERIAL

Supplemental material can be found online at http://www.mcpiqojournal.org. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

Abbreviations and Acronyms: **BMI** = body mass index; **CKD** = chronic kidney disease; **FGF23** = fibroblast growth factor 23; **GDUL** = Genetic Determinants of Urinary Lithogenicity; **GENOA** = Genetic Epidemiology Network of Arteriopathy; **GWAS** = Genome-wide association study; **HPFS** = Health Professionals Follow-up Study; **NHS** = Nurses' Health Study; **PREVEND** = Prevention of Renal and Vascular End-stage Disease; **QQ** = quantile-quantile; **SNP** = single nucleotide polymorphism

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