

Heritability of Urinary Traits That Contribute to Nephrolithiasis

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Abstract

Background and objectives Kidney stones and their risk factors aggregate in families, yet few studies have systematically estimated heritabilities and genetic correlations of the numerous urinary traits associated with risk of kidney stones.

Design, setting, participants, & measurements Twenty-four-hour urine samples were collected from the Genetic Epidemiology Network of Arteriopathy cohort of families in Rochester, Minnesota, to measure urinary determinants of supersaturation. Diet was assessed using the Viocare food frequency questionnaire. Heritabilities and genetic correlations among the urinary traits were estimated using variance components methods.

Results Samples were available from 811 individuals (344 men, 467 women; mean age 66 ± 9 years). Age, sex, and weight were significantly correlated with the majority of urinary traits. Many urine excretions (calcium, magnesium, citrate excretion) had strong evidence for heritability ($P < 0.01$) both before and after adjusting for the identified covariates. Among significantly heritable urinary traits, genetic factors explained 20%–36% of inter-individual variation after adjustment for covariates. Urinary calcium excretion was significantly genetically correlated with urinary magnesium and with urinary citrate excretion ($P < 0.05$). Although eGFR influenced many urinary traits, controlling for eGFR did not greatly affect estimated heritabilities.

Conclusions Evidence from this cohort suggests a strong heritable component to many urinary nephrolithiasis risk factors. Further study of genetic influences on urinary traits relevant for kidney stone pathogenesis is warranted.

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Introduction

Kidney stones are common, affecting up to 10% of individuals over their lifetime (1). Human urine is often supersaturated with respect to stone-forming salts, which is a key contributing factor in kidney stone pathogenesis (2). Although evidence suggests that the risk of kidney stones aggregates in families, the heritability of the many key urinary measurements used to assess kidney stone risk has not been estimated. Greater understanding of this potential heritability could provide a rationale for identifying underlying genetic factors, which in turn could suggest new pathogenic and therapeutic targets.

The primary objective of this study was to estimate the heritabilities of urinary nephrolithiasis risk factors in a population not selected for stone disease. Our secondary objective was to determine whether heritable urinary risk factors are influenced by the same genetic factors by estimating the genetic correlations between them, implying that a single gene might influence stone risk *via* multiple pathways. We took advantage of sibships in the Genetic Epidemiology Network of Arteriopathy (GENOA) cohort from Rochester, Minnesota, for whom detailed phenotypes and family structures are available (3,4). Adult sibships are

ideal for studying the contributions of genes to urinary nephrolithiasis risk factors because their dietary patterns have stabilized and siblings usually live in separate households, thus minimizing a shared environment. Heritability provides an overall estimate of additive genetic influences (reflecting the genome-wide contribution of allelic variation shared by siblings) on trait variation. Significant genetic correlations between traits reflect the extent to which common underlying genetic factors affect both traits.

Materials and Methods

This study was approved by the Mayo Clinic Institutional Review Board.

GENOA Cohort

GENOA, a member of the Family Blood Pressure Program, recruited non-Hispanic white hypertensive sibships from Rochester, Minnesota, for linkage and association studies to investigate the genetic underpinnings of hypertension in phase I (1996–2001) (4). In phase II (2000–2004), 1241 Rochester participants were successfully re-recruited to measure potential target organ damage as a result of hypertension (5).

The Genetic Determinants of Urinary Lithogenicity (GDUL) study (2006–2012) is an ancillary study of the phase III GENOA Genetics of Chronic Kidney Disease study conducted in GENOA participants (5). Participants in the Rochester GENOA cohort were invited to participate in this study, which consisted of 24-hour urine collections and a diet food frequency questionnaire (FFQ; Viocare Technologies, Princeton, NJ) completed at 1–3 visits between the CKD and GDUL studies. Participants were excluded from this study if they were in end stage renal failure (stage 5 CKD).

Study Visit

After they provided informed consent, participants completed one or preferably two 24-hour urine collections (6,7) and the FFQ. A total of 333, 295, and 183 participants had a total of one, two, or three urine collections, respectively. For individuals with two or three urine collections, values used were averaged. The mean time between the earliest (CKD) and last (GDUL) urine collections was 1.73 years (range, 0.9–3.6 years). The average time between the two GDUL collections was 22 days. Intraclass correlation coefficients (ICCs) for urine factors across collections revealed that the majority of urine measures were relatively stable across time. Of the urine factors, chloride had the lowest ICC (0.41) and calcium had the highest ICC (0.73).

Participants also completed a detailed kidney stone questionnaire, and data from the GENOA CKD study questionnaire were also available.

Urine Collection

Urine was collected with toluene as a preservative. Twenty-four-hour urinary concentrations of oxalate, calcium and other determinants of supersaturation were measured in the Mayo Clinic Renal Testing Laboratory. Supersaturation was calculated using the EQUIL2 program (8).

Total Urine Protein Isolation

An aliquot of a 24-hour urine collection was spun at 3600 rpm, 4°C, for 10 minutes to remove cells and debris and stored at –80°C until use. At the time of inhibition assays, the sample was thawed, spun at 1400 rpm at 4°C for 5 minutes to further remove cells and debris, and rinsed twice with a Vivaspin concentrator (5000 molecular weight cut-off; Sartorius, Goettingen, Germany) using PBS containing Roche Complete protease inhibitor (one tablet per 1000 ml). The protein concentration in the final concentrate (approximately 200 μ l) was measured using a bicinchoninic acid assay kit (Pierce, Rockford, IL) and PBS added to achieve a final concentration of 1 mg/ml.

Crystal Growth Inhibition Assay

Calcium oxalate (CaOx) monohydrate crystal growth inhibition was measured using a modification of the seeded crystal growth assay described by Nakagawa and colleagues (9). The upper limit of metastability (ULM) of CaOx and calcium phosphate (CaP) in human urine was measured using a modification of the method of Asplin and colleagues (10). Sequential aliquots of calcium chloride (CaP ULM) or 5 μ l oxalic acid solution (CaOx ULM) were added to urine in a temperature-controlled cuvette

under constant stirring, and the point of precipitation was assessed at a wavelength of 620 nm using a Cary Bio 50 UV-Visible spectrophotometer (Agilent Technologies, Inc., Santa Clara, CA). Supersaturation for CaOx and CaP in the native urine sample and at the final concentration at which the threshold absorbance was reached or exceeded (ULM) were calculated using EQUIL2 (8). To reduce within-patient variability, values for replicate measures were averaged. Crystallization measures were only performed at the two GDUL visits.

Statistical Analyses

Data management and statistical analyses were conducted in SAS software (version 9.3; SAS Institute, Inc., Cary, NC) and R software (version 12.1.2; R Core Development Team, Vienna, Austria) (11). Most urine and dietary measures appeared to have relatively normal distributions; thus, no variable transformations were applied. ULM CaP and ULM-supersaturation CaP were not normally distributed and were thus transformed by taking the natural logarithm of the variable plus one before analyses; values that were <0 were also removed before analysis ($n=28$ for ULM-supersaturation CaP). Values that were ≥ 4 SDs from the mean of any urine or diet measure were removed. We conducted *t* tests for the urine and diet measures to test whether there were significant differences between men and women.

Correlation and Association Testing

Correlations were estimated using Pearson's product moment correlation on the full GENOA sample ($N=811$). Because we tested for correlation in many pairs of traits, we used the Benjamini–Hochberg method to account for multiple testing in the correlation analysis (12). This technique controls the proportion of falsely rejected null hypotheses (false discovery rate) by adjusting the *P* values for each test based on the number of tests conducted as well as the dependency structure between the test statistics.

Next, we conducted linear mixed models that included age, sex, height, and weight as predictor variables to explore the relationships between these variables and each urine and diet measure. Linear mixed-effects (LME) modeling (13) with family as a random intercept was used to account for the sibship structure among GENOA participants while retaining a valid type I error rate. LME correlated data will have less variability than uncorrelated data and statistical tests of the regression coefficients will have inflated *P* values, resulting in a higher type I error rate. Removing the variability due to family structure adjusts the degrees of freedom so that the SEMs of the regression coefficients represents the number of independent observations, thus stabilizing the type I error rate. This technique is optimized under a balanced design (when the number of siblings per sibship is the same across all sibships). In our study, 47% of the sibships were composed of singletons, whereas 53% of the sibships had at least two siblings. R^2 values for the LME models were calculated based on the likelihood ratio (14). The modeling was repeated by including the same predictor variables plus eGFR, calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation (15).

Biometrical Genetic Modeling

We used variance components modeling to estimate the heritability of urinary factors, both before and after accounting for covariates (age, sex, height, and weight). After accounting for phenotypic variation due to the covariates, the phenotypic covariance between sibling pairs was partitioned into additive genetic covariance and variance not explained by additive genetic effects (error covariance), as follows:

$$\Omega = 2\Phi\sigma_g^2 + I\sigma_e^2,$$

where σ_g^2 represents the genetic variance due to additive genetic factors and σ_e^2 is the error variance. The kinship matrix, Φ , represents the Mendelian expectation that sibling pairs, on average, share one half of their genetic variation. Sequential oligogenic linkage analysis routines (SOLAR) modeling (16) was used to implement the variance component modeling based on maximum likelihood estimation. Heritability ($h^2 = \sigma_g^2 / \sigma_p^2$), the proportion of total phenotypic variance that is attributable to genetic variance, was tested for significance by comparing the log-likelihood of the model in which heritability is estimated to that of the model in which heritability is fixed to 0. All GENOA participants ($N=811$) were included in the SOLAR modeling. Although the 208 singletons are not used by SOLAR to estimate the genetic contribution to trait variation, they are used for estimation of overall trait variation. Because we tested the heritabilities of several traits, we used the Benjamini–Hochberg method to account for multiple testing in the heritability analysis (12). We performed sensitivity analysis by reestimating heritability for urinary traits that demonstrated significant heritability after adjustment for age, sex, height, and weight. For these traits, we additionally adjusted for dietary factors that were significant predictors of the urinary trait. Finally, we estimated heritability for these traits in the subset of the sample that had no history of kidney stones ($n=585$).

SOLAR was also used to perform bivariate analysis for pairs of traits that had significant heritabilities after accounting for age, sex, height, and weight (h^2 ; $P<0.05$). In this bivariate modeling framework, the phenotypic covariance between two traits is decomposed into genetic correlation due to additive genetic effects influencing both traits and correlation due to environmental effects influencing both traits, according to the following model:

$$\Omega_{12} = 2\Phi\rho_g\sigma_{g1}\sigma_{g2} + \rho_e\sigma_{e1}\sigma_{e2}$$

where 1 and 2 are the two traits of interest, ρ_g is the additive genetic correlation between the traits and ρ_e is the correlation due to unmeasured environmental effects. The genetic correlation provides an estimate of the proportion of genetic effects shared between the two traits. SOLAR estimates phenotypic correlation using family relationships among the participants. The formula for calculating total phenotypic correlation is as follows:

$$\rho_p = \rho_g\sqrt{h_1^2h_2^2} + \rho_e\sqrt{(1-h_1^2)(1-h_2^2)}$$

where ρ_p is the phenotypic correlation between traits 1 and 2, h_1^2 is the heritability of trait 1, and h_2^2 is the heritability of

trait 2. The genetic and environmental correlations between the traits estimated in SOLAR, ρ_g and ρ_e , were tested for significance by comparing the log-likelihood of the model in which the parameter of interest is estimated to the log-likelihood of the model in which the parameter is fixed to 0.

Results

A total of 811 individuals from 446 sibships participated in this study. The sibship structure of the sample was as follows: 208 singletons, 164 sibpairs, 49 sibships with 3 siblings, and 25 sibships with 4 or more siblings. The mean age was 66 ± 9 years and 57.6% of participants were women. Most of the 24-hour urine traits and diet measures summarized in Table 1 differed significantly between men and women. Only 619 of the 811 participants (76%) completed the FFQ. The sex distribution did not differ substantially between those who did or did not provide diet information, although those who completed the questionnaire were slightly younger (mean age 65.3 versus 66.9 years; $P=0.04$), and certain urine analytes subtly differed in a direction consistent with the overall effect of age on specific factors (e.g., urine calcium of 160 mg/d [completed questionnaire] versus 141 mg/d [did not complete questionnaire]; $P<0.001$).

Many urinary traits and dietary measures were also influenced by additional demographic factors, including age and weight (Supplemental Table 1). Those participants with a history of kidney stones ($n=88$; 13.1%) tended to have a higher body weight, urine calcium excretion, and lower dietary calcium intake (Supplemental Table 2). Several individual dietary components, including sodium, total protein, and net alkali absorption (calculated by the method of Oh (Urine(Na+K+Ca+Mg)–(Cl+1.8 Pi)) (17), also influenced many urinary traits (Table 2). However, dietary fructose (18) did not significantly correlate with key urinary measures including urine pH, oxalate, or uric acid, but was weakly correlated with calcium ($r=0.10$; $P<0.05$) and citrate ($r=0.08$; $P<0.05$). After correction for multiple testing, 39 of the 42 significant correlations ($P<0.05$) remained significant. Many urinary traits correlated with each other (e.g., calcium with magnesium, and calcium with citrate) (Supplemental Table 3).

Estimated heritabilities are listed in Table 3. Heritabilities were statistically significantly different from zero and moderate in magnitude for urinary excretion of calcium ($h^2=0.41$; SEM 0.10), magnesium ($h^2=0.34$; SEM, 0.09), pH ($h^2=0.35$; SEM, 0.10), citrate ($h^2=0.39$; SEM, 0.09), and volume ($h^2=0.30$; SEM, 0.11) (all $P<0.01$), and lower in magnitude for urinary sulfate excretion ($h^2=0.15$; SEM, 0.08) ($P<0.05$). Covariates accounted for 8%–32% of the variance in these urinary measures; however, after adjustment, heritabilities for six of seven quantitative urinary traits remained statistically significant. In addition, heritabilities remained significant after Benjamini–Hochberg adjustment for multiple testing (Supplemental Table 4). Heritabilities did not differ between the entire GENOA cohort and the subset of nonstone formers (Supplemental Table 5). Estimates of the heritabilities of urinary crystallization measures were not significantly different from 0 after adjustment for age, sex, height, and weight.

To assess the effect of diet, analyses were repeated for those urine factors that had a significant heritability ($P<0.05$)

Trait	Total (N=811)		Men (n=344; 42.4%)		Women (n=467; 57.6%)		P Value ^a
	n	Mean±SD	n	Mean±SD	n	Mean±SD	
Demographics							
Age, yr	811	66±9	344	67±9	467	65±9	0.004
Height, cm	811	168±10	344	176±7	467	162±6	<0.001
Weight, kg	811	88±19	344	97±18	467	81±18	<0.001
24-h urine measures							
Calcium, mg/d	808	156±88	343	162±95	465	151±83	0.09
Magnesium, mg/d	811	108±41	344	120±45	467	99±36	<0.001
pH	811	6.2±0.5	344	6.1±0.5	467	6.3±0.6	<0.001
Osmolality, mOsm/kg	811	515±193	344	592±193	467	458±172	<0.001
Sodium, mEq/d	807	139±58	341	167±60	466	119±48	<0.001
Potassium, mEq/d	811	59±23	344	69±23	467	51±19	<0.001
Chloride, mEq/d	807	130±55	341	156±57	466	110±45	<0.001
Phosphorus, mg/d	809	678±290	342	836±292	467	563±229	<0.001
Sulfate, mmol/d	811	19±7	344	22±8	467	16±6	<0.001
Citrate, mg/d	806	567±324	344	624±351	462	524±296	<0.001
Oxalate, mmol/d	805	0.30±0.10	342	0.34±0.10	463	0.27±0.09	<0.001
Uric acid, mg/d	809	443±172	342	523±183	467	384±137	<0.001
Creatinine, mg/d	810	1133±425	343	1453±388	467	897±268	<0.001
Net alkali absorption, mEq/d	809	63±24	343	72±25	466	56±21	<0.001
Urine volume, ml/d	811	1958±700	344	1973±727	467	1947±680	0.59
ULM CaOx, D.G.	698	24.2±8.5	292	23.7±8.6	406	24.7±8.4	0.14
ULM-SS CaOx, D.G.	699	21.3±8.1	292	20.8±8.0	407	21.8±8.1	0.10
ULM CaP, D.G.	696	3.9 (2.0, 6.0)	292	4.2 (2.5, 6.2)	404	3.5 (1.8, 6.0)	0.03
ULM-SS CaP, D.G.	673	3.2 (1.3, 5.4)	285	3.5 (1.6, 5.6)	388	2.8 (1.1, 5.3)	0.05
Crystal growth inhibitory activity, %	572	0.40±0.09	240	0.38±0.09	332	0.42±0.09	<0.001
Dietary measures							
Total protein, g/d	619	81±35	261	89±38	358	76±31	<0.001
Animal protein, g/d	619	53±26	261	60±30	358	49±22	<0.001
Calcium, mg/d	619	1073±560	261	1081±596	358	1068±531	0.74
Oxalate, mg/d	610	219±130	261	218±138	349	221±123	0.85
Fructose, g/d	615	21±12	259	21±12	356	21±12	0.69
Sucrose, g/d	616	38±22	259	37±23	357	39±22	0.43

Data are presented as n, mean±SD, or median (1st quartile, 3rd quartile). ULM, upper limit of metastability; CaOx, calcium oxalate; D.G., delta Gibbs free energy term; SS, supersaturation; CaP, calcium phosphate.

^aP value from a t test of means between men and women.

in adjusted models. Incorporating those dietary factors that correlated with urinary traits (Table 2) into the model had little effect on the estimated heritabilities (Table 4). Many urine analytes also correlated with eGFR calculated using the CKD-EPI creatinine equation (15), including calcium, magnesium, and citrate (results not shown). However, when heritabilities were re-estimated controlling for eGFR, age, sex, height, and weight they did not appreciably change (Supplemental Table 6).

Genetic correlations were examined for urinary traits with highly significant heritabilities after accounting for covariates ($P<0.01$). Moderately positive genetic correlations were observed between urinary magnesium and calcium excretions ($\rho_g=0.56$; $P<0.05$) and between urinary magnesium and citrate excretions ($\rho_g=0.44$; $P<0.05$). Positive environmental correlations were observed among four urinary traits: calcium, magnesium, pH, and citrate ($\rho_e=0.18$ – 0.48 ; $P<0.05$) (Table 5).

Discussion

This study estimated heritabilities of a large panel of urinary measures that are commonly used to assess risk of kidney stones, and demonstrated that many but not all conventional measures of urinary lithogenicity have statistically significant and substantial heritable components. Urinary calcium and magnesium excretions were most notable for strong heritability, whereas urine oxalate excretion was most notable for lack of heritability. These results suggest that efforts to understand the genetics underlying stone risk factors should focus on those urinary nephrolithiasis risk factors with greatest heritability. Such efforts could help elucidate kidney stone pathogenesis and reveal new targets for prevention efforts.

Kidney stones develop approximately 3-fold more frequently in individuals with a positive family history, and a family history of nephrolithiasis has been reported in 4%–12% of healthy controls compared with 16%–37% of

Table 2. Correlation matrix of dietary measures and key 24-hour urine measures

Trait (24-h Urine Measures)	Net Alkali Absorption	Dietary Total Protein	Dietary Animal Protein	Dietary Calcium	Dietary Oxalate	Dietary Fructose	Dietary Sucrose
Calcium	0.28 ^a	0.20 ^a	0.20 ^a	0.17 ^a	0.07	0.10 ^b	0.07
Magnesium	0.48 ^a	0.20 ^a	0.17 ^a	0.13 ^a	0.11 ^c	0.13 ^c	0.05
pH	0.37 ^a	−0.05	−0.09 ^{b,d}	0.06	0.05	0.03	0.13 ^c
Osmolality	0.02	0.15 ^a	0.21 ^a	0.04	−0.04	0.04	−0.09 ^{b,d}
Sodium	0.59 ^a	0.15 ^a	0.17 ^a	−0.02	−0.01	−0.07	−0.15 ^a
Sulfate	0.45 ^a	0.36 ^a	0.39 ^a	0.19 ^a	0.12 ^c	0.06	−0.06
Citrate	0.50 ^a	0.12 ^c	0.13 ^c	0.07	0.06	0.08 ^{b,d}	0.03
Oxalate	0.39 ^a	0.13 ^c	0.09 ^b	−0.03	0.10 ^b	0.04	−0.01
Uric acid	0.60 ^a	0.19 ^a	0.19 ^a	0.03	0.07	0.02	−0.03
Volume	0.35 ^a	0.14 ^a	0.09 ^b	0.10 ^b	0.14 ^a	0.01	0.06

^a $P < 0.001$.
^b $0.01 < P < 0.05$.
^c $0.001 < P < 0.01$.
^dNot significant at $P < 0.05$ after adjustment for multiple testing.

Table 3. Heritabilities of 24-hour urine measures

Trait (24-h Urine Measures)	Unadjusted		Proportion of Variance of Measure Explained by Covariates (%) ^a	Adjusted for Age, Sex, Height, and Weight	
	h^2	P Value		h^2	P Value
Calcium, mg	0.41 ^b	<0.001	15.9	0.25 ^c	0.003
Magnesium, mg	0.34 ^b	<0.001	14.3	0.25 ^b	<0.001
pH	0.35 ^b	<0.001	8.5	0.27 ^c	0.001
Osmolality, mOsm/kg	0.26 ^c	0.002	21.1	0.20 ^d	0.02
Sodium, mEq	0	0.50	24.0	0.07	0.23
Potassium, mEq	0	0.50	17.6	0.005	0.47
Chloride, mEq	0	0.50	25.1	0.007	0.47
Phosphorus, mg	0	0.50	26.1	0.08	0.18
Sulfate, mmol	0.15 ^d	0.03	32.1	0.04	0.34
Citrate, mg	0.39 ^b	<0.001	8.3	0.36 ^b	<0.001
Oxalate, mmol	0.11	0.10	17.3	0.12	0.06
Uric acid, mg	0.04	0.33	21.9	0.08	0.15
Creatinine, mg	0	0.50	50.8	0	0.50
Net alkali absorption, mEq	0	0.50	11.7	0	0.50
Urine volume, ml	0.30 ^c	0.002	2.0	0.24 ^d	0.01
ULM CaOx, D.G.	0.18 ^d	0.04	1.4	0.14	0.09
ULM-SS CaOx, D.G.	0.22 ^d	0.02	3.2	0.14	0.10
ln ULM CaP, D.G.	0.68 ^b	<0.001	29.2	0.16	0.08
ln ULM-SS CaP, D.G.	0.63 ^b	<0.001	28.4	0.12	0.15
Crystal growth inhibitory activity, %	0.29 ^d	0.02	11.2	0.18	0.11

Adjusted models included age, sex, height, and weight.
^aPercent of variance of the trait explained by adjustment variables.
^b $P < 0.001$.
^c $0.001 < P < 0.01$.
^d $0.01 < P < 0.05$.

affected individuals (19–22). Two published estimates of the heritability (h^2) for stone disease are $63\% \pm 14\%$ and $46\% \pm 9\%$ (21,22). Indeed, the familial clustering index, one epidemiologic indicator of disease heritability, is greater for nephrolithiasis (2.5–4) than for diabetes mellitus (2.0) or hypertension (2.0) (23). A study of isolated

Croatian island groups provided additional evidence of a strong genetic component due to differences in stone prevalence between low (1.5%), moderate (2.3%), and high (5.4%) inbreeding villages (24).

Hypercalciuria is the best established risk factor for calcium urolithiasis, present in up to 50% of cases (25,26).

Table 4. Heritabilities of 24-hour urine measures adjusted for diet

Trait (24-h Urine Measures)	h^2 Unadjusted	h^2 Unadjusted <i>P</i> Value	h^2 Adjusted for Age, Sex, Height, and Weight	h^2 Adjusted for Age, Sex, Height, Weight, and Dietary Covariates ^a
Calcium, mg	0.41 ^b	<0.001	0.25 ^c	0.27 ^d
Magnesium, mg	0.34 ^b	<0.001	0.25 ^b	0.27 ^c
pH	0.35 ^b	<0.001	0.27 ^c	0.30 ^c
Citrate, mg	0.39 ^b	<0.001	0.36 ^b	0.32 ^c
Osmolality, mOsm/kg	0.26 ^c	0.002	0.20 ^d	0.26 ^d
Urine volume, ml	0.30 ^c	0.002	0.24 ^d	0.35 ^c

^aDietary covariates included for each outcome measure were based on the correlations between the urinary measures and the dietary measures (Table 2). Dietary covariates are as follows: for urinary calcium, dietary protein, animal protein, calcium, and fructose; for urinary magnesium, dietary protein, animal protein, calcium, oxalate, and fructose; for pH, animal protein and sucrose; for citrate, dietary protein, animal protein, and fructose; for osmolality, dietary protein, animal protein, and sucrose; for urine volume, dietary protein, animal protein, calcium, and oxalate.

^b $P < 0.001$.

^c $0.001 < P < 0.01$.

^d $0.01 < P < 0.05$.

Table 5. Genetic and environmental correlations among pairs of urine traits with highly significant heritabilities

Trait (24-h Urine Measures)	Calcium, mg	Magnesium, mg	pH	Citrate, mg
Calcium, mg	0.25 ^a	0.38 ^{b,c}	0.22 ^{b,d}	0.48 ^{b,c}
Magnesium, mg	0.56 ^{d,e}	0.25 ^a	0.18 ^{b,d}	0.19 ^{b,d}
pH	-0.20 ^e	0.08 ^e	0.27 ^a	0.37 ^{b,c}
Citrate, mg	0.35 ^e	0.44 ^{d,e}	-0.35 ^e	0.36 ^a

All biometric models included age, sex, height, and weight.

^aHeritabilities from univariate polygenic analysis (h^2).

^bEnvironmental correlations (ρ_e).

^c $P < 0.001$.

^d $0.01 < P < 0.05$.

^eGenetic correlations (ρ_g).

The incidence of hypercalciuria in first-degree relatives of hypercalciuric stone formers was 43%, with a suggested autosomal dominant pattern of inheritance (27). Overall, the heritability of urinary calcium excretion has been estimated at approximately 40% (28). Data from the male Health Professionals Follow-Up Study (29) and a French Canadian nephrolithiasis cohort (30) also strongly support hypercalciuria as a heritable stone risk factor. Among the French Canadian kidney stone families, heritability attributed to a single major gene for calcium excretion was 0.58 (31). Data from other populations suggest that hypercalciuria is a polygenic disorder (32), but the exact molecular defects contributing to it remain incompletely defined. In a smaller twin study (12 sets), heritability was relatively high for calcium, but was also ≥ 0.90 for oxalate, uric acid, and citrate (33). Other family studies support a genetic contribution to urinary oxalate (34), citrate (35), and uric acid (20) excretion, albeit of a lesser magnitude.

In this study, adjusted models found evidence for significant heritability of citrate (0.36) and calcium (0.25), but not for oxalate or uric acid. The estimated heritabilities were much less than those in the twin study (33) or in the French Canadian study (31). However, for the first time,

our study also suggested a heritable component for urinary magnesium excretion (0.34). Indeed, the thick ascending limb is a site of robust calcium and magnesium reabsorption (36), and a previous genome-wide association study implicated *CLDN14* in kidney stone risk and reduced bone density (although urinary calcium excretion data were not available) (37). To the best of our knowledge, this is also the first study to suggest a genetic component for urinary pH regulation in a sampling of the population without a systemic acid base disorder (*e.g.*, renal tubular acidosis). Importantly, both extremes of urinary pH increased propensity for kidney stones, but of different types (*i.e.*, low urinary pH is associated with uric acid stones, whereas high urinary pH is associated with calcium phosphate stones). In addition, we detected significant heritability for urinary osmolality and volume, which could implicate genetic regulation of thirst. This may not be entirely surprising, because low urinary volume has been a consistent risk factor for stone disease in many studies over the years (38).

Evidence suggests that urinary macromolecular crystallization inhibitors might be defective in certain stone formers, thereby explaining their disease (39–41). We found

some evidence for heritability of the two indices of urinary inhibition we studied in this cohort, a seeded CaOx crystal growth assay and the ULM. However, the heritability was not present in models after adjustment for age, sex, and weight. Although it is possible that other indices of crystal growth inhibition might have stronger heritable components, the role of inhibitors as a genetically influenced risk for stone disease remains unclear.

The sibship structure of our sample also allowed us to mathematically estimate the portion of the correlation between urinary traits that was a result of apparent genetic effects, with the remainder assumed to be environmental (Table 4). There was moderate genetic correlation between urinary calcium and magnesium excretion ($\rho_g=0.56$; $P=0.03$). Genetic variation of renal proteins important for the reabsorption of both divalent cations in the thick ascending limb may be logical candidates to implicate (36). Environmental correlations between urinary calcium, magnesium, pH, and citrate (ρ_e from 0.18 to 0.48; P values from 0.03 to 1.5×10^{-6}) may represent the joint effect of dietary patterns (e.g., low vegetable/fruit intake with high meat intake).

Our study also has potential weaknesses. Human epidemiologic studies have demonstrated an association between hypertension and kidney stones (42–44). Moreover hypercalciuria, a common risk factor for nephrolithiasis, has also been associated with high BP in humans (45–47), implicating altered calcium metabolism as a common feature. Because GENOA cohort members were selected on the basis of hypertensive sibships, their stone risk may be higher and they may also be enriched for nephrolithiasis risk factors such as the ones we studied. In addition, the participants were limited to Caucasians and of a relatively older age when studied. Thus, findings may not be directly transferable to the general population.

In conclusion, evidence from this relatively large cohort suggests a strong heritable component to many urinary traits that impart nephrolithiasis risk. This study supports an underlying genetic component to stone disease that could act *via* physiologic control of urinary composition.

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Disclosures

None.

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