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A Twin Study of Genetic Influences on Nephrolithiasis in Women and Men

David S. Goldfarb[1], Ally R. Avery[2], Lada Beara-Lasic[1], Glen E. Duncan[2,3] and Jack Goldberg[4]

[1]Nephrology Division, Department of Medicine, NYU Langone Health, and NYU School of Medicine, New York, New York, USA; [2]Washington State Twin Registry, Washington State University, Spokane, Washington, USA; [3]Elson S. Floyd College of Medicine, Department of Nutrition and Exercise Physiology, Washington State University, Spokane, Washington, USA; and [4]Department of Epidemiology, University of Washington, Seattle, Washington, USA

Background: Nephrolithiasis is a complex phenotype influenced by both genetic and environmental factors. Previously we found a genetic component to stone disease using a sample of male twin pairs. We now report on the genetic contribution to stones in a sample of female and male twin pairs.

Methods: We conducted a classic twin study of kidney stones using the Washington State Twin Registry. Data were collected by questionnaire to obtain self-reported history of kidney stones. Univariate structural equation modeling was used to determine the relative contributions of additive genetics, common environment, and unique environment.

Results: There were 7053 same-sex pairs with kidney stone data. The mean age of the sample was 39 years, similar in women and men. The prevalence of stones was 4.9% of women and 6.2% of men. We found significant contributions from genetics and the unique environment ($P < 0.05$ for both) for the risk for stone disease in women and men. There was no significant contribution of the common environment for either sex. After adjusting for age, heritability was 46% (95% confidence interval 0.36–0.56) in women and 57% (0.46–0.68) in men, which was significantly different ($P < 0.05$).

Conclusions: Nephrolithiasis in women has a heritable component less than that we again demonstrate in men. This finding may in part explain why more stone formers are men than women. Women twins demonstrated a greater effect of the unique environment on stone prevalence. The specific environmental risk factors that account for this effect are not currently known.

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Nephrolithiasis is a complex phenotype influenced by both genetic and environmental factors.[1,2] Previously we found a significant genetic component to stone disease using a middle-aged sample of male twin pairs. In that study of the Vietnam Era Twin (VET) Registry, the proband concordance prevalence in monozygotic (MZ) twins (32.4%) was significantly greater than that in dizygotic (DZ) twins (17.3%) ($P < 0.001$), consistent with an important genetic influence.[3] The heritability of the risk for stones was estimated to be 56%. That study is one of the strongest indicators that nephrolithiasis is a heritable trait in the general population, although the genetic basis for most kidney stones, most of which are composed of insoluble calcium salts, remains unknown. Other studies, also lacking stone composition, have demonstrated higher rates of stones occurring among first-degree relatives of previous stone formers.[4,5] Well-known genetic diseases with autosomal recessive inheritance, such as cystinuria and primary hyperoxaluria, or with x-linked recessive inheritance, such as Dent disease, are too rare to account for these findings.

Like many epidemiologic studies, our prior twin study lacked stone composition, and we assumed that most stones affecting participants were composed of calcium salts. The most common urinary abnormality identified in 24-hour urine collections from calcium stone formers is higher urine calcium excretion. Some evidence demonstrates that greater degrees of calciuria are heritable.[6] Analysis of candidate genes for higher urinary calcium excretion, and genome-wide association studies of stone formers, have identified some potential genetic contributors but have yet to elucidate genotypes responsible for the relatively high

Correspondence: David S. Goldfarb, New York University School of Medicine, Nephrology Section/111G, New York DVAMC, 423 East 23 Street, New York, New York 10010, USA. E-mail: david.goldfarb@nyumc.gov

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heritability demonstrated in the VET classic twin study.[7] Recent studies using sequencing of putative candidate genes for stones of any composition find explicatory monogenic causes of calcium stones among fewer than 10% of affected participants.[6]

Kidney stones affect more men than women with a ratio of less than 2:1, with recent data showing this ratio continuing to narrow. Recent analysis of the National Health and Nutrition Examination Survey (NHANES) data demonstrate that stones are becoming more prevalent in both men and women.[8] The reasons why stones are more prevalent in men remains a topic of speculation, with differences in solute excretion or urine volume hypothesized. However, it is also possible that women and men differ in the degree to which genetics contributes to the kidney stone–forming phenotype. Because the VET Registry study of stones did not include women, we sought to perform another classic twin study that included women. In this study, we now report on the genetic contribution to stones in a sample of both female and male twin pairs.

METHODS

All twin pairs in this study were same-sex members of the Washington State Twin Registry, a community-based registry of twins primarily identified through the Washington State Department of Licensing. Details of the construction and characteristics of the Registry have been described elsewhere.[9,10] All members complete an enrollment survey that collects basic information about sociodemographics, health, and lifestyle behaviors. Overall, the twins in this sample were young (39.0 $\pm$ 18.1 years), and predominately white (87.7%). The Washington State Twin Registry has been approved for human subject participation by the Washington State University Institutional Review Board.

Demographic information used in the analysis consisted of age, sex, zygosity, race (dichotomized as white or nonwhite), education (categorized into less than high school, high school graduate, or at least some college), and income (dichotomized as more than or less than $60,000 per year). Standard questions about childhood similarity were used to classify twins as identical (MZ) or fraternal (DZ). When compared with DNA-based methods, these questions correctly determine zygosity with greater than 90% accuracy.[11,12]

To ascertain lifetime history of kidney stones, we used a single question from the enrollment survey that asks all twins if a doctor or medical professional has ever diagnosed them with kidney stones.

Statistical analyses were conducted using R version 3.3.1 (R Core Team, Vienna, Austria). Descriptive characteristics were calculated as means and SDs for continuous variables and percentages for categorical variables. We estimated the genetic and environmental influences on kidney stones using structural equation modeling in twins. Our analysis starts with a descriptive analysis of twin correlations with 95% confidence intervals for self-reported kidney stones stratified by zygosity. Comparing the within-pair twin correlations provides an initial indication of the genetic and environmental influence on stone prevalence. An MZ correlation that is larger than the DZ correlation suggests a genetic influence on kidney stones. The rationale for this inference is that MZ twins share 100% of their genetic material, whereas DZ twins share only 50% on average. A correlation that is similar in MZ and DZ pairs indicates a role for common family environment. The absence of correlations in either MZ or DZ pairs suggests that the variation in kidney stones is due primarily to unique environmental influences.

To formally estimate genetic and environmental effects on kidney stones, we used structural equation modeling based on the twin correlations.[13] A model, fit to the raw data overall and then stratified by sex, estimated the percentage of phenotypic variance due to additive genetic (A), common environmental (C), and unique environmental (E) components.[14] The full ACE model, as well as reduced models (i.e., AE, CE), were fit to the data. The best-fitting model was determined by a likelihood ratio χ^2 test comparing the full ACE model with reduced models that did not include all A, C, and E effects (e.g., AE). A non–statistically significant χ^2 test between the full and reduced models indicates that the reduced model is a better, more parsimonious fit to the data. Akaike's[15] information criterion was used as a global measure of goodness of model fit, with lower values being an indication of a better-fitting model. We also tested differences in heritability in the best-fitting models in men and women. All structural equation modeling analyses were conducted using the OpenMx package for R.[13,16]

RESULTS

There were 7053 same-sex pairs with kidney stone data, which included 1684 MZ male pairs, 841 DZ male pairs, 3069 MZ female pairs, and 1459 DZ female pairs. Descriptive information is provided in Table 1. The overall prevalence of kidney stones was 6.2% in men and 4.9% in women. Data on concordance, discordance, and correlations by zygosity and sex for kidney stones are shown in Table 2. The tetrachoric correlations show a higher concordance rate for stone disease among MZ men compared with DZ men, and among MZ women compared with DZ women. In

Table 1. Demographic characteristics of all twins by zygosity and sex in the Washington State Twin Registry

	MZ men (n = 3368)	DZ men (n = 1682)	MZ women (n = 6138)	DZ women (n = 2918)
Age, yr, mean (SD)	38.4 (18.5)	40.6 (19.3)	37.9 (17.2)	41.1 (18.5)
Race, % white	88	90	86	91
Education, %				
Less than high school	6	7	3	4
High school graduate	19	21	18	19
At least some college	75	72	79	77
Marital status, %				
Single	44	42	36	32
Married	43	44	44	46
Widowed/divorced/ separated	7	9	12	15
Living with partner	6	5	8	7
Income more than $60,000, %	58	56	51	50

DZ, dizygotic; MZ, monozygotic.

addition, the rates for concordance are greater in MZ men compared with MZ women.

After adjusting for age, both heritability and the unique environment contributed to the prevalence of stone disease in men and women (Table 3); however, there was no contribution from the common environment for either sex. In the best-fitting AE model, the heritability was 57% in men and 46% in women; this difference in heritability was statistically significant ($P < 0.05$).

DISCUSSION

We report the results of the first classic twin study of nephrolithiasis including both women and men. In men, we confirm the result of our previous study of male twins, demonstrating a similar contribution of heritability. These results contrast with our findings in women. Although nephrolithiasis in women, as in men, is heritable, the genetic influence is lower, with a concomitant increase in the influence of the unique environment.

In our previous study of the VET Registry middle-aged male twins, we found a similar kidney stone prevalence of 6.4%.[3] In that article, model fitting yielded a heritability of 56%. In the current study's cohort of men, we observed a similar heritability of 57% for kidney stones.

Table 2. Kidney stones among twins by zygosity and sex in the Washington State Twin Registry

	MZ men	DZ men	MZ women	DZ women
Total pairs	1684	841	3069	1459
Concordant for stones, n pairs	40	12	29	9
Discordant for stones, n pairs	123	86	231	140
Tetrachoric correlation	0.68	0.41	0.43	0.21

DZ, dizygotic; MZ, monozygotic.

As universally reported in observational studies of stones, we report a higher prevalence of stones among men than among women, although the difference yields a ratio of men:women of only 1.3, slightly less than the value of 1.5 reported in the most recent NHANES data.[8] The basis for this difference in prevalence between men and women has frequently been speculated on but has not been definitively explained. Although heritability did contribute to the prevalence of stones among female twins, we present here for the first time the evidence that stone disease is more heritable among men than among women (57% vs. 46%, $P < 0.05$) who demonstrate a greater influence of the unique environment. The unique environment result, as contrasted with the common environment result, implies that there have been events and exposures that affected one twin and not the other, and that tend to make twins in a pair different from each other. In this classic twin study analysis, we are not able to be more specific about what specific exposures are responsible for the results.

The basis for the difference in stone prevalence among men and women has long been a topic for study. There are only limited attempts to investigate the effect of family history on stone prevalence. In one survey of 380 stone formers in Sweden, a positive family history among first-degree relatives was more common in female (64.7%) than in male individuals (51.0%).[5]

That the recent NHANES data show that the difference has been narrowing is taken as evidence of nongenetic influences on stone formation.[8] Changes in dietary and lifestyle choices are considered likely to play a critical role in this changing epidemiology of stone disease. Similarly, increased rates of stones from earlier, to later, NHANES cohorts, particularly in black, non-Hispanic, and Hispanic individuals, is further evidence of nongenetic causes contributing. Nongenetic variables thought to be associated with increasing stone prevalence include declining intake of dairy and increased prevalence of higher body mass index and the consequent metabolic syndrome.[17,18] The Nurses Health Studies I and II, in which the participants are all women, do not demonstrate identical effects of dietary intakes on stone incidence or prevalence, as compared with the Health Professionals Follow-up Study, in which the participants are all men.[19,20] Perhaps the most striking difference between women and men in those studies, in part accounting for differences in stone prevalence, is that men have higher urinary oxalate excretion.[21,22] This difference may be more attributable to differences in endogenous oxalate production than to dietary intake. As reported recently, analysis of the 2007–2012 NHANES data indicated strong association of kidney stones in women younger than 50 years with prior pregnancies.[23] Women of reproductive

Table 3. Estimated genetic and environmental parameters for kidney stones in the Washington State Twin Registry adjusted for age

Estimates of variance components (95% confidence intervals)[a]				Test of model fit			
Model[b]	Additive genetics (A)	Common environment (C)	Unique environment (E)	$\Delta\chi^2$	df	ΔP	**ΔAIC**[c]
All twins							
ACE	0.52 (0.32–0.59)	0.00 (0.00–0.18)	0.48 (0.41–0.55)	—	—	—	—
AE	**0.52 (0.45–0.59)**	**—**	**0.48 (0.41–0.55)**	**0.0**	**1**	**1.0**	**−2.0**
CE	—	0.44 (0.37–0.50)	0.56 (0.50–0.63)	17.0	1	<0.05	15.0
Male/Male pairs							
ACE	0.57 (0.23–0.68)	0.00 (0.00–0.31)	0.43 (0.32–0.54)	—	—	—	—
AE	**0.57 (0.46–0.68)**	**—**	**0.43 (0.32–0.54)**	**0.0**	**1**	**1.0**	**−2.0**
CE	—	0.48 (0.37–0.58)	0.52 (0.42–0.63)	8.8	1	<0.05	6.8
Female/Female pairs							
ACE	0.46 (0.17–0.56)	0.00 (0.00–0.25)	0.54 (0.44–0.64)	—	—	—	—
AE	**0.46 (0.36–0.56)**	**—**	**0.54 (0.44–0.64)**	**0.00**	**1**	**1.00**	**−2.0**
CE	—	0.38 (0.29–0.47)	0.62 (0.53–0.71)	8.2	1	<0.05	6.2

[a]Proportion of variance explained by additive genetics, common environment, and unique environment according to each model.
[b]ACE refers to the model including additive genetics (A), common environment (C), and unique environment (E). AE includes only additive genetics and unique environment, and CE common and unique environment; reduced models are compared with ACE.
[c]Akaike's information criterion (AIC) is a global measure of goodness of fit; best-fitting models are shown in bold.

age who had been pregnant had more than twice the odds of developing stones, compared with women who had never been pregnant. The prevalence of kidney stones increased with increasing number of pregnancies. We have not yet analyzed pregnancy data for our female twins.

Another hypothesis of note is that women are "better" at replacing the transdermal salt and water losses associated with summertime increases in ambient temperature, and have lesser seasonal declines in urine volume than men.[24] We have also recently demonstrated that following days with higher ambient wet-bulb temperatures, men were at significantly greater risk for emergent kidney stone presentations than women (Gregory Tasian, MD, unpublished observations, 2018). Again, whether exposure to temperature or different diets accounts for our result showing a greater effect of the unique environment on women's kidney stone prevalence is beyond the data we have available.

As in our prior study of stones in the VET Registry, the Washington State Twin Registry does not collect information regarding stone composition. Because roughly 75% to 80% of stones are composed of calcium oxalate and calcium phosphate, we assume that the proportion of stones in this report are of that same composition, and that the heritability we report occurs among calcium stone formers.[25] However, stone composition may differ among men and women. In young women ages 20 to 39, the prevalence of stones composed of hydroxyapatite, a crystalline form of calcium phosphate, sharply increases to 40% to 45%, much above the average prevalence of 20% in the male population of all ages or in older or younger women.[25] Because the basis for the increased prevalence of calcium phosphate stones in young women is uncertain, we cannot be certain if differences in heritability of calcium oxalate versus calcium phosphate contribute to the difference in heritability seen in our study between men and women.

As uric acid accounts for only 10% to 15% of all stones, far behind calcium, and largely causes stones in an older population, it is very unlikely that the effects we report are accounted for by any stone crystal form other than calcium. Stones of other crystal compositions, caused by genetic disorders, such as cystine or xanthine, are far too rare to be relevant in this report. Although recessive disorders, such as renal tubular acidosis, primary hyperoxaluria, and Dent disease (X-linked), do cause calcium oxalate and calcium phosphate stones that are themselves indistinguishable from those affecting the general population, these disorders are also rare enough that they cannot make any measurable contribution to stone prevalence in this study.[26]

The genetic basis for stone disease in the general population, both in men and women, has not been established, so that the genes responsible for the significant heritability seen in twins cannot currently be delineated. Given the prior twin data and family history studies, stone formers have been the subjects of a variety of attempts to elucidate the heritability of stone disease. Because among calcium stone formers, the most common urinary abnormality is higher urine calcium excretion, this phenotype has served as the basis for selecting candidate genes that could affect it. One study demonstrated heritability of increased calcium excretion in a collection of French-Canadian families, but the genetic basis for that finding has not been reported.[27] Another series of calcium stone formers with higher urine calcium excretion had linkage disequilibrium for *ADCY10*, which codes for a bicarbonate-sensitive adenylate cyclase; the physiologic importance or effects of this enzyme remain undescribed.[28] Although single nucleotide polymorphisms of *CASR*, *TRPV5*, and other putative

determinants of urine calcium excretion may have an influence among some families, they fail to account for any significant prevalence of stones.[29,30] In addition, attempts to find rare allelic variants among people with low and high urinary calcium excretion have failed to yield convincing evidence of major gene-disease associations and have therefore not significantly expanded the list of candidate genes.[7] Studies that have genotyped all stone formers, both young and old, presenting to specialty kidney stone clinics, have revealed monogenic causes of stones, including autosomal recessive causes, that cannot account for the high heritability we have shown in our twin studies.[6,31] None of these studies made discoveries that varied among men and women, and to our knowledge, no studies of the genetic basis for kidney stones has demonstrated differences in heritability before.

One limitation of our study is that the history of stones is based on self-reporting in response to a questionnaire. We have not validated the accuracy of these patient reports; however, the ability of patients to self-report stones accurately has previously been demonstrated.[32] In addition, because radiologic imaging of participants was not performed, we cannot know if any asymptomatic stones in participants who had never experienced renal colic were not reported; we would not expect a difference in false-negative reports in MZ compared with DZ twin pairs, or in women compared with men. We do not know at what age these surveyed individuals first developed kidney stones. Finally, although the ACE model can estimate the contributions from genes and the environment, it is unable to identify specific genes or environments involved, and does not measure any interactions occurring between genes and the environment.[14,33]

In conclusion, we have demonstrated using a classic twin study that the heritability of stone disease in women is less than that of men. These findings may in part explain why stones are less prevalent in women than in men. We also have confirmed our previous finding that heredity is an important factor in the development of kidney stones in men.

DISCLOSURE

DSG is a consultant at Retrophin, Alnylam, and Allena and a patent holder and owner of The Ravine Group. All the other authors declared no competing interests.

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