



Genome-wide association study of nephrolithiasis in an Eastern European population

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Abstract

Purpose Nephrolithiasis is a urological pathology that occurs at high rates and carries a great burden in terms of costs. The probability of recurrence is significant, necessitating improvements in prophylaxis and understanding of the disease mechanism. Despite the high heritability of this disease, only five genome-wide association studies (GWAS) of nephrolithiasis have been published.

Methods We selected 335 unrelated confirmed nephrolithiasis cases from two major sample collection projects (blood and buccal swabs) in Romania. DNA was extracted from whole blood and buccal swabs at deCODE Genetics (Reykjavik, Iceland) and genotyped.

Results Single-nucleotide polymorphisms identified from this GWAS implicated biological pathways and gene ontologies involving solute transport, renal physiology, and calcium homeostasis. Three loci especially emerged as candidates with a highly significant association with nephrolithiasis: RS10917682 in *Regulator of G protein signaling 5*, which has crucial roles in mRNA regulation and has been linked to renal cell carcinoma; RS1118528 in *Solute carrier family 25 member 24*, which encodes a mitochondrial ATP-Mg/phosphate carrier protein that likely influences a variety of important cellular pathways; and the *TOX2*-associated locus rs4437026, because *TOX2* is upregulated in several tumor types and linked to tumor progression.

Conclusion This study is the largest kidney stone-related GWAS reported in an Eastern European population and the first GWAS performed in a Romanian population to investigate the genetic risk factors for nephrolithiasis. We identified several loci that warrant further investigation for a better understanding of this highly heritable condition.

Keywords Nephrolithiasis · Kidney stone · GWAS · Romania · Eastern Europe

Introduction

The prevalence of nephrolithiasis is increasing, not only in developed countries but also in the rest of the world. Contributing factors include a shift from more traditional

diets towards a western way of eating and the increasing prevalence of obesity, diabetes, and a more sedentary lifestyle. The many advances of the modern world come with a cost to health, including metabolic dysfunction, cardiovascular pathologies, and nephrolithiasis. Since the 1960s, when the first major epidemiological studies were undertaken, clinicians and statisticians witnessed an increase in the prevalence of kidney stones. For example, in the 1964–1972 period, the prevalence of nephrolithiasis in the United States was 2.62%. This rate increased to 3.84% during 1976–1980. After the 1980s, the prevalence stabilized at 5.2–5.4%, where it has largely stayed, since [1] the lifetime risk of kidney stones is 8.8% in the United States [2], with an estimated recurrence rate of 14% after 1 year and 35% after 5 years [3], placing a significant burden on the health care system. Another reason for the increased prevalence is broader access to medical facilities, allowing for discovery of many incidental kidney stones after a routine

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ultrasound or a computed tomography scan performed for another condition.

In addition to environmental factors such as diet, kidney stone formation has a heritable component, with almost 65% of kidney stones forming in patients with a family history of the condition. Both twin and genealogy studies have reported a strong heritability for kidney stone disease [4], and twin studies have identified a heritability of >45% for stone disease and >50% for hypercalciuria. A strong family history of urolithiasis, including in a parent or a sibling, results in a standard incidence ratio for stone formation of >50, in contrast to a standard incidence ratio of 1.29 in spouses with no family history.

Five genome-wide association studies (GWAS) of nephrolithiasis have been published, identifying 15 loci associated with disease; however, no transethnic studies have been undertaken [5]. The five existing studies have been conducted in countries including Japan, the UK, and Iceland, but to date, no data have been published regarding the genetic variants linked to kidney stones in Romania. Here, we performed what is, to date, the largest kidney stone-related GWAS in an Eastern European population to uncover gene variants associated with the condition.

Materials and methods

Study population

We selected 335 unrelated confirmed nephrolithiasis cases from the ROMCAN and ProMark projects sample collection, a hospital-based sample set of 5434 nephrolithiasis cases and controls recruited from five major hospitals in Bucharest between 2008 and 2017. Blood samples and buccal swabs were collected for the ROMCAN and ProMark, respectively [6]. Both ProMark and ROMCAN are large-scale genetic epidemiological studies investigating the profile of prostate, colorectal, breast, and lung cancers. All participants included in this study as cases were cancer-free. A description of relevant epidemiological and clinical information can be found in the Table 1—ESM Annex 1.

Ethics statement

All participants gave written informed consent prior to enrollment and accepted the use of personal and clinical data and biological samples for genetic research. To collect personal data, trained interviewers performed face-to-face interviews using standardized questionnaires. The Bioethical Committee of the Romanian College of Physicians approved the study, and the study protocols were approved by the National Ethical Board of the Romanian Medical Doctors Association in Romania.

Genotyping and analysis of single-nucleotide polymorphism (SNP) data

DNA was extracted from whole blood for the ProMark samples and buccal swabs for the ROMCAN samples, respectively, at deCODE Genetics (Reykjavik, Iceland) and genotyped using Infinium OmniExpress-24 bead chips (Illumina). DNA samples were prepared and hybridized following the manufacturer's instructions. The genotype data were filtered using Plink! v1.07. A total of 104,390 markers met the filtering criteria. An association test was performed between the 104,390 markers and a phenotype represented by the confirmed nephrolithiasis diagnosis. The association test was calculated using Plink! v1.07 (6), with a single binary variable as a response. All reported *P* values are two-sided. After filtering, only 329 cases were included in the study because of genotyping failures.

Results

Of 5434 included participants (4234 men, 1200 women), there are 329 with stones and 5105 controls. The distribution of association *P* values (Manhattan plot) for this trait is shown in Fig. 1.

The ten top scoring SNPs for association with nephrolithiasis are shown in Table 1. The SNP with the lowest *P* value (1.59×10^{-5}) for this trait was rs1980221, located on chromosome 5. This intronic SNP lies in the uncharacterized gene *LOC105374686* with similar minor allele frequencies (GG=0.044828-, TOPMED; G=0.04018, GnomAD). Other interesting variants from the top 10 scoring SNPs included the intronic variants rs2637748 in the uncharacterized gene *LOC107986098*, rs4437026 in the *TOX2* (*TOX high-mobility group-box family member 2*) gene, and rs124899 in the *SMIM15-AS1* (*SMIM15 antisense RNA 1*) gene, along with rs10917682, a 3' untranslated region variant in the *RGS5* (*Regulator of G protein signaling 5*) gene, and RS1118528 in the *SLC25A24* (*Solute carrier family 25 member 24*) gene. Despite the possible biological implications of these variants, neither of these SNPs showed genome-wide significance for association with nephrolithiasis.

Of those in the top 10, the *RGS5* RS10917682 variant showed a phenotype association with a *P* value of 7.94×10^{-5} , an odds ratio of 1.634 for the tested allele (G), and a minor allele frequency of 0.1204. The region of this variant is linked to crucial roles in gene expression by influencing the localization, stability, export, and translation efficiency of mRNA.

The *SLC25A24* RS1118528 variant showed a phenotype association with a *P* value of 9.54×10^{-5} and an

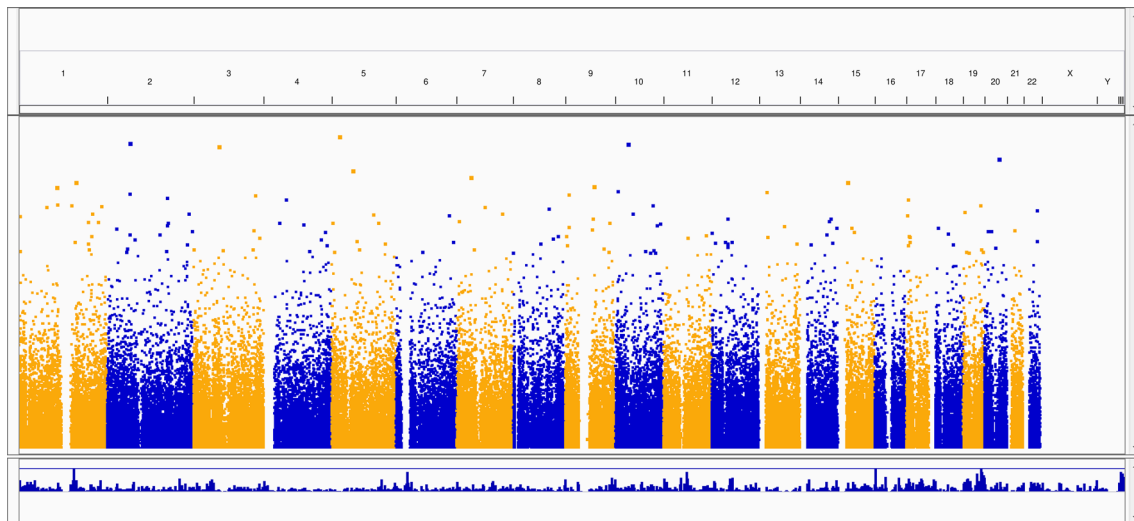


Fig. 1 Manhattan plot of all P values for associations of SNPs with the kidney stone phenotype

Table 1 Top associated SNPs for nephrolithiasis as a binary trait

SNP	CHR	Base pair	Tested allele	MAF	P value	OR
rs1980221	5	23,056,728	G	0.03517	1.59E−05	2.588
rs7583682	2	65,007,479	C	0.08537	2.01E−05	0.5493
rs708408	10	35,947,847	T	0.09146	2.04E−05	1.82
rs2637748	3	73,096,887	G	0.2021	2.25E−05	1.531
rs4437026	20	44,011,864	T	0.05015	3.50E−05	2.155
rs124899	5	61,172,856	C	0.3079	5.32E−05	1.423
rs10271006	7	42,317,215	G	0.2295	6.69E−05	0.6855
rs10917682	1	163,142,414	G	0.1204	7.94E−05	1.634
rs3101649	15	27,688,976	A	0.1368	8.09E−05	1.59
rs4877214	9	81,545,927	A	0.08055	9.23E−05	1.789

SNP single-nucleotide polymorphism, CHR chromosome, MAF minor allele frequency, OR odds ratio

odds ratio of 0.71 for the G allele. The mitochondrial ATP-Mg/phosphate carrier protein that this gene encodes may affect mitochondrial adenine-nucleotide-dependent enzymes that regulate gluconeogenesis from lactate, urea synthesis, mitochondrial DNA replication, transcription, and protein synthesis, among others [7]. In addition, *TOX2* is involved in a variety of relevant pathways, so we further analyzed the results for the *TOX2*-related rs4437026.

Given the lack of GWAS studies investigating this phenotype and the limitations of our study, we did not follow up our GWAS with a replication study. Despite this issue, we identified 567 markers as associated with nephrolithiasis at $P < 0.005$ (Table 2—Annex 1 is considered for future replication studies).

Discussion

Our study represents the largest kidney stone-related GWAS to date from an East European population. The analysis integrates data from 329 cases involving kidney stones and 5105 controls of Romanian ancestry, providing a genetic profile of associations with nephrolithiasis. The genes implicated by our GWAS are linked to biological pathways and gene ontologies involving solute transport, renal physiology, and calcium homeostasis.

Among the loci falling in the top 10 for association strength, the *RGS5*-associated locus RS10917682 occurs at a higher frequency in the Romanian population compared with results based on TOPMED [8] ($G = 0.060780$) and

GnomAD [9] ($G = 0.05805$), making this variant a strong candidate for future research. *RGS5* expression levels are reported to be above average in the kidney, at 110.7 transcripts per million of total transcripts (pTPM) reported by HPA [10] and 44.2 pTPM reported by GTEx, respectively [11]. In one recent study, *RGS5* was found to have a significant role in renal cell carcinoma (RCC) and to show a significant association at high expression levels with low serum calcium and increased white blood cell count. The RGS family regulates cellular signaling events downstream of G protein-coupled receptors, which are associated with the initiation and progression of multiple cancers [12]. Gene expression thus might be prognostic and have a significant role in initiation and progression of RCC, as first hypothesized in 2004 with findings linking *RGS5* to G protein-mediated signaling in tumor vessels in RCC [13]. Later work in animal models indicated that *RGS5* is a potent GTPase-activating protein for $G_{i\alpha}$ and $G_{q\alpha}$, which are expressed in vascular smooth muscle, and has been considered as a marker for pericytes, which express *RGS5* at high levels [14]. Indeed, this protein is expressed at levels sevenfold higher in renin cells compared with the total kidney cortex, by the smooth muscle cells of the developing kidney arterioles, and by mesangial cells in the adult animal [15].

Considering these previous findings and our current association of this allele with kidney stone formation, the pathological implication of this variant in renal physiopathology is of interest. The 3' prime untranslated location of RS10917682 appears to have a crucial role in gene expression by influencing the localization, stability, export, and translation efficiency of mRNA.

The *SLC25A24*-associated RS1118528 allele also was in the top 10 candidates showing a significant association with kidney stone diagnosis. *SLC25A24* has previously been identified among 21 genes with the highest expression increase during the stone elimination period [15]. The mitochondrial ATP-Mg/phosphate carrier protein encoded by *SLC25A24* is broadly expressed and facilitates the exchange of adenine nucleotides, including ATP-Mg, ATP, ADP, and AMP, and phosphate between mitochondrial matrix and cytosol. As noted, this transporter regulates adenine-nucleotide concentrations in the mitochondrial matrix and may influence many crucial cellular processes [16]. Expression of this gene in kidney varies from 8.4 pTPM, as reported by HPA, to 4.0 pTPM, as reported by GTEx [11].

A third allele that warranted a closer look was rs4437026, an intronic variant in the *TOX2* gene, which modifies chromatin structure and is almost identical to high-mobility group-box DNA-binding domains [17]. A recent study showed that *TOX* and *TOX2* are targets of the calcium/calmodulin-regulated transcription factor NFAT [18]. In addition, *TOX* expression is frequently upregulated in diverse

types of human tumors, and its overregulation often is associated with tumor progression and with the control of apoptosis, cell growth, metastasis, and DNA repair. Because of this diversity of roles for *TOX2*, we also singled it out from our results for the top 10 loci most significantly associated with kidney stone diagnosis.

Conclusions

The pathophysiology of nephrolithiasis is complex, with strong evidence pointing to a genetic predisposition toward stone formation. Although GWAS can give clues to the underlying biological mechanism and despite the heritability of this disease, only five GWAS of nephrolithiasis have been published. This study is the first in a Romanian population and the largest in an Eastern European population to use GWAS to investigate genetic risk factors for nephrolithiasis. The results of the association test identified 567 markers with an observed $P < 0.005$ for this phenotype. In addition, we highlighted three especially promising association loci in the top 10 that were most significantly linked to nephrolithiasis, and we expect closer analysis of these candidates to facilitate a better understanding of the biological processes underlying this disease.

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Compliance with ethical standards

Conflict of interest The authors confirm that there are no conflicts of interest.

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