Prevalence and characteristics of genetic disease in adult kidney stone formers

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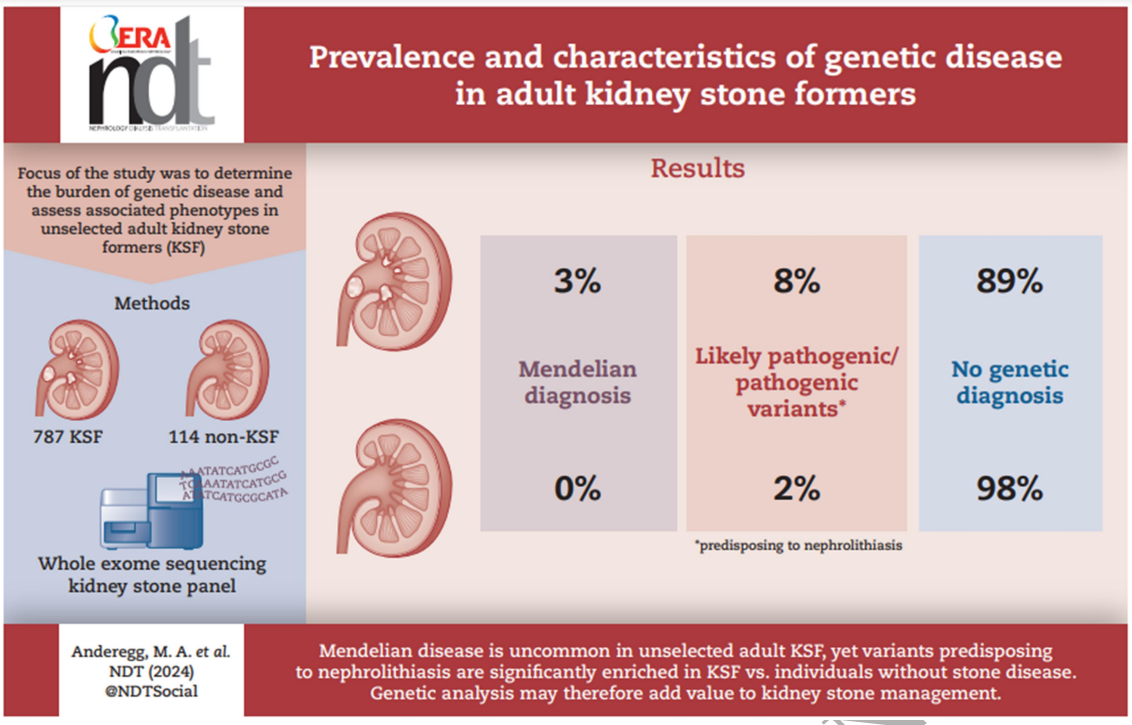
GRAPHICAL ABSTRACT



ABSTRACT

Background. Molecular mechanisms of kidney stone formation remain unknown in most patients. Previous studies showed high a heritability of nephrolithiasis, but data on prevalence and characteristics of genetic disease in unselected adults with nephrolithiasis are lacking. This study was conducted to fill this important knowledge gap.

Methods. We performed whole exome sequencing in 787 participants of the Bern Kidney Stone Registry, an unselected cohort of adults with ≥ 1 past kidney stone episode (KSF), and 114 non-stone-forming individuals (NKSF). An exome-based panel of 34 established nephrolithiasis genes was analyzed and variants assessed according to ACMG criteria. Pathogenic (P) or likely pathogenic (LP) variants were considered diagnostic.

Results. Mean age of KSF was 47±15 years, and 18 % were first time KSF. A Mendelian kidney stone disease was present in 2.9% (23 of 787) of KSF. The most common genetic diagnoses were cystinuria (*SLC3A1*, *SLC7A9*; n=13), Vitamin D-24 hydroxylase deficiency (*CYP24A1*; n=5) and primary hyperoxaluria (*AGXT, GRHPR, HOGA1*; n=3). 8.1% (64 of

787) of KSF were monoallelic for LP/P variants predisposing to nephrolithiasis, most frequently in *SLC34A1/A3* or *SLC9A3R1* (n=37), *CLDN16* (n=8) and *CYP24A1* (n=8). KSF with Mendelian disease had a lower age at the first stone event (30±14 years vs. 36±14 years, p=0.003), were more likely to have cystine stones (23.4 % vs. 1.4 %) and less likely to have calcium oxalate monohydrates stones (31.9 % vs. 52.5 %) compared to KSF without genetic diagnosis. The phenotype of KSF with variants predisposing to nephrolithiasis was subtle and showed significant overlap with KSF without diagnostic variants. In NKSF, no Mendelian disease was detected, and LP/P variants were significantly less prevalent compared to KSF (1.8 % vs. 8.1%).

Conclusion. Mendelian disease is uncommon in unselected adult KSF, yet variants predisposing to nephrolithiasis are significantly enriched in adult KSF.

Keywords: kidney stones, mendelian, monogenic, nephrolithiasis, whole exome sequencing

KEY LEARNING POINTS

What was known:

- Kidney stone formation is strongly influenced by genetic factors (positive family history in 30-60%) and >30 Mendelian forms of kidney stone disease were described, explaining 6.8 - 29.4% of stones in selected populations.
- However, the diagnostic utility of whole exome sequencing in unselected adults with nephrolithiasis has not been established.
- Current strategies to manage this common and relapsing disease are inadequate.

This study adds:

- Mendelian disease in unselected adults with nephrolithiasis is rare and far less common than previously reported.

- Genetic variants predisposing to kidney stone formation are significantly enriched in individuals with nephrolithiasis, yet the associated phenotypes display a significant overlap with individuals without diagnostic variants.
- While many Mendelian forms of nephrolithiasis can be detected with thorough phenotypic analysis, genetic testing can ameliorate diagnosis in adult stone formers, where phenotypes are often less pronounced.

Potential impact:

- Whole exome sequencing allows accurate assessment of Mendelian disease and identification of predisposing variants in adult individuals with nephrolithiasis.
- Our study highlights the potential of genetic testing to direct patients to personalized therapies and clinical trials.

INTRODUCTION

Nephrolithiasis is a common global healthcare problem [1]. Kidney stones recur frequently and cause substantial morbidity, reduced quality of life and enormous cost [2-4]. A comprehensive phenotypic examination is paramount for the detection of urinary abnormalities and provides an efficient way for the identification of underlying Mendelian disease. Still, in many adult patients with kidney stones, the molecular pathogenesis of clinical traits associated with kidney stone formation (e.g. hypercalciuria) remains unknown. Consequently, undifferentiated dietary and pharmacological preventive measures are initiated, but many patients continue to form stones [5,6]. Hence, current strategies in managing this very common and debilitating disease are clearly inadequate. There is an unmet need for novel diagnostic and therapeutic approaches.

Kidney stone formation is strongly influenced by genetic factors: a positive family history is present in 30-60% of individuals with kidney stones [7], and both twin [8] and genealogy [9] studies revealed a high heritability of nephrolithiasis. More than 30 Mendelian (also called monogenic) causes of nephrolithiasis have been described thus far [10]. Recent studies have also highlighted the importance of intermediate effect size / incomplete penetrance variants in increasing the risk for nephrolithiasis[11-14]. Identification of patients with Mendelian forms of nephrolithiasis remains a major challenge, and no clear consensus exists on clinical parameters to guide genetic testing. Clinical phenotypes can be diagnostic for many Mendelian diseases – if phenotypic screening is diligently performed – and usually prompt personalized genetic testing. Yet, many cases of genetic forms of nephrolithiasis are missed and incorrectly labelled “idiopathic”.

Whole exome sequencing (WES) is widely applied as diagnostic tool for rare diseases and the detection of pathogenic variants in cancer [15,16]. In contrast, the diagnostic utility of WES has not been established for most constitutional disorders, such as nephrolithiasis. In small, selected cohorts of early-onset or familial nephrolithiasis, a Mendelian cause was identified in

6.8 - 29.4% of cases [10,17-19]. However, the frequency and phenotypic spectrum of genetic disease in sporadic adult-onset nephrolithiasis, by far the most common type encountered in clinical routine, is unknown. To address these important knowledge gaps, we analyzed a WES-based gene panel in a deeply phenotyped, little selected European cohort of 787 adult kidney stone formers (KSF) and in 114 non-stone forming individuals (NKSF).

MATERIALS AND METHODS

Study cohort

The study was conducted with participants enrolled in the Bern Kidney Stone Registry (BKSR), an observational cohort of adult kidney stone formers described previously and in the SI[20-23]. Inclusion criteria for the BKSR are i) written informed consent, ii) age ≥ 18 years, and iii) ≥ 1 past kidney stone episode.

Inclusion criteria for NKSF were i) written informed consent, ii) age ≥ 18 years, and iii) no history of past kidney stones and no evidence of asymptomatic nephrolithiasis or nephrocalcinosis on ultrasound at enrolment.

Whole exome sequencing and variant calling

Isolation of genomic DNA, exome capture, high-throughput sequencing and bioinformatic analysis including joint variant calling using Genome analysis Toolkit v3.8 (GATK) according to GATK best practices recommendations[24,25] and annotation using Ensembl Variant Effect Predictor Release 106 (VEP) [26] was performed using established methods, described in more detail in Supplemental Methods. After filtering for predicted consequences and genome aggregation database (gnomAD) minor allele frequency (<1% in all populations) [27], variants in 34 genes [10,17-19,28] previously implicated in Mendelian kidney stone disease were examined (Fig. 1, lower panel). Further variant stratification was performed according to the

recommendations of the American College of Medical Genetics and Genomics (ACMG)[29] after manual review of phenotype data. Only variants classified as likely pathogenic (LP) or pathogenic (P) applying the ACMG criteria were defined as diagnostic variants leading to a genetic diagnosis. Separation into Mendelian or LP/P variants predisposing to nephrolithiasis (LP/P variants) was conducted depending on evidence available from this study and/or from published case/control studies, described in more detail in the SI.[11,12,30,31] (Fig. 1 and Supplemental Methods).

Statistical Analysis

Continuous variables were reported as medians with 25^{th}-75^{th} percentiles or means with standard deviations and categorical variables were reported as counts with percentages, as appropriate. Different statistical methods were employed to analyze differences between independent groups, depending on the nature of the variables involved. Mann-Whitney U test was used for non-normally distributed continuous variables, while Student's t-test was applied for normally distributed variables. For categorical variables, the Fisher's Exact Test was implemented. Findings from these analyses were summarized and presented as descriptive tables. Statistical tests were two-sided and a p-value < 0.05 was considered statistically significant. Statistical analyses were performed using Stata, version 16 (Statacorp, TX, USA). Pie-charts and dot plots were generated with GraphPad Prism, version 8.4.3 (GraphPad Software, San Diego, CA, USA).

RESULTS

Study cohort

We performed WES in 901 individuals, including 787 KSF and 114 NKSF (Fig. 1). Clinical characteristics of the study cohort, separated in KSF and NKSF, are shown in Table 1. Mean age ± standard deviation (SD) was 46.6±14.5 years in KSF and 42.4±14.9 years in NKSF, respectively. The majority of KSF were male (71.8 %, n=565), whereas the percentage of men was lower in NKSF (54 %, n=61). Consistent with previous reports, KSF were significantly more likely to report a positive family history of kidney stones compared to NKSF (43.6 % vs. 6.8 %, Table 1) [32,33], and lumbar spine bone mineral density (BMD) was lower in KSF compared to NKSF (Table 1) [34,35].

Genetic analysis

Among 787 unrelated, unselected adult KSF, we discovered in 23 patients (2.9 %) a Mendelian form of nephrolithiasis, i.e., pathogenic (P) or likely pathogenic (LP) variants in genes associated with Mendelian forms of nephrolithiasis respecting the relevant mode of inheritance (autosomal dominant and/or autosomal recessive and X-linked recessive) (Table 2, Fig. 1, Fig. 2). Mendelian disease was detected in nine of 34 analyzed genes (n= number of patients): *AGXT* (n=1), *ATP6V1B1* (n=1), *CYP24A1* (n=5), *GRHPR* (n=1), *HOGA1* (n=1), *SLC3A1* (n=9), *SLC4A1* (n=1), *SLC7A9* (n=4), *SLC12A1* (n=1).

A total of 66 individuals (8.4%), 64 (8.1%) of which were KSF, presented with LP/P variants not fulfilling our stringent criteria for Mendelian disease, but predisposing for nephrolithiasis (Suppl. Table 1). LP/P variants were detected in nine of 34 genes (n=number of patients): *ADCY10* (n=1), *CASR* (n=4), *CLDN16* (n=8), *CYP24A1* (n=8), *SLC4A1* (n=1), *SLC7A9* (n=3), *SLC34A1* (n=15), *SLC34A3* (n=17) and *SLC9A3R1* (n=8). Of these variants, 18 % (12 of 66) were novel, previously unreported in ClinVar or HGMD (Suppl. Table 2).

Prior to WES, a Mendelian form of nephrolithiasis was known or suspected in 18 of 23 individuals (78 %) with a post WES Mendelian diagnosis, despite each case being reviewed by a kidney stone expert. Therefore, in five of 23 individuals (22 %) with Mendelian disease (that is 5/787, 0.6 % of total KSFs) genetic analysis established a new or corrected a suspected *a priori* diagnosis. The diagnostic yield was similar between first-time and recurrent stone formers (Supplementary Table 3), and between men and women (Table 3, Suppl. Table 4).

Genotype-phenotype correlation

Mean age of first kidney stone event (23.5±12.3 years vs. 35.9±13.8 years, p<0.001) and mean age of presentation for metabolic-work up (39±15.8 years vs. 47.2±14.3 years, p=0.009) were lower in KSF with Mendelian diagnosis compared to KSF without, with intermediate results for patients with LP/P variants predisposing for nephrolithiasis (Table 3, Suppl. Fig. 3). Furthermore, cystine stones were significantly more common (64.7 % vs. 1.4 %) and calcium oxalate monohydrates stones less common (23.5 % vs. 52.5 %) in KSF with Mendelian diagnosis compared to KSF with LP/P variants or without genetic diagnosis, in line with higher urinary cystine and urine pH in KSF with Mendelian diagnosis (Table 3, Suppl. Fig. 2). The prevalence of a positive family history of kidney stone disease was similar in all three groups (43.1 % vs. 49.2% vs. 46.0 %). Additionally, KSF with Mendelian disease had more past kidney stone events [5 (2,5) vs. 3 (2,4), p=0.052] than KSF with LP/P variants. In contrast, KSF with LP/P variants had significantly lower plasma phosphate (Table 3). Lumbar spine BMD was higher in KSF with Mendelian disease compared to KSF without genetic disease or LP/P variants predisposing for nephrolithiasis (Table 3, Suppl. Fig. 6).

Mendelian disease

The most common Mendelian diagnosis was cystinuria (n=13) due to biallelic diagnostic variants in *SLC3A1* (type A cystinuria, n=9) or *SLC7A9* (type B cystinuria, n=4). Urine cystine excretion, where available, was increased in all KSF with genetic diagnosis of cystinuria. One patient had biallelic diagnostic variants in *SLC3A1* and a monoallelic LP/P variant in *SLC7A9*. KSF with biallelic diagnostic *SLC3A1* or *SLC7A9* variants all had cystine stones.

Five patients had biallelic diagnostic variants in *CYP24A1,* which encodes the Vitamin-D inactivating enzyme Vitamin D-24 hydroxylase. Interestingly, all KSF with biallelic variants in *CYP24A1* (but no KSF with a monoallelic *CYP24A1* variant) displayed cystic kidney disease, as reported previously [31,36]. Common misdiagnoses for patients with biallelic variants in *CYP24A1* (n=3) were polycystic kidney disease or medullary sponge kidney. Two patients with biallelic *CYP24A1* variants underwent parathyroidectomy for suspected primary hyperparathyroidism (PHPT), without phenotype alleviation postoperatively.

We furthermore identified three patients with primary hyperoxaluria (PH) in our cohort. The diagnosis was already established in the two patients with primary hyperoxaluria type 1 (PH1) and primary hyperoxaluria type 2 (PH2), respectively. The diagnosis was previously unknown in the patient with primary hyperoxaluria type 3 (PH3) due to a homozygous pathogenic *HOGA1* variant. This patient had his first kidney stone episode at age 36. Metabolic work-up at age of 67 showed CKD stage 2 and a urine oxalate in the upper normal range on free-choice diet, but a strong increase after an instructed one-week diet low in calcium and sodium.

No Mendelian form of nephrolithiasis was detected in the 114 NKSF included in this study (Fig. 1, Fig. 2, Suppl. Table 5).

LP/P variants predisposing to nephrolithiasis

The most common identified genetic predisposition to nephrolithiasis was renal phosphate wasting due to monoallelic variants in the genes encoding the renal sodium/phosphate co-transporters NaPi-2a and NaPi-2c, *SLC34A1* (n=15), *SLC34A3* (n=18) or in the gene *SLC9A3R1,* encoding the regulatory interaction protein for NaPi-2a/2c, NHERF, (n=7). LP/P variants in *SLC34A1*, *SLC34A3*, but not in *SLC9A3R1,* were enriched in KSF compared with controls (NKSF and gnomAD, Supplementary Table 5). KSF with LP/P variants in *SLC34A1/A3* or *SLC9A3R1* had similar plasma phosphate and TmP/GFR compared to KSF without genetic diagnosis, but urine calcium in individuals with LP/P variants in *SLC34A3* was higher compared to KSF without genetic diagnosis (Suppl. Fig. 4).

We also identified eight patients carrying the same previously described monoallelic LP/P variant (c.458A>G, p.Asn153Ser) in *CLDN16*, encoding the tight junction protein claudin-16. Biallelic pathogenic variants in *CLDN16* cause familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC)[37], and an increased prevalence of nephrolithiasis has been observed in monoallelic carriers of pathogenic *CLDN16* variants [30,38]. Individuals with the monoallelic *CLDN16* variant presented with hypercalciuria and calcium oxalate stones but normal renal function and plasma magnesium. The identified *CLDN16* variant was more prevalent in KSF vs. NKSF and vs. all LP/P-variants in *CLDN16* in gnomAD-NFE, respectively (Suppl. Table 5).

Additionally, eight patients carried monoallelic LP/P variants in *CYP24A1,* and the respective variants were five to eight times more prevalent in KSF vs. NKSF and vs. gnomAD-NFE, respectively (Suppl. Table 5). Overall (i.e., considering the prevalence of all variants), *CYP24A1* variants were not enriched in our cohort compared to gnomAD-NFE (Supplementary Table 5). KSF with mono- and biallelic *CYP24A1* variants both displayed hypercalciuria, but hypercalcemia and/or suppressed parathyroid hormone (PTH) or

nephrocalcinosis were only present in KSF with biallelic variants. Urine calcium was higher in KSF with biallelic variants, but KSF with monoallelic variants had a higher number of past stone events (Supplemental Fig. 5). BMD and the prevalence of osteoporosis / osteopenia were similar in patients with genetic variants predisposing to urinary phosphate or calcium wasting compared to KSF without genetic diagnosis (Suppl. Fig. 6 and Suppl. Table 7).

We furthermore identified six patients with monoallelic LP/P variants in *SLC7A9*. Risk variants were either previously described to cause autosomal-dominant cystinuria (c.544G>A p.(Ala182Thr)) [39,40] [41,42], or enriched in KSF vs NKSF and gnomAD (c.313G>A p.(Gly105Arg)). In contrast to biallelic variants, the six KSF with monoallelic *SLC7A9* variants presented with calcium-containing kidney stones without cystine content, similar to previously reported cases [43-45]. KSF with monoallelic *SLC7A9* variants had elevated urine cystine, albeit lower levels compared to KSF with biallelic variants in *SLC3A1*/*SLC7A9*. No significant differences in stone number or age at first stone event were detected between the two groups of patients (Suppl. Fig. 5). In seven individuals with cystine-containing kidney stones and two individuals with increased urine cystine, no diagnostic variants in *SLC3A1* or *SLC7A9* could be identified (Suppl. Table 6).

Four KSF had LP/P variants in the gene encoding the calcium-sensing receptor, *CASR*, which is associated with familial hypocalciuric hypercalcaemia type 1 (FHH1), or autosomal-dominant hypocalcemia. The frequency of *CASR* LP/P variants in KSF was significantly higher than LP/P variants in all gnomAD-NFE participants (Supplementary Table 5). The phenotype of these patients was variable: two patients presented with hypercalciuria but normal plasma calcium, phosphate and PTH. One patient had hypercalcemia with a low-normal PTH and low urine calcium. Another patient presented with elevated PTH, hypercalcemia, pronounced hypophosphatemia, hypercalciuria and calcium oxalate dihydrate stones, compatible with the diagnosis of PHPT. Parathyroidectomy led to a complete normalization of the phenotype.

In the 114 NKSF included in this study, exome sequencing revealed a LP/P variant in two individuals, corresponding to a prevalence of variants predisposing to nephrolithiasis of 1.8 % (compared with 8.13% for KSF) (Fig. 1, Fig. 2, Suppl. Table 5).

DISCUSSION

In this exome-based targeted panel study in a large, unselected European cohort of 787 adult KSF, we detected a Mendelian kidney stone disease in 23 patients (2.9 %) using stringent diagnostic criteria. This diagnostic yield is significantly lower compared to previous studies conducted in selected groups of KSF [10,17,46]. Yet the fraction of additional individuals (8.13%) with LP/P variants predisposing to nephrolithiasis is substantial, especially when considering the broad inclusion criteria, the high prevalence of the disease and the potential rate of false-negatives due to stringent molecular genetic diagnosis criteria and technical limitations of exome sequencing, such as inability to detect deep intronic or difficulty in reliably calling copy-number variants. In fact, if Mendelian disease and variants predisposing to nephrolithiasis are combined, the overall diagnostic yield is 11 % (87 of 787 individuals), comparable to neurometabolic disorders or cancer, where exome sequencing is routinely used [16,47,48]. Interestingly, the diagnostic yield was not different between first time and recurrent stone formers and did not differ when stratified by sex. In NKSF, no Mendelian kidney stone disease was detected, and LP/P variants in nephrolithiasis genes were significantly less prevalent compared to KSF (1.8 % vs. 8.1 %).

Cystinuria due to biallelic diagnostic variants in *SLC3A1* or *SLC7A9* was the most common Mendelian disease in our cohort (57 %; n=13 of 23 individuals with Mendelian disease), and an additional six individuals carried monoallelic LP/P variants in *SLC7A9*. We confirmed a high prevalence of CKD in KSF with cystinuria [49]. Yet, this applied only to KSF with biallelic diagnostic variants. We were unable to provide a genetic diagnosis in nine KSF with a

cystinuria phenotype, including seven individuals with cystine-containing kidney stones and two individuals with elevated urinary cystine excretion. While this could at least partially be due to technical limitations, such as the inability to detect deep-intronic variants or missed CNVs [39], it may also indicate that the genetic architecture of cystinuria is not yet completely deciphered. Of note, the prevalence of phenotypic cystinuria (including patients with elevated urinary cysteine but no cysteine stones) in the BKSR (22/787 KSF; 2.8 %) was only marginally higher than described for adult stone formers (1-2%), but significantly lower than in pediatric cohorts (6-10%)[50,51].

Of the LP/P variants detected predisposing to nephrolithiasis, by far the most common (63 %; n=40 of 64) were variants in *SLC34A1/3* or *SLC9A3R1*, highlighting the pathophysiological importance of renal phosphate loss in increasing risk for kidney stone formation [12,52]. However, the phenotype of KSF with LP/P variants in these genes on a free choice diet was very subtle and mostly indistinguishable from KSF without predisposing variants. Future studies need to determine if the phenotype of KSF with LP/P variants in these genes can be unmasked by dietary interventions (e.g., by a low phosphate diet) for diagnostic purposes and mitigated for prevention of future stone events (e.g., by phosphate supplementation).

We only detected one KSF with a monoallelic LP/P variant in *ADCY10,* indicating that variants in *ADCY10* are not contributing significantly to the overall nephrolithiasis risk, at least in individuals of European descent [43,53]. Further, we identified four patients with *CASR* LP/P variants in our cohort with very variable phenotypes. Atypical characteristics such as chondrocalcinosis or kidney stones have been described in patients with FHH1 but complicate the separation from primary hyperparathyroidism (PHPT) [54-56]. Yet, separation is of critical importance: parathyroidectomy is the first-line treatment in PHPT but surgery is ineffective in FHH1. Additional complexity arises from the fact that PHPT and FHH1 can co-exist, as observed in patient# 6023 [57,58]. Interestingly, the diagnostic rate in uric acid stone formers was very low, with no Mendelian disease and only one monoallelic LP/P variant (in *ADCY10*)

found in 38 uric acid stone formers, suggesting no significant contribution of Mendelian disease in uric acid stone disease.

Genetic diagnoses in KSF can have important prognostic implications and therapeutic consequences. However, while the pathophysiology is well delineated, the effectiveness of therapeutic interventions has not been studied systematically in most forms of Mendelian nephrolithiasis. The widespread use of genetic testing will facilitate the inclusion of KSF with Mendelian disease and LP/P variants predisposing to nephrolithiasis in prospective registries and clinical trials to study disease evolution and evaluate tailored therapeutic interventions. Currently, KSF are only subjected to genetic testing if a specific disease is strongly suspected. While clinical signs suggesting an inherited disorder have been proposed [59], Mendelian disease was missed or misclassified in five of 23 (22%) KSF in our cohort, despite individual review of each case by a kidney stone expert. The situation is even far more difficult in KSF with LP/P variants predisposing to nephrolithiasis: the associated phenotypes were discrete and variable with a large phenotype overlap with KSF without LP/P variants. Yet, the low prevalence of LP/P variants detected in NKSF suggests a rather high penetrance of LP/P variants predisposing to nephrolithiasis in adults, with the limitation of a small NKSF sample size. Together, these results preclude the definition of clear phenotypic criteria to reliably prioritize genetic testing in adult KSF, with the notable exception of Mendelian forms of nephrolithiasis with clear and unequivocal phenotypes.. In addition, LP/P variants present with their own challenges in clinics and considerations for clinical reporting of LP/P variants and risk alleles have recently been suggested [14].

Strengths of our study include very minor patient pre-selection with broad inclusion criteria, the large sample size, availability of ethnically matched non-stone forming controls and the detailed phenotype. Our study also has limitations, such as the inability to detect deep intronic and copy-number variants, lack of family recruitment for segregation analyses, monocentric recruitment, overrepresentation of men and a limited ethnic diversity.

In conclusion, our study shows that Mendelian disease is far less common in adult KSF than previously suggested, but simultaneously reveals a high prevalence of LP/P variants predisposing to nephrolithiasis in adult KSF. Our study also highlights the potential of genetic testing in unselected adult KSF for accurate assessment of Mendelian disease and identification of variants predisposing to nephrolithiasis to direct patients to tailored therapies and clinical trials.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

DGF, AS and JAS conceptualized the study. DGF and MAA acquired financial support. MAA, EGO and DGF designed data analysis plans. MAA, EGO and MB performed data analysis. MAA, EGO, MB, AS and DGF performed data analysis and data interpretation. MAA wrote the first draft of the manuscript. All authors contributed to discussion and editing of text and approved the final version of the manuscript.

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DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon request. Stratified genetic data will be accessible upon publication at European Genome-Phenome Archive (EGA) (https://ega-archive.org/).

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Table 1 | Characteristics of the study cohort. Characteristics are indicated separately for participants with and without kidney stone disease. Categorical variables are described by number of participants N (%), continuous variables are described by their mean (SD) or median (25^{th}-75^{th} percentile). eGFR, estimated glomerular filtration rate; BSA, body surface area; PTH, parathyroid hormone; SD, standard deviation.
* The Non kidney stone formers (NKSF) with LP/P variants (#5948, #5500) have been removed for phenotypic analyses.

Characteristics	Kidney stone formers (N=787)	Non kidney stone formers (N=112) *	*p*-values
Age, years	46.64 (14.57)	42.41 (14.88)	0.007
Males	565 (71.8%)	61 (54.0%)	<0.001
Body mass index, kg/m^2	26.83 (5.08)	26.34 (4.55)	0.37
Hypertension	255 (36.0%)	28 (35.9%)	1.00
Diabetes	24 (3.1%)	1 (1.1%)	0.50
Obesity, BMI ≥ 30 kg/m^2	168 (22.4%)	14 (15.1%)	0.11
Hyperuricemia	346 (44.0%)	54 (47.8%)	0.48
Family history of kidney stone disease	317 (43.6%)	3 (6.8%)	<0.001
Kidney stone recurrence, > 1 stone event	614 (82.2%)		
Age at first stone event, years	35.41 (13.85)		
Total number of stone events	3.00 (2.00, 4.00)		
Blood parameters			
Total calcium, mmol/L	2.36 (0.12)	2.30 (0.12)	<0.001
Ionized calcium, mmol/L	1.21 (1.19, 1.23)	1.19 (1.18, 1.22)	0.002
Phosphorus, mmol/L	1.01 (0.17)	1.01 (0.16)	0.89
Magnesium, mmol/L	0.83 (0.07)	0.86 (0.08)	<0.001
Uric acid, umol/L	328.96 (169.56)	308.44 (71.51)	0.26
Intact PTH, ng/L	39.06 (30.00, 51.00)	37.50 (30.50, 48.00)	0.56
25-OH Vitamin D_3, nmol/L	40.18 (27.00, 59.00)	48.50 (32.72, 72.44)	0.017
eGFR creatinine Equation CKD-EPI 2009, mL/min per 1.73 m2 BSA	94.18 (21.08)	96.31 (21.88)	0.36
Urine parameters			
Total urine volume, L/24h	1.98 (1.47, 2.54)	2.10 (1.49, 2.65)	0.41
Urine pH	5.94 (5.39, 6.60)	6.00 (5.50, 6.75)	0.14
Urine sodium / creatinine ratio, mmol/mmol/24h	13.62 (10.97, 16.98)	13.76 (9.92, 16.21)	0.37
Urine potassium / creatinine ratio, mmol/mmol/24h	4.70 (3.77, 5.82)	5.56 (4.43, 6.55)	<0.001
Urine uric acid / creatinine ratio, mmol/mmol/24h	0.61 (0.10)	0.38 (0.27)	<0.001
Urine calcium / creatinine ratio, mmol/mmol/24h	0.43 (0.30, 0.58)	0.33 (0.21, 0.44)	<0.001
Urine magnesium / creatinine ratio, mmol/mmol/24h	0.30 (0.24, 0.38)	0.32 (0.27, 0.42)	0.059
Urine citrate / creatinine ratio, mmol/mmol/24h	0.19 (0.13, 0.27)	0.24 (0.18, 0.30)	0.003
Urine phosphate / creatinine ratio, mmol/mmol/24h	2.18 (0.54)	2.24 (0.57)	0.41
Urine oxalate / creatinine ratio, mmol/mmol/24h	0.03 (0.02, 0.04)	0.03 (0.02, 0.05)	0.062
Stone phenotypes			
Calcium oxalate dihydrate	73 (12.9%)		
Calcium oxalate monohydrate	287 (50.8%)		
Calcium phosphate	130 (23.0%)		
Uric acid	38 (6.7%)		
Cystine	18 (3.2%)		
Struvite	15 (2.7%)		
Bone Mineral Density			
Lumbar Spine BMD, g/cm^2	1.02 (0.14)	1.06 (0.13)	0.011
Lumbar Spine T-Score	-0.57 (1.26)	-0.11 (1.20)	0.005
Lumbar Spine Z-Score	-0.11 (1.38)	0.29 (1.29)	0.024
Femoral neck BMD, g/cm^2	0.84 (0.13)	0.86 (0.12)	0.21
Femoral neck T-Score	-0.55 (1.02)	-0.31 (0.95)	0.076
Femoral neck Z-Score	0.11 (0.99)	0.24 (0.86)	0.30

Table 2: Genotype and phenotype of patients with Mendelian diagnosis. Acc.N°: RefSeq accession number, ACMG: American College of Medical Genetics, AF: allele frequency, AR: autosomal recessive, AD: autosomal dominant, dbSNP: Reference SNP number of variant, het: heterozygous, hom: homozygous, , LP: likely pathogenic, P: pathogenic, tot: total, Novel: mutation detected for the first time in this study/not previously described, NS: nonsense, Family history: N= no, Y=yes, M: male, F: female, mo: months, NL: Nephrolithiasis, NC: Nephrocalcinosis, dRTA: distal renal tubular acidosis, PHPTH: primary hyperparathyroidism, CKD: chronic kidney disease. Stone analysis: CaOx: Calcium oxalate, COM: Calcium oxalate monohydrate, COD: Calcium oxalate dihydrate, CaP: Calcium phosphate, CA: Carbonate apatite, Brushite: Calcium hydrogen phosphate dihydrate, MAP: Magnesium ammonium phosphate (=Struvite), UA: Uric acid.

ID	Gene	Inheri-tance	Allelic state	Nucleotide change	Amino acid change	ACMG Class	ACMG criteria	AF gnoMAD (allel/tot/hom) gnoMAD (NFE)	Acc. N° dbSNP	Sex	Age at first stone	Number of past stone events	Stone composition analysis (%)	Family history	Key Phenotype	Clinical diagnosis before WES	Genetic diagnosis after WES
	AGXT – Primary Hyperoxaluria Type 1 (PH1)																
5011	*AGXT*	AR	hom	c.466G>A	p.Gly156Arg	P	PS1, PM5, PM1, PP2, PM2, PP3, PP5	7/226.862/0 5/101.706/0	NM_000030.3 rs121908530	M	4	8	1) 80 COM, 20 COD 2) 80 COM, 20 COD	N	CaOx NL, normal kidney function Hyperoxaluria (1100)	PH1	PH1
	ATP6V1B1 – Distal renal tubular acidosis with sensorineural hearing loss/deafness (AR dRTA)																
5775	*ATP6V1B1*	AR	hom	c.242T>C	p.Leu81Pro	P	PP3s, PM2, PP5	1/250.636/0 0/113.172/0	NM_001692.4 rs121964880	M	N/A	>5	100 CA	N	CaP NL, dRTA, medullary NC, sensorineural deafness	AR-dRTA	AR-dRTA
	CYP24A1 – (Infantile) HC/NL, 1,25-OH vitamin D-24 hydroxylase deficiency																
5033	*CYP24A1*	AR	hom	c.1186C>T	p.Arg396Trp	P	PM2, PM5, PP3m, PP5vs	167/251.228/1 124/113.676/1	NM_000782.5 rs114368325	F	24	1	N/A	Y	NL, osteoporosis, single kidney cyst. Hypercalcemia (total 2.64; ionized 1.4) Normal PTH (47) Normal 25-Vit D (83) Normal 1,25-Vit D (105) Hypercalciuria (7.7)	Idiopathic, infantile HC, NL, s/p parathyreo-idectomy	(Infantile) HC/NL
5052	*CYP24A1*	AR	hom	c.1186C>T	p.Arg396Trp	P	PM2, PM5, PP3m, PP5vs	167/251.228/1 124/113.676/1	NM_000782.5 rs114368325	M	N/A	1	N/A	Y	NL, Nephrocalcinosis, multiple kidney cysts, CKD stage 4, Osteopenia, Chondrocalcinosis. Hypercalcemia (total 2.8; ionized 1.41) Low PTH (4) Low 25-Vit D (16) Normal 1,25-Vit D (112) Hypercalciuria (7.2)	Infantile HC/NL, CKD IV° due to NC, s/p parathyreo-idectomy	(Infantile) HC/NL
5152	*CYP24A1*	AR	het	c.1186C>T	p.Arg396Trp	P	PM2, PM5, PP3m, PP5vs	167/251.228/1 124/113.676/1	NM_000782.5 rs114368325	M	26	2	N/A	N	NL, multiple kidney cysts. Normocalcemia (total 2.4; ionized 1.26) Low PTH (10) Normal 25-Vit D (87) Normal 1,25-Vit D (177) Hypercalciuria (19)	Idiopathic NL, polycystic kidney disease	(Infantile) HC/NL

			het	c.428_430 del	p.Glu143del	P	PM2, BS2, PM4, PP5vs	133/251.266/1 119/113.708/1	rs777676129								
5334	*CYP24A1* *(NB* also CLDN16 monoallelic)*	AR	het	c.1226T>C	p.Leu409Ser	LP	PM2, PP5vs	188/251.066/0 153/113.656/0	NM_000782.5 rs6068812	M	21	3	1) 90 COM, 10 COD; 2) 80 COM, 20 COD	Y	CaOx NL, osteopenia, multiple kidney cysts. Normal plasma calcium (total 2.48; ionized 1.27) Normal plasma magnesium (0.72) Low PTH (9) Normal 25-Vit D (59) Normal 1,25-Vit D (93) Hypercalciuria (14.3)	Idiopathic NL, polycystic kidney disease	(Infantile), HC/NL
			het	c.400T>G	p.Trp134Gly	LP	PM2, PP3m, PP5s	4/251.146/0 3/113.702/0	rs1170841548								
5794	*CYP24A1*	AR	hom	c.428_430del	p.Glu143del	P	PM2, BS2, PM4, PP5vs	133/251.266/1 119/113.708/1	NM_000782.5 rs777676129	M	61	2	N/A	N	NL, NC, multiple kidney cysts, incomplete dRTA Normal plasma calcium (total 2.49; ionized 1.25) Low PTH (12) Low 25-Vit D (26) Normal 1,25-Vit D (73) Hypercalciuria (10.2)	Medullary sponge kidney	(Infantile), HC/NL
	GRHPR – Primary hyperoxaluria, type 2 (PH2)																
5432	*GRHPR*	AR	hom	c.103del	p.Asp35ThrfsTer11	P	PVS1, PM2, PP5vs	59/248.776/0 57/111.550/0	NM_012203.2 rs80356708	M	28	8	1) 80 COM, 20 COD 2): 90 COM, 10 COD 3): 90 COM, 10 COD	Y	CaOx NL, CKD stage 3 Hyperoxaluria (1796) Low urine glycolate/ high urine glycerate Hypocitraturia (1.3)	PH2, start dialysis at age 57	PH2
	HOGA1 –Primary hyperoxaluria, type 3 (PH3)																
5595	*HOGA1*	AR	hom	c.700+5G>T	p.?	P	PM2, PP3m, PP5vs	312/251.412/1 239/113.698/1	NM_138413.4 rs185803104	M	36	5	80 COM, 20 COD	N	CaOx NL, CKD stage 2 Normal urine calcium (2.88) Normal urine oxalate random diet (428-463), high urine oxalate low Ca/Na diet (1090)	Idiopathic NL	PH3

	SLC3A1 – Cystinuria type A															
5030	*SLC3A1*	AR	het	c.1400T>C	p.Met467Thr	P	PM1, PP2, PM2, PM5, PP3, PP5vs, PP4	627/251.156/4479/113.498/3	NM_000341.4 rs121912691	F	18	5	80 Cystine, 20 CA	Y	Cystine NL, CKD stage 3, osteoporosis. Urine cystine (115-254 mmol/mol Creatinine) Norm: <30)	Cystinuria	Cystinuria type A
			het	c.1617+1G>A	p.?	LP	PVS1s, PM2, PP4	4/250.972/0	rs558461213								
5092	*SLC3A1*	AR	het	c.851A>G	p.Asp284Gly	LP	PP3s, PM2, PP2, PP4	NF / NF	NM_000341.4 -	M	17	1	100 Cystine	Y	Cystine NL, Urine cystine (263 mmol/mol Creatinine) Norm: <30)	Cystinuria	Cystinuria type A
			het	c.1617+5G>A	p.?	LP	PS4m, PM2, PP3m, PP4	NF / NF	-								
5094	*SLC3A1*	AR	het	c.1400T>C	p.Met467Thr	P	PM1, PP2, PM2, PM5, PP3, PP5vs, PP4	627/251.156/4479/113.498/3	NM_000341.4 rs121912691	M	54	5	1) 90 CA 10 COM 2) 100 cystine	Y	Cystine NL, CKD stage 2, kidney cysts Urine cystine (247 mmol/mol Creatinine) Norm: <30)	Cystinuria	Cystinuria type A
			het	c.809G>A	p.Arg270Gln	LP	PP3s, PM2, PP2, PP4	2/251.430/0 0/113.720/0	rs142358712								
5156	*SLC3A1*	AR	hom	c.1400T>C	p.Met467Thr	P	PM1, PP2, PM2, PM5, PP3, PP5vs, PP4	627/251.156/4479/113.498/3	NM_000341.4 rs121912691	M	22	5	100 cystine	Y	Cystine NL Urine cystine (193-235 mmol/mol Creatinine) Norm: <30)	Cystinuria	Cystinuria type A
5162	*SLC3A1*	AR	het	c.1400T>C	p.Met467Thr	P	PM1, PP2, PM2, PM5, PP3, PP5vs, PP4	627/251.156/4479/113.498/3	NM_000341.4 rs121912691	F	24	5	100 cystine	N	Cystine NL, CKD stage 3 Urine cystine (267-939 mmol/mol Creatinine) Norm: <30)	Cystinuria	Cystinuria type A
			het	c.787A>C	p.Ser263Arg	LP	PP3m, PM2, PP2, PP4	NF / NF	-								
5168	*SLC3A1*	AR	het	c.851A>G	p.Asp284Gly	LP	PP3s, PM2, PP2, PP4	NF / NF	NM_000341.4 -	F	18	5	1) 100 cystine 2) 100 cystine 3) 90 cystine, 10 CA	Y	Cystine NL Urine cystine (213-435 mmol/mol Creatinine) Norm: <30)	Cystinuria	Cystinuria type A
			het	c.1617+5G>A	p.?	LP	PS4m, PM2, PP3, PP4	NF / NF	-								

5479	*SLC3A1*	AR	het	c.1400T>C	p.Met467Thr	P	PM1, PP2, PM2, PM5, PP3, PP5vs, PP4	627/251.156/4479/113.498/3	NM_000341.4 rs121912691	F	22	3	1) 100 cystine 2) 100 cystine	Y	Cystine NL, CKD stage 2 Urine cystine (191-232 mmol/mol Creatinine) Norm: <30)	Cystinuria	Cystinuria type A
			het	c.1094G>T	p.Arg365Leu	P	PM1, PP2, PM2, PM5, PP3m, PP5, PP4	2/251.376/0 1/113.678/0	rs567478582								
	SLC7A9	AD/AR	het	C.544G>A	p.Ala182Thr	P	PM2, PM1supp, PP2, PP5vs	727/282810/2 504/113.736/2	NM_014270.5 rs79389353				See above		See above		
5288	*SLC3A1*	AR	hom	c.647C>T	p.Thr216Met	LP	PP3s, PM2, PP2, PP5, PP4	23/251.414/0 15/113.710/0	NM_000341.4 rs369641941	F	19	5	1)-5) 100 cystine	N	Cystine NL, CKD stage 3 Urine cystine (291-306 mmol/mol Creatinine) Norm: <30)	Cystinuria	Cystinuria type A
5578	*SLC3A1*	AR	hom	c.833T>C	p.Phe278Ser	LP	PP3s, PM2, PP2, PP4	1/251.432/0 1/113.722/0	NM_000341.4 rs762218116	M	20	18	N/A	N	Cystine NL, CKD stage 3 Urine cystine (145-239 mmol/mol Creatinine) Norm: <30)	Cystinuria	Cystinuria type A
	SLC7A9 – Cystinuria, type B																
5038	*SLC7A9*	AR	het	c.1316A>G	p.Tyr439Cys	LP	PM2, PP3m, PP2, PP4	NF / NF	NM_014270.5	F	17	28	1) 60 cystine, 40 CA 2) 100 cystine	N	Cystine NL, CKD stage 3 Urine cystine (301 mmol/mol Creatinine) Norm: <30)	Cystinuria	Cystinuria type B
			het	c.775G>A	p.Gly259Arg	LP	PP3m, PM2, PP2, PP5, PP4	2/251.260/0 2/113.626/0	rs121908483								
5160	*SLC7A9*	AR	hom	c.313G>A	p.Gly105Arg	P	PM1, PP2, PM5supp, PP3m, PP5, PP4	96/251.028/1 64/113.436/1	NM_014270.5 rs121908480	M	1	4	100 cystine	Y	Cystine NL Urine cystine (286 mmol/mol Creatinine) Norm: <30)	Cystinuria	Cystinuria type B
5412	*SLC7A9*	AR	hom	c.313G>A	p.Gly105Arg	P	PM1, PP2, PM5supp, PP3m, PP5, PP4	96/251.028/1 64/113.436/1	NM_014270.5 rs121908480	M	44	5	100 cystine	N	Cystine NL Urine cystine (223 mmol/mol Creatinine) Norm: <30)	Cystinuria	Cystinuria type B

5716	*SLC7A9*	AR	hom	c.313G>A	p.Gly105Arg	P	PM1, PP2, PM5supp, PP3m, PP5, PP4	96/251.028/164/113.436/1	NM_014270.5 rs121908480	F	16	2	100 cystine	Y	Cystine NL Urine cystine (273 mmol/mol Creatinine) Norm: <30)	Cystinuria	Cystinuria type B
	SLC12A1 – Bartter syndrome, type 2																
5769	*SLC12A1*	AR	het	c.1493C>T	p.Ala498Val	VUS-LP	PP3s, PM2, PP4	4/152.154/00/112.752/0	NM_000338.3 rs1366101480	F	22	1	1) 100 CA 2) 20 COD, 20 Struvite, 60 CA 3) 20 COD, 20 Struvite, 60 CA	N	CaP/CaOx NL, NC, CKD stage 2 Low Plasma K (3.3) High plasma bicarbonate (28) High urine pH (7.0) High PTH (88) Low 25-Vit D (22) Normal 1,25-Vit D (114) Hypercalciuria (5.31) Hypocitraturia (1.8) Normal urine oxalate (219)	Idiopathic NL and NC, CKD, incomplete dRTA	Bartter syndrome, type 2
			het	c.1878G>A	p.Trp626Ter	LP	PVS1, PM2, PP4	1/251.116/01/113.462/0	rs768765027								

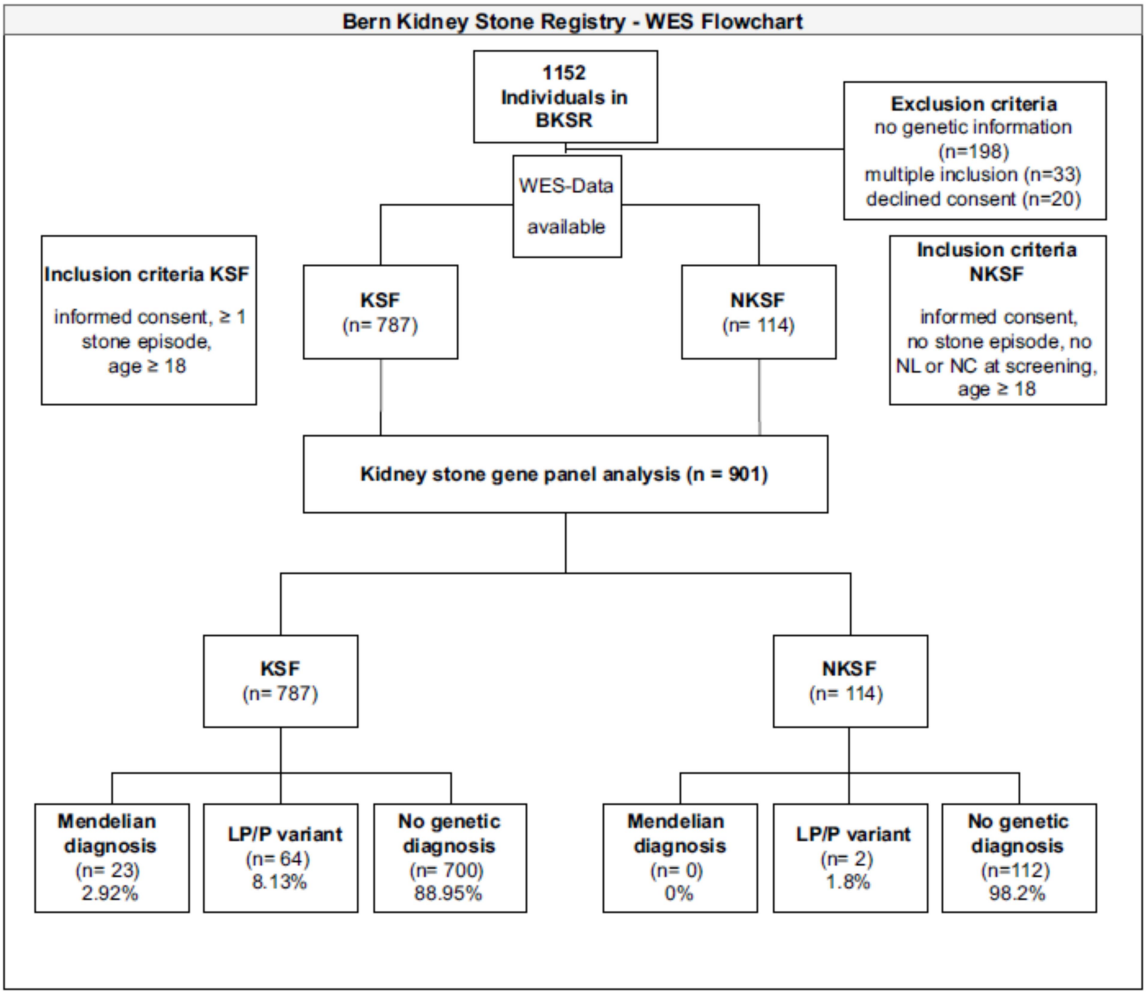
Normal/pathologic values laboratory parameters:

Plasma calcium (mmol/l): 2.15-2.5
Plasma magnesium (mmol/l): 0.66-1.07
Plasma phosphate (mmol/l): 0.81-1.45
PTH (pg/ml): 15-65
25-Vit D (nmol/l): 50-135
1,25-Vit D (pmol/l): 48-190

Hypercalciuria (mmol/24h): >5
Hyperoxaluria (µmol/24h): >500
Hypocitraturia (mmol/24h): <1.65
Urine magnesium (mmol/24h): 3-5
Urine phosphate (mmol/24h): 13-32

Table 3 | Characteristics of stone formers with and without pathogenic gene variants. Characteristics are indicated separately for stone formers with Mendelian disease and with/without LP/P predisposing gene variants**.** Categorical variables are described by number of participants N (%), continuous variables are described by their mean (SD) or median (25^{th}-75^{th} percentile). eGFR, estimated glomerular filtration rate; BSA, body surface area; PTH, parathyroid hormone; SD, standard deviation.

Characteristics	No genetic diagnosis (N=700)	LP/P variants (N=64)	Mendelian disease (N=23)	*p*-values
Age, years	47.18 (14.30)	43.73 (15.78)	39.13 (15.83)	0.009
Males	502 (71.7%)	50 (78.1%)	14 (60.9%)	0.27
Body mass index, kg/m^2	26.94 (5.12)	26.56 (4.91)	24.42 (3.15)	0.074
Hypertension	223 (35.4%)	23 (41.1%)	9 (40.9%)	0.57
Diabetes	23 (3.4%)	1 (1.6%)	0 (0.0%)	0.86
Obesity, BMI ≥30 kg/m^2	152 (22.8%)	15 (24.2%)	1 (4.8%)	0.12
Hyperuricemia	306 (43.7%)	28 (43.8%)	12 (52.2%)	0.75
Family history of kidney stone disease	278 (43.1%)	29 (49.2%)	9 (40.9%)	0.66
Kidney stone recurrence, > 1 stone event	547 (82.5%)	49 (79.0%)	18 (81.8%)	0.73
Age at first stone event, years	35.96 (13.76)	33.89 (13.47)	23.48 (12.29)	<0.001
Total number of stone events	3.00 (2.00, 4.00)	3.00 (2.00, 4.00)	5.00 (2.00, 5.00)	0.14
Blood parameters				
Total calcium, mmol/L	2.35 (0.12)	2.38 (0.12)	2.40 (0.11)	0.075
Ionized calcium, mmol/L	1.21 (1.19, 1.23)	1.22 (1.19, 1.25)	1.23 (1.21, 1.25)	0.17
Phosphorus, mmol/L	1.01 (0.17)	0.96 (0.14)	1.07 (0.13)	0.037
Magnesium, mmol/L	0.83 (0.07)	0.84 (0.07)	0.81 (0.07)	0.40
Uric acid, umol/L	328.87 (177.47)	325.73 (74.03)	342.85 (78.56)	0.93
Intact PTH, ng/L	40.00 (30.00, 52.00)	35.60 (27.80, 44.00)	36.00 (22.00, 55.00)	0.069
25-OH Vitamin D_3, nmol/L	40.00 (27.00, 59.00)	45.00 (29.50, 60.00)	32.45 (22.00, 73.00)	0.71
eGFR creatinine Equation CKD-EPI 2009, mL/min per 1.73 m2 BSA	96.42 (81.53, 109.15)	99.10 (83.07, 110.36)	93.52 (71.42, 97.96)	0.28
Urine parameters				
Total urine volume, L/24h	1.96 (1.47, 2.50)	2.05 (1.37, 2.59)	2.54 (2.38, 3.30)	<0.001
Urine pH	5.89 (5.35, 6.52)	6.29 (5.60, 6.70)	6.82 (6.63, 7.16)	<0.001
Urine sodium / creatinine ratio, mmol/mmol/24h	13.58 (10.97, 16.79)	12.39 (10.70, 17.20)	18.78 (15.27, 20.41)	<0.001
Urine potassium / creatinine ratio, mmol/mmol/24h	4.69 (3.75, 5.78)	4.26 (3.71, 5.56)	6.52 (5.18, 7.57)	<0.001
Urine uric acid / creatinine ratio, mmol/mmol/24h	0.62 (0.10)	0.59 (0.10)	0.60 (0.14)	0.19
Urine calcium / creatinine ratio, mmol/mmol/24h	0.43 (0.30, 0.58)	0.47 (0.36, 0.60)	0.40 (0.24, 0.53)	0.13
Urine magnesium / creatinine ratio, mmol/mmol/24h	0.30 (0.24, 0.38)	0.28 (0.23, 0.35)	0.38 (0.28, 0.47)	0.039
Urine citrate / creatinine ratio, mmol/mmol/24h	0.19 (0.13, 0.27)	0.20 (0.14, 0.27)	0.22 (0.13, 0.31)	0.72
Urine phosphate / creatinine ratio, mmol/mmol/24h	2.19 (0.55)	2.15 (0.47)	2.15 (0.46)	0.82
Urine oxalate / creatinine ratio, mmol/mmol/24h	0.03 (0.02, 0.04)	0.02 (0.01, 0.04)	0.04 (0.02, 0.05)	0.12
Urine cystine / creatinine ratio, mmol/mol/24h	4.00 (3.00, 5.00)	4.00 (3.00, 6.00)	204.00 (3.00, 263.00)	<0.001
Stone phenotypes				
Calcium oxalate dihydrate	66 (13.1%)	7 (15.9%)	0 (0.0%)	0.24
Calcium oxalate monohydrate	264 (52.4%)	19 (43.2%)	4 (23.5%)	0.035
Calcium phosphate	115 (22.8%)	13 (29.5%)	2 (11.8%)	0.35
Uric acid	37 (7.3%)	1 (2.3%)	0 (0.0%)	0.40
Cystine	7 (1.4%)	0 (0.0%)	11 (64.7%)	<0.001
Struvite	12 (2.4%)	3 (6.8%)	0 (0.0%)	0.17
Bone Mineral Density				
Lumbar Spine BMD, g/cm^2	1.01 (0.14)	1.00 (0.11)	1.11 (0.13)	0.022
Lumbar Spine T-Score	-0.58 (1.28)	-0.71 (1.01)	0.32 (1.05)	0.022
Lumbar Spine Z-Score	-0.11 (1.40)	-0.28 (1.14)	0.64 (1.15)	0.086
Femoral neck BMD, g/cm^2	0.83 (0.13)	0.84 (0.10)	0.89 (0.21)	0.37
Femoral neck T-Score	-0.57 (1.02)	-0.48 (0.87)	-0.14 (1.50)	0.26
Femoral neck Z-Score	0.11 (1.00)	0.09 (0.79)	0.34 (1.39)	0.67



Kidney Stone Gene Panel

Calcium Metabolism		Renal Tubular Acidosis		Uric Acid Metabolism		Oxalate Metabolism	
ADCY10	AD	*ATP6V0A4*	AR	*HPRT1*	XLR	*AGXT*	AR
CASR	AR	*ATP6V1B1*	AR	*SLC2A9*	AR	*GRHPR*	AR
CLCN5	XLR	*ATP6V1C2*	AR	*SLC22A12*	AD/AR	*HOGA1*	AR
CLDN16	AR	*CA2*	AR	**Bartter Syndrome**		*SLC26A1*	AR
CLDN19	AR	*FOXI1*	AR	*CLCNKB*	AR	**Other**	
CYP24A1	AR	*SLC4A1*	AR	*KCNJ1*	AR	*APRT*	AR
HNF4A	AD (p.R76W)*	*WDR72*	AR	*SLC12A1*	AR	*FAM20A*	AR
OCRL	XLR	**Phosphate Metabolism**		**Cystinuria**		*XDH*	AR
VDR	AD	*SLC9A3R1*	AD	*SLC3A1*	AR		
*specific SNV associated with both MODY and FRTS		*SLC34A1*	AR	*SLC7A9*	AR		
		SLC34A3	AR				

Figure 1. Upper Panel: Flowchart of patient inclusion, exclusion and genetic analysis in the Bern Kidney Stone Registry (BKSR). Data of 1152 individuals recruited into the BKSR were analyzed. After exclusion of individuals without genetic information available, declined consent or multiple inclusions, the analyzed cohort consisted of 901 individuals (787 stone

formers (KSF) and 114 non stone forming controls (NKSF). Before inclusion in the BKSR, NKSF underwent ultrasound imaging to exclude nephrolithiasis (NL) and/or nephrocalcinosis (NC). Genetic analysis was performed in a standardized prioritization pathway in 34 known kidney stone genes. N: number of individuals. Genetic Diagnosis: Likely pathogenic or pathogenic variant according to ACMG criteria. LP/P Variant: monoallelic LP/P variant, predisposing to nephrolithiasis. WES: whole exome sequencing.

Lower Panel: Kidney stone disease gene panel used for genetic analysis. Panel of 34 known nephrolithiasis genes used with their inheritance mode, as accepted for classification as "Mendelian disease" in this manuscript, grouped by phenotypes. AD: autosomal dominant, AR: autosomal recessive, XLR: X-linked recessive, MODY: maturity-onset diabetes of the young, FRTS: Fanconi renal tubular syndrome.

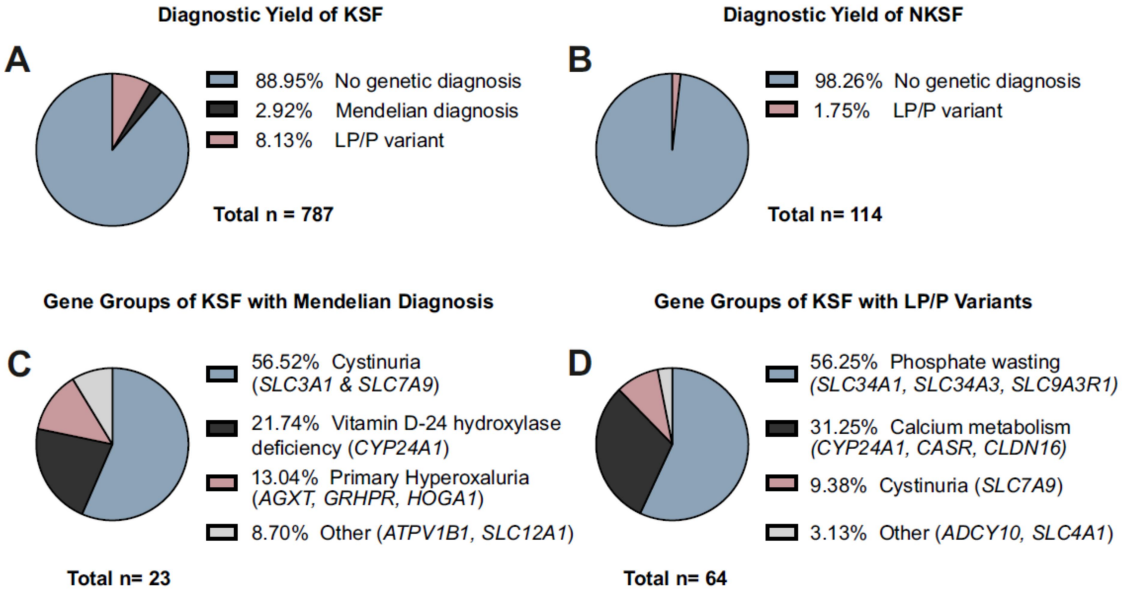


Figure 2. Overall yield of genetic diagnoses (A) Diagnostic yield (Mendelian vs. LP/P variants predisposing to nephrolithiasis)) in kidney stone formers (KSF), (B) Diagnostic yield in non-stone forming controls (NKSF), C) Overview of Mendelian diagnoses in KSF, sorted by phenotype groups, (D) Overview of LP/P variants predisposing to nephrolithiasis in KSF, sorted by phenotype groups.