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3 **The V-ATPase B1 subunit polymorphism p.E161K is associated with impaired urinary**  
4 **acidification in recurrent stone formers**  
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**ABSTRACT**

Mutations in the V-ATPase B1 subunit gene *ATP6V1B1* cause autosomal-recessive dRTA. We previously identified a single nucleotide polymorphism (SNP) in the human B1 subunit (c.481G>A; p.E161K) that displayed greatly diminished pump function *in vitro*. To investigate the impact of this SNP on urinary acidification, we conducted a genotype-phenotype analysis of recurrent stone formers in the Dallas and Bern kidney stone registries. 32 of 555 (5.8 %) of the patients examined were heterozygous for the p.E161K SNP, the remaining 523 patients (94.2 %) carried two wild-type alleles. Adjusted for sex, age, BMI and dietary acid and alkali intake, p.E161K SNP carriers had a tendency to higher urinary pH on a random diet (6.31 versus 6.09; p=0.089). Under an instructed low Ca and Na diet, urinary pH was higher in p.E161K SNP carriers (6.555 versus 6.005; p<0.005). Kidney stones of p.E161K carriers were more likely to contain CaP than stones of wild-type patients. In acute NH<sub>4</sub>Cl loading, p.E161K carriers displayed a higher trough urinary pH (5.34 vs 4.89; p=0.01) than wild-type patients. 14.58 % of wild-type patients and 52.38% of p.E161K carriers were unable to acidify their urine below 5.3 and thus can be considered to have incomplete dRTA. In summary, our data indicate that recurrent stone formers with the V-ATPase B1 subunit p.E161K SNP exhibit a urinary acidification deficit with an increased prevalence of CaP containing kidney stones. The burden of E161K heterozygosity may be a *forme fruste* of dRTA.

## INTRODUCTION

Urinary acidification is achieved by  $H^+$  secretion from  $\alpha$ -intercalated cells in the collecting duct [1]. In these cells,  $H^+$  furnished by the cytosolic carbonic anhydrase is translocated against a concentration gradient by the ATP-driven V-ATPase into the urine. Contemporaneous with apical  $H^+$  secretion, carbonic anhydrase liberates  $HCO_3^-$  ions which exit the  $\alpha$ -intercalated cell basolaterally via the AE1 chloride-base exchanger to complete transepithelial acid-base transport. Dysfunction of  $\alpha$ -intercalated cells causes dRTA, which is characterized by metabolic acidosis in the presence of unduly alkaline urine, growth retardation, rickets, nephrolithiasis, nephrocalcinosis, hypokalemia, and progressive renal failure [2]. dRTA can be acquired or inherited. Mutations in the a4 and B1 subunits of the V-ATPase, the AE1 chloride-base exchanger and the carbonic anhydrase type II enzyme were thus far identified as causes of inherited dRTA in humans [3-6]. Mutations in a4 and B1 subunits cause early onset, autosomal-recessive disease whereas AE1 mutations cause autosomal-dominant, later onset disease. Partial impairment of distal tubular acidification is known as incomplete dRTA and has been associated with osteoporosis and nephrolithiasis [7-9]. In contrast to dRTA which is characterized by overt metabolic acidosis, blood pH and  $HCO_3^-$  are normal in incomplete dRTA but maximal urinary acidification is impaired when challenged [7, 10]. The etiology of incomplete dRTA is unknown, but may be in part due to allelic variants of genes involved in  $H^+$  secretion in  $\alpha$ -intercalated cells.

The V-ATPase consists of two multi-subunit complexes, the  $V_1$  (head) and  $V_0$  (membrane anchored) subunits [11]. The 640 kDa  $V_1$  subunit is composed of subunits A, B, C, D, E, F, G and H. Mammals have two B subunits, the ubiquitous B2 isoform and the B1 isoform which is restricted to specialized epithelia of the inner ear, epididymis and intercalated cells [12]. Numerous homozygous and compound heterozygous missense, nonsense, frameshift and splice-site mutations along the B1 subunit gene *ATP6V1B1* have been reported in individuals

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3 with congenital dRTA [3, 13].

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5 We previously investigated disease-causing missense B1 subunit mutations *in vitro* [14]. In B  
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7 subunit-defective yeast, wild-type human B1 but not mutant B1 subunits rescued the growth  
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9 phenotype by functional complementation. With one exception (p.G316E mutation), all  
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11 mutant B1 subunits studied caused disrupted V-ATPase assembly in cell-based and  
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13 biochemical assays; i.e. the failure to assemble in mammalian cells and inability to  
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15 complement in yeast were concordant features. The extension of our analysis to common  
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17 *ATP6V1B1* SNPs unexpectedly revealed that the SNP (c.481G>A; p.E161K) exhibits greatly  
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19 diminished pump function *in vitro* with the yeast growth assay despite intact assembly in  
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21 mammalian cells [14]. This lack of concordance between pump assembly and function is  
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23 unique and raises the question of whether this is truly a functional variant or some  
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25 phenomenon specific to yeast hosts. If this variant allele affects urinary acidification *in vivo* is  
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27 currently unknown. To address this question we conducted a genotype-phenotype analysis in  
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29 recurrent stone formers (SF).  
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## 37 **RESULTS**

### 38 **Screening and identification of p.E161K carriers**

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41 To identify p.E161K carriers, we conducted a search in the kidney stone registries at the  
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43 Charles and Jane Pak Center of Mineral Metabolism and Clinical Research, UT Southwestern  
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45 Medical Center, Dallas, TX and at the Division of Nephrology, Hypertension and Clinical  
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47 Pharmacology, University Hospital of Bern, Switzerland. The kidney stone registry in Bern  
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49 was initiated in 2004 with the Dallas registry as a template. Thus, both stone registries contain  
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51 patients that underwent the same detailed metabolic work-up protocols, including a one week  
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53 controlled Ca (15-20 mmoles/day) and Na (100 mEq/day) diet intervention protocol (for  
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3 details see Concise Methods). Patients in the Bern registry also underwent dual-energy X-ray  
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5 absorptiometry (DEXA) analysis of bone mineral density (BMD). 555 patients in both  
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7 registries met the inclusion criteria (age  $\geq$  18 years, written informed consent, at least one  
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9 episode of kidney stone disease) and had no exclusion criterion (known medications and  
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11 disease states interfering with urinary acidification, for details see Concise Methods). All 555  
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13 patients were genotyped for the c.481G>A; p.E161K SNP using bidirectional Sanger  
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15 sequencing of exon 6 of *ATP6V1B1*. To exclude additional mutations that could confound the  
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17 analysis, we sequenced the coding regions and intro-exon boundaries of the two genes  
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19 associated with autosomal-recessive dRTA in humans (*ATP6V1B1* and *ATPV0A4*) in all  
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21 p.E161K heterozygous SF included in the study. To distinguish benign from likely disease-  
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23 causing variants, we evaluated each variant individually on the basis of strict criteria as  
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25 described in Concise Methods. The sequencing of the *ATP6V1B1* and *ATPV0A4* genes  
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27 revealed no additional mutations and no additional, likely disease-causing variants.  
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### 35 **Baseline characteristics of study population**

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38 Characteristics of the patients included in the analysis are depicted in Table 1. 32 of the 555  
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40 recurrent SF included in the analysis were heterozygous (5.8 %) for the p.E161K SNP. None  
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42 of the 555 recurrent SF analyzed were homozygous for the SNP. The frequency of p.E161K  
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44 heterozygosity was similar in both registries (5.5 % in the Dallas registry, 5.9 % in the Bern  
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46 registry). As expected for recurrent SF, there were twice as many males than females overall  
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48 in the two cohorts. There were no significant sex differences between wild-type and  
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50 heterozygous patients (74.2 % males versus 68.8% males respectively;  $p=0.50$ ). However,  
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52 heterozygous SF were younger at first presentation at the stone clinic (median 38.3 yr versus  
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54 42.7 yr;  $p=0.042$ ), had a lower BMI (median 25.0 kg/m<sup>2</sup> versus 26.5 kg/m<sup>2</sup>;  $p=0.016$ ) and  
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56 were more likely to have a positive family history of kidney stone disease (60.7% versus  
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3 41.3%,  $p=0.044$ ). Furthermore, calculi of p.E161K carriers were significantly more likely to  
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5 contain CaP (70.0% versus 38.6%;  $p=0.005$ ).  
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7 DEXA analysis revealed no relevant differences in BMD between the two groups of patients.  
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9 The prevalence of osteoporosis and osteopenia was equal in both groups.  
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### 12 **Blood and urinary biochemistries on random outpatient diet**

  
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18 Blood and urinary biochemistries of patients on a random outpatient diet are depicted in Table  
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20 2. There were no differences in plasma electrolytes or renal function between the two groups.  
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22 Random blood glucose was higher in wild-type patients (median 5.0 mmol/l versus 4.8  
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24 mmol/l;  $p=0.0159$ ) and plasma bicarbonate was lower in heterozygous patients (mean 25.2  
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26 mmol/l versus 26.1 mmol/l;  $p=0.0178$ ). Heterozygous SF displayed non-significant trends to  
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28 higher urinary pH (mean 6.31 versus 6.09;  $p=0.089$ ) and a lower 24 hr citrate excretion  
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30 (median 2.01 versus 2.79 mmol/24 hr;  $p=0.08$ ). Normalization to K, K minus P and net  
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32 gastrointestinal absorption (NGIA), all as surrogates of alkali intake, also showed numerically  
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34 lower citrate values in heterozygotes that did not reach statistical significance. 24 hr sulfate  
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36 excretion as a measure of animal protein intake, 24 hr urinary ammonium ( $\text{NH}_4$ ), net acid  
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38 excretion (NAE) and NGIA were comparable between the two groups of patients.  
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### 45 **Blood and urinary biochemistry on an outpatient diet low in Na and Ca**

  
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49 Blood and urinary biochemistries on an instructed outpatient diet low in Na and Ca are  
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51 depicted in Table 3. This diet is not meant as therapy but rather to control extrinsic dietary  
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53 factors allowing one to observe the endogenous characteristics of the subjects [15]. Compared  
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55 to the random outpatient diet, there were clear reductions in both groups of patients in 24 hr  
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57 urinary Na and Ca excretion on the low Ca and Na diet, indicating adherence to the dietary  
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3 instructions. With the exception of plasma P which was higher in the heterozygous patients  
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5 (mean 1.01 versus 0.92 mmol/l;  $p=0.042$ ), blood chemistries revealed no relevant differences  
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7 between the two groups. Heterozygous patients exhibited a significantly higher 24 hr urine pH  
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9 (median 6.555 versus 6.005;  $p=0.0065$ ), however there was no difference in 24 hr citrate  
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11 excretion when diet was controlled (median 2.372 versus 2.653 mmol/24 hr;  $p=0.32$ ). As was  
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13 the case under random diet, 24 hr sulfate excretion, 24 hr urinary  $\text{NH}_4$ , NAE and NGIA were  
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15 comparable between the two groups under the low Na and Ca diet.  
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## 22 **Multivariate analyses**

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25 In the unadjusted analysis, heterozygous carriers of the p.E161K SNP exhibit signs of  
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27 impaired urinary acidification. This includes increased CaP content of calculi, lower plasma  
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29 bicarbonate on a random outpatient diet and a lower urinary pH on a more controlled diet. We  
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31 next performed a multivariate analysis to adjust for known predictors of acid-base  
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33 homeostasis, including sex, age, BMI and dietary acid and alkali intake taking, taking  
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35 between-center variability into account [16-18]. As shown in Table 4, after this adjustment,  
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37 urinary pH on low Ca and Na diet remained significantly different between the two groups of  
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39 patients. However, plasma bicarbonate under random outpatient diet was no longer different  
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41 between the two groups. Due to the relatively low number of heterozygotes, logistic  
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43 regression for stone analysis could not be performed with inclusion of all covariates. Table 5  
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45 shows associations between p.E161 carriers and kidney stone type as odds ratios based on  
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47 mixed effects logistic regression models, alternatively adjusting for sex, age and BMI or for  
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49 combinations of two of these variables, taking center variability into account. E161K  
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51 heterozygosity remains significantly associated with an increased CaP content of calculi.  
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### Response to acute acid loading

We next challenged the renal acid excretory capacity in wild-type (n=48) and heterozygous (n=21) SF by performing the standard NH<sub>4</sub>Cl loading test [7]. There were no significant age and sex differences between the two groups (Table 6). Urinary pH was measured at baseline and hourly for 6 consecutive hours after ingestion of NH<sub>4</sub>Cl gelatin capsules (0.1 g / kg body weight). As shown in Fig. 1A, there was no difference in the median fasting urinary pH at the beginning of the test (5.78 in wild-type versus 5.87 in heterozygous patients; p=0.71). However, we observed a clear difference in the trough urinary pH reached between the two groups of patients (median 4.89 in wild-type versus 5.34 in heterozygous patients; p=0.01; Mann-Whitney U test) (Fig. 1B). This significant difference in trough urinary pH persisted even after adjustment for sex and age (Fig. 1C). 7 of 48 wild-type (14.58%) and 11 of 21 heterozygous patients (52.38%) were unable to acidify their urine below 5.3 and thus had incomplete dRTA (p=0.001, Chi-square test). Baseline and peak urinary NH<sub>4</sub> concentration and urinary NH<sub>4</sub> excretion profiles (AUC) were not different between the two groups of patients (Fig. 1D-F).

### Blood and urinary biochemistry in non SF with the p.E161K SNP

To assess the impact of the p.E161K SNP on urinary pH in non SF, we performed an analysis of blood and urinary parameters of wild-type and p.E161K heterozygous non SF. Biochemical data and DNA of these individuals are deposited in the Dallas stone registry as non SF controls. Inclusion and exclusion criteria for non SF were identical to the ones used for SF with the exception that history of renal stone disease was an exclusion criterion. We identified a total of 148 wild-type and 14 p.E161K heterozygous non SF in the database that met the inclusion criteria and no exclusion criterion. There was no difference between the two groups



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3 with regards to median age (39.9 years versus 35.7 years;  $p=0.37$ ) or sex (40.5% men versus  
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5 28.6% men;  $p=0.64$ ). Blood and 24 hr urinary biochemistries of non SF on a random  
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7 outpatient diet are depicted in Table 7. There were no differences in plasma electrolytes or  
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9 renal function between the two groups. Median plasma bicarbonate tended to be lower in  
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11 heterozygous than in wild-type non SF (24 mmol/l versus 27 mmol/l), but the difference did  
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13 not reach statistical significance ( $p=0.15$ ). Median urinary pH in heterozygous non SF was not  
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15 different from wild-type non SF on a random outpatient diet (6.2 versus 6.1;  $p=0.44$ ).  
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17 However, similar to the observation made in SF, heterozygous non SF displayed a  
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19 significantly higher 24 hr urine pH than wild-type non SF (median 6.7 versus 6.2;  $p=0.048$  )  
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21 on an instructed diet (Table 8). This difference in 24 hr urine pH persisted even after  
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23 adjustment for sex and age ( $p=0.05$ ).  
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## DISCUSSION

Classically, congenital dRTA has been considered an autosomal-recessive disease. A recent study demonstrated that heterozygous carriers in a large family with a B1 truncation mutation (p.Phe468fsX487) were not normal but exhibited features of urinary acidification deficit and elevated stone risk [19]. The phenotype in heterozygotes of this peculiar mutation was attributed to haploinsufficiency since *in vitro* studies were not compatible with negative dominance of this mutation. The authors also failed to find evidence of negative dominance *in vitro* of other known B1 missense mutations and two common SNPs, including p.E161K. If the other known B1 subunit missense mutations also cause a detectable deficit in urinary acidification in a heterozygous state is currently unknown. The functional role of the B1 subunit p.E161K SNP has not been studied *in vivo*.

The goal of the current study was to investigate the phenotype of recurrent SF with the B1 subunit p.E161K SNP. To our knowledge, this is the first study with a comprehensive analysis of the effect of a V-ATPase B1 SNP on urinary acidification. By combining patient data of the Dallas and Bern stone registries, we identified 32 heterozygous p.E161K carriers in a total of 555 recurrent SF, corresponding to an allele frequency of 5.8 %. This is slightly lower compared to a previous study that found the c.481G>A; p.E161K SNP to be in Hardy-Weinberg equilibrium with an allele frequency of 10% [13]. We identified no homozygous patients in our cohort of 555 recurrent SF. Assuming Hardy-Weinberg equilibrium, the observed frequency of heterozygous patients in our cohort would correspond to a homozygosity frequency of 0.000883 %. Thus, ~1100 recurrent SF would be needed for the identification of one homozygous p.E161K carrier.

Hildebrandt and colleagues recently studied a cohort of recurrent SF by high throughput mutation analysis of 30 genes known to cause kidney stone disease [20]. They detected in 14.9 % of cases likely causative mutations in 14 of the 30 genes analyzed, indicating that monogenetic causes of recurrent nephrolithiasis are more prevalent than currently appreciated.

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3 Interestingly, one patient in that analysis with recurrent nephrolithiasis, hypercalciuria and  
4 diminished plasma bicarbonate was found to be homozygous for the p.E161K SNP.  
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7 Our analysis indicates that heterozygous p.E161K SF also exhibit subtle defects in urinary  
8 acidification. p.E161K heterozygous SF had a higher 24h urinary pH under a diet low in Ca  
9 and Na and a tendency to higher urinary 24 h urinary pH under random outpatient diet than  
10 wild-type SF. In support of these findings, p.E161K heterozygous non SF also exhibited a  
11 higher urinary pH under a diet low in Ca and Na than wild-type non SF.  
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17 Challenging renal acid excretory capacity by acute acid loading unmasked the suspected  
18 acidification deficit in p.E161K heterozygous SF and unveiled a high prevalence of  
19 incomplete dRTA in this group of patients. Compatible with the findings of an increased  
20 urinary pH, the CaP content of calcareous stones was significantly higher in p.E161K SNP  
21 carriers. An alkaline urine favors CaP stone formation but decreases the risk of uric acid  
22 precipitation. Using computer-based methods (EQUIL2 [21] and JESS [22]) as well as  
23 empirical physicochemical methods, Pak and coworkers showed that raising urinary pH from  
24 6.0 to 6.5, such as we have observed in our two groups, will increase the brushite relative  
25 supersaturation ratio, saturation index and concentration product ratio from 2.1 to 4.5, 1.8 to  
26 2.3, and 1.4 to 2.5 respectively, thereby escalating risk of calcium phosphate stones [23].  
27 Thus, the p.E161K SNP may augment the individual risk for development of renal calculi.  
28 However, due to the opposing effect on CaP and uric acid stone risk, the prevalence of the  
29 p.E161K SNP in the general population may not greatly differ from the one observed in  
30 recurrent SF. Clearly, additional studies will be needed to answer this question.  
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49 In addition to defects in urinary acidification, B1 subunit mutations are associated with  
50 sensorineural hearing loss [3, 13]. In this study, we did not detect obvious hearing  
51 abnormalities in recurrent SF with the p.E161K carriers, but audiometric investigations were  
52 not performed and thus the impact of the p.E161K SNP on inner ear function remains  
53 unknown at the moment.  
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3 Patients with overt dRTA exhibit low BMD mainly due to low bone formation [24]. The case  
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5 may be different in patients with a mild impairment in urinary acidification and normal  
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7 systemic acid-base balance. A recent study detected no difference in BMD in recurrent SF  
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9 with incomplete dRTA when compared with SF without RTA [25]. We also did not observe  
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11 differences in BMD between p.E161K heterozygous and wild-type SF. This may be related to  
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13 the fact that these subjects do not have sustained systemic acidosis to alter bone biology.  
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16 In summary, our data indicate that recurrent SF with the V-ATPase B1 subunit p.E161K SNP  
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18 exhibit a urinary acidification deficit with an increased prevalence of CaP containing kidney  
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20 stones. The burden of E161K heterozygosity may be a *forme fruste* of distal RTA.  
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## CONCISE METHODS

### Patients and study protocol

The study was conducted with patients recruited at the Division of Nephrology, Hypertension and Clinical Pharmacology at the University Hospital of Bern, Switzerland and at the General Clinical Research Center at the University of Texas Southwestern Medical Center, Dallas, TX with approval by the Institutional Review Board (Dallas) or by the Ethical Committee of the Kanton Bern (Bern). All participants provided written informed consent and the study was conducted in accordance with the Declaration of Helsinki. Patients in the registries were seen at the clinic between March 2004 and March 2014, suffered from at least one stone episode and underwent a three visit mineral metabolism work-up. Mineral metabolism work up included a 24 hr urine on a random outpatient diet and one 24 hr urine after one week under an instructed low Ca (15-20 mmoles/day) and Na (100 mEq/day) diet according to a protocol first established by Pak et al. [26, 27]. Urine and blood analysis were performed at the Central Laboratory of the University Hospital of Bern, Switzerland or at the Center of Mineral Metabolism and Clinical Research University of Texas Southwestern Medical Center, Dallas, TX using standard laboratory methods. Blood gas analysis was done on venous blood samples. Estimated GFR was calculated according to CKD-EPI [28]. Osteodensitometry was performed at the Department of Osteoporosis at the University Hospital of Bern, Switzerland by dual-energy X-ray absorptiometry (DEXA; Hologic QDR 4500A, Hologic, Bedford, MA, USA) at the lumbar spine, the non-dominant femoral neck, the proximal femur, the distal tibial diaphysis and epiphysis. Charts of all patients in the registry were reviewed manually for this study. Inclusion criteria were: informed consent and at least one stone episode. Exclusion criteria for analysis were: Absence of informed consent, genetic diseases causing distal renal tubular acidosis, cystinuria, primary hyperoxaluria, primary or secondary hyperparathyroidism, autoimmune diseases, renal diseases, malignancy, hypo- or

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3 hyperthyroidism, liver diseases, short bowel syndrome or bariatric surgery, chronic urinary  
4 tract infection, pregnancy, mineralocorticoid deficiency, anorexia nervosa, metabolic acidosis,  
5 diarrhoea or medications interfering with urinary acidification during the investigation.  
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9 Biochemical data and DNA of non-stone forming individuals used in the study are deposited  
10 in the Dallas stone registry as non SF controls. Inclusion and exclusion criteria for non SF  
11 included in this study were identical to the ones used for SF with the exception that history of  
12 renal stone disease was an exclusion criterion.  
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### 17 18 19 20 21 **DNA extraction and genotyping**

22 Genomic DNA was extracted from peripheral blood leucocytes using a Nucleospin<sup>®</sup> Blood L  
23 (Macherey-Nagel, Switzerland) DNA extraction kit. In all subjects included in the study (SF  
24 and non SF), exon 6 with adjacent exon-intron boundaries of the *ATP6V1B1* gene was  
25 individually amplified by PCR (AmpliTaq Gold system, Applied Biosystems) using primers  
26 described previously [3]. The DNA sequence of both strands was determined by Sanger  
27 sequencing at Microsynth AG, Switzerland. In addition, in all of the 32 p.E161K  
28 heterozygous SF, coding sequences and intron-exon boundaries of *ATP6V0A4* and *ATP6V1B1*  
29 were sequenced using TruSeq Custom Amplicon v1.5 (Illumina) on a MiSeq (Illumina). Data  
30 were analysed using integrated MiSeq Reporter v2.5.1. Mutations were considered to be  
31 known if they were deposited in the Human Gene Mutation Database  
32 (<http://www.hgmd.cf.ac.uk/ac/index.php>). Nonsynonymous variants were considered as likely  
33 disease-causing according to the following inclusion and exclusion criteria [29]: Inclusion  
34 criteria (1) truncating mutation (stop gained, abrogation of start or stop codon, abrogation of  
35 obligatory splice site, or frame shift) or (2) missense mutation if one of the following is  
36 applied: (1) *in silico* prediction by Polyphen2-HumVar with a score > 0.90, suggesting a  
37 probably damaging effect on the protein level [30] or (2) the given disease-causing allele is  
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3 supported by functional data. Exclusion criteria: (1) allele is present in healthy controls of the  
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5 Exome Variant Server database with a minor allele frequency of > 5.0%.  
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### 8 9 **Acute acid loading**

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11 All p.E161K carriers were invited to participate in the acute acid loading test. Recruitment of  
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13 wild-type SF for NH<sub>4</sub>Cl loading was as follows: all patients referred to our stone clinic for  
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15 metabolic work up of stone disease that met the inclusion criteria but no exclusion criterion  
16  
17 (see section “Patients and Study protocol”) were consecutively enrolled, irrespective of  
18  
19 baseline urinary pH, sex or age. Genotyping was performed after acid loading. Acid loading  
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21 was performed using the short NH<sub>4</sub>Cl loading test as described [7, 31]. NH<sub>4</sub>Cl gelatin  
22  
23 capsules (0.1 g / kg body weight) were given to fasting subjects at 0800 hrs with water in the  
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25 presence of the nursing staff. During the test, fluid intake was ad libitum. Venous blood  
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27 samples were obtained for chemistry, pH and blood gases at 0800, 1000, 1200 hrs. Urine was  
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29 collected hourly from 0800 to 1400 hrs. Urine pH was measured immediately after collection  
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31 with an electrode pH meter that was calibrated every day. Urinary ammonium was assessed  
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33 enzymatically by the glutamate dehydrogenase method.  
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### 41 **Statistical analysis**

42  
43 Continuous variables with normal distribution are expressed as the mean±SD and in case of  
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45 skewed distribution as the median (25<sup>th</sup>-75<sup>th</sup> percentile). Categorical variables are expressed  
46  
47 as numbers and frequencies in %. The shape of the distribution of each continuous variable  
48  
49 was visually inspected and square root, log or inverse transformations were applied to ensure  
50  
51 normality for statistical analyses. For a few number of variables no appropriate transformation  
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53 was found. Welch’s t-test, Mann–Whitney U or chi-square tests were performed where  
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55 appropriate to compare baseline characteristics for continuous and categorical variables. The  
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3 statistical tests were two-sided and a  $p$ -value  $< 0.05$  was considered to indicate a statistically  
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5 significant difference.  
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8 In order to estimate the associations between the p.E161K SNP and acid-base parameters or  
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10 kidney stone type, we first applied univariable linear or logistic regression analyses taking the  
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12 between-center variability into account where available. We next analyzed the independent  
13  
14 associations of the p.E161K SNP with the response variables and assumed that sex, age, BMI,  
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16 dietary acid and alkali intake are most likely to influence these parameters and selected them  
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18 as explanatory co-variables. We therefore performed a multivariate analysis of the influence  
19  
20 of the p.E161K SNP adjusted for the main effects sex, age, BMI, urinary sulfate excretion and  
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22 net gastrointestinal alkali absorption using linear regression and mixed effects linear and  
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24 logistic regression models. We considered the presence of non-linear effects and of  
25  
26 interactions and therefore included quadratic terms and interactions involving up to three  
27  
28 different factors into the full model. Age and urinary sulfate excretion were square root  
29  
30 transformed and BMI was transformed as its negative inverse. All continuous explanatory  
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32 variables were centered to zero to address the potential problem of co-linearity that may be  
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34 induced by adding squared terms and interaction terms to the models. No important co-  
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36 linearities between the prediction variables were detected upon inspection of scatterplot and  
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38 correlation matrices and variance inflation factors. Backward selection was carried out to  
39  
40 eliminate interaction terms and quadratic terms with a  $p$ -value of 0.10 or higher. All parent  
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42 terms of significant higher order terms were kept in the model. All main effects were also kept  
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44 in the model irrespective of their significance. Due to the small number of heterozygotes the  
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46 mixed effects logistic regression models did not converge when applying the full model.  
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48 Therefore, for binary outcomes of kidney stone parameters, mixed effects logistic regression  
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50 models were run with the p.E161K SNP as the only fixed covariate, while adjusting for at  
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52 most two alternating additional covariates at a time and taking the between-center variability  
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3 into account. Estimates for logistic regression with more than three fixed effects and with one  
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5 random effect were not available because of a noninvertible singular Hessian (the matrix of  
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7 second derivatives). Models were validated graphically if appropriate for 1.) homogeneity by  
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9 plotting residuals versus fitted values, for 2.) normality by a histogram of the residuals and a  
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11 quantile-quantile (QQ-) plot, for 3.) homoskedasticity by plotting residuals against each  
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13 explanatory variable used in the final models. These visual inspections did not reveal any  
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15 obvious deviations from homoskedasticity or normality. The statistical analysis was performed  
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17 by using the R Statistics software version 3.0.2 [32].  
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**FIGURE LEGENDS****Figure 1. Acute acid loading by standard NH<sub>4</sub>Cl test in wild-type and p.E161K heterozygous SF.**

48 wild-type (WT) and 21 p.E161K heterozygous (E161K) SF underwent NH<sub>4</sub>Cl loading. 68.8 % of WT and 71.4 % of E161K heterozygous SF tested were male ( $p=0.82$ ). Median age of WT and E161K patients was 41.8 years (IQR 34.5-50.6) and 39.5 years (IQR 25.4-44.8), respectively ( $p=0.17$ ).

A) Urinary pH at beginning of the test before ingestion of NH<sub>4</sub>Cl capsules (time 0). B) Trough urinary pH (nadir pH) reached during the test. C) Trough urinary pH reached during the test, adjusted for sex and median age. For this conditional plot a linear regression model adjusted for sex and age was used. Age was set to the median of 41.8 years and sex to the most common category, men. D) Urinary NH<sub>4</sub> at beginning of the test before ingestion of NH<sub>4</sub>Cl capsules (time 0). E) Peak urinary NH<sub>4</sub> reached during the test. F) Urinary NH<sub>4</sub> excretion profile (area under the curve, AUC). Urinary NH<sub>4</sub> levels were not available in all test participants. Between group differences are determined by Mann–Whitney U test or chi-square test where appropriate and the corresponding  $p$ -value is indicated.

## TABLES

Table 1. Baseline patient characteristics according to E161K heterozygosity (N=555)

Characteristic	Wild-type		Heterozygote		p
Total stone patients	523	94.2% (of total)	32	5.8% (of total)	
Center Bern	351	67.11%	22	68.75%	0.85
Center Dallas	172	32.89%	10	31.25%	
Age at first presentation (yr)	514	42.65 (33.9-53.0)	31	38.30 (32.2-46.3)	0.042
Male sex	382	74.2%	22	68.8%	0.50
BMI (kg/m <sup>2</sup> )	479	26.5 (23.4-29.6)	29	25.0 (22.4-27.1)	0.015
Positive family history of stones	179	41.3%	17	60.7%	0.044
Patients with stones available for analysis	369	71.3%	20	62.5%	0.29
Stones containing calcium oxalate	346	94.0%	18	90.0%	0.47
Stones containing calcium phosphate	142	38.6%	14	70.0%	0.005
Stones containing uric acid	19	5.2%	0	0%	0.30
T-Score LWS	303	-0.51±1.05	20	-0.48±1.61	0.94
T-Score Femur neck	300	-0.54±1.05	20	-0.57±0.97	0.92
T-Score Tibia diaphysis	301	0.40±1.08	19	0.38±0.91	0.93
T-Score Tibia epyphysis	300	-0.58±0.96	19	-0.65±0.87	0.74
Osteoporosis present <sup>a</sup>	21	6.9%	1	4.8%	0.71
Osteopenia present <sup>a</sup>	162	53.1%	13	61.9%	0.44

The number of stone formers is indicated for each characteristic stratified by the genotype. Categorical variables are further described by % and continuous variables by their mean ± standard deviation or by their median (25<sup>th</sup>-75<sup>th</sup> percentile). Between group differences are determined by Welch's t-test, Mann-Whitney U or chi-square test as appropriate and the corresponding p-value is indicated.

<sup>a</sup>Osteoporosis was defined as T-Score < -2.5 and Osteopenia as T-Score < -1.0 and > -2.5



**Table 2. Blood and urine parameters according to E161K heterozygosity on random outpatient diet.**

Characteristic	Normal range	Unit	N	Wild-type	N	Heterozygote	p
Plasma Na	132 - 142	mmol/l	480	140.5±2.2	29	140.6±2.1	0.87
Plasma K	3.5 - 4.7	mmol/l	480	3.9 (3.7-4.1)	29	4 (3.8-4.1)	0.28
Plasma Cl	97 - 108	mmol/l	475	104 (102-106)	29	105 (102-107)	0.25
Plasma Ca total	2.10 - 2.55	mmol/l	481	2.4 (2.3-2.4)	29	2.4 (2.3-2.5)	0.13
Plasma Ca ionized	1.13 - 1.30	mmol/l	343	1.2 (1.2-1.2)	22	1.2 (1.2-1.2)	0.14
Plasma P	0.84 - 1.45	mmol/l	481	1±0.2	29	1.1±0.2	0.11
Plasma Mg	0.75 - 1.00	mmol/l	401	0.8 (0.8-0.9)	24	0.8 (0.8-0.9)	0.23
Plasma Creatinine	59 - 104	µmol/l	481	80 (70-89)	29	80 (69-88)	0.52
eGFR CKD-EPI	>90	ml/min/per 1.73 m <sup>2</sup>	481	98.1 (83.8-109.9)	28	99 (87.5-114.1)	0.39
Plasma urea	3.2 - 8.1	mmol/l	348	5.3 (4.5-6.3)	20	4.9 (4.3-5.6)	0.10
Plasma uric acid	202 - 416	µmol/l	475	325±79.3	29	308.6±81.4	0.30
Plasma random glucose	3.33 - 5.55	mmol/l	474	5.0 (4.6-5.6)	28	4.8 (4.4-5)	0.0159
Blood pH	7.35 - 7.45	—	344	7.392±0.029	22	7.398±0.032	0.38
Plasma Bicarbonate	18.0 - 29.0	mmol/l	469	26.1±2.4	30	25.2±2	0.0178
Plasma anion gap	8 - 16	mmol/l	317	10.3±2.5	21	9.8±2.2	0.31
Serum PTH	15 - 65	pg/ml	427	38 (30-46)	26	34 (23.8-60.8)	0.74
Serum 25-OH-Vitamin D	49 -134	nmol/l	222	40 (24.3-56)	10	35.5 (26.3-40.5)	0.29
Serum 1,25-OH-Vitamin D	48 - 160	pmol/l	343	97 (72.5-125.5)	21	91 (70-132)	0.64
Plasma Alkaline phosphatase	35 - 105	U/l	350	65 (55-76)	21	63 (57-74)	0.80
Urine Dpd/creatinine	2.5 - 9.0	nmol/mmol	311	4 (3.4-4.9)	19	4.8 (3.8-5.5)	0.14
Urine pyridinium crosslinks	—	nmol/l	316	37.3 (23.4-56)	20	33 (19.8-49.1)	0.54
Serum TSH	0.27-4.20	mU/l	347	1.3 (0.9-1.8)	22	1.3 (0.8-1.5)	0.55
Urinary volume	—	ml	502	1998 (1493-2618)	30	1887 (1609-2111)	0.67
Urine pH	—	—	476	6.09±0.64	30	6.31±0.68	0.089
Urine anion gap	—	mmol/l	329	41.3±14.3	22	41.3±12.8	0.98
Urine Na	40 - 220	mmol/24 hr	501	184 (141-236)	30	171.8 (130.1-230.6)	0.35
Urine K	25 - 125	mmol/24 hr	501	60.5 (46-77)	30	58.3 (43.5-66.4)	0.077
Urine Cl	110 - 250	mmol/24 hr	472	172.3 (128.4-225.5)	27	141 (117.5-209.7)	0.14
Urine Ca	2.50 - 7.50	mmol/24 hr	500	6.26 (4.46-8.82)	30	6.82 (5.55-8.88)	0.52
Urine P	13.0 - 42.0	mmol/24 hr	500	30.8 (24.1-38.9)	30	30.1 (26.1-36.1)	0.52
Urine Mg	2.50 - 8.50	mmol/24 hr	497	4.34 (3.29-5.42)	30	4.35 (3.8-4.93)	0.59
Urine uric acid	< 5900	µmol/24 hr	500	3500 (2935-4270)	30	3216 (2633-3594)	0.0039
Urine urea	96 - 556	mmol/24 hr	349	410.6 (329.3-509.3)	22	364.4 (288.6-513.2)	0.21
Urine creatinine	8600 - 19400	µmol/24hr	502	14334±4046	30	13519±3300	0.20
Urine citrate	1.65 - 6.6	mmol/24 hr	487	2.79 (1.81-4.02)	28	2.01 (1.36-3.09)	0.080
Urine citrate/K	—	(mmol/24hr)/ (mmol/24hr)	487	0.046 (0.032-0.064)	28	0.041 (0.023-0.05)	0.12
Urine K-P	—	mmol/ 24 hr	500	28.4 (17.3-41.9)	30	22.6 (14.3-37.1)	0.083
Urine citrate/K-P	—	(mmol/24hr)/ (mmol/24hr)	485	0.098 (0.063-0.173)	28	0.094 (0.043-0.162)	0.41
NGIA	—	mmol/24 hr	468	30.76±29.67	27	26.51±28.62	0.46
Urine SO <sub>4</sub>	—	mmol/24 hr	487	22.2 (16.3-28.5)	28	20.4 (15.5-28)	0.74
Urine oxalate	<500	µmol/24 hr	492	382.4 (286.1-559.4)	28	358 (268.8-482.8)	0.079
Urine glycolate	150 - 600	µmol/24 hr	331	371.5 (148.5-574.9)	20	357.5 (182.4-499.3)	0.56
Urine NH <sub>4</sub>	10 - 107	mmol/24 hr	96	36.8±15.9	4	31.9±4.7	0.32
NAE	—	mmol/24 hr	96	90.1 (63.3-103.8)	4	74.2 (67.32-80.9)	0.20
Urine NH <sub>4</sub> /SO <sub>4</sub>	—	(mmol/24hr)/ (mmol/24hr)	96	1.82 (1.41-2.18)	4	1.9 (1.77-2.9)	0.46
Urine NH <sub>4</sub> /NAE	—	(mmol/24hr)/ (mmol/24hr)	96	0.405±0.092	4	0.447±0.125	0.56

The number of stone formers is indicated for each characteristic stratified by the genotype. Continuous variables are further described by their mean ± standard deviation or median (25<sup>th</sup>-75<sup>th</sup> percentiles) as appropriate. Between group differences are determined by Welch's t-test or Mann-Whitney U test as appropriate and the corresponding p-value is indicated. Abbreviations: PTH, parathormone; TSH, thyroid stimulating hormone; NGIA, net gastrointestinal alkali intake; NH<sub>4</sub>, ammonium; SO<sub>4</sub>, sulfate; NAE, net acid excretion; p, p-value.

Table 3. Blood and urine parameters according to E161K heterozygosity on a low Ca and Na diet.

Characteristic	Normal range	Unit	N	Wild-type	N	Heterozygote	p
Plasma Na	132 - 142	mmol/l	317	139.2±1.8	21	139.4±1	0.39
Plasma K	3.5 - 4.7	mmol/l	317	3.813±0.262	21	3.848±0.186	0.43
Plasma Cl	97 - 108	mmol/l	214	101.7±2.3	10	101.1±1.2	0.16
Plasma Ca total	2.10 - 2.55	mmol/l	311	2.31 (2.25-2.39)	21	2.34 (2.3-2.44)	0.12
Plasma Ca ionized	1.13 - 1.30	mmol/l	303	1.194±0.048	19	1.206±0.041	0.23
Plasma P	0.84 - 1.45	mmol/l	316	0.92±0.174	21	1.012±0.191	0.042
Plasma Mg	0.75 - 1.00	mmol/l	212	0.845 (0.8-0.89)	10	0.825 (0.775-0.848)	0.16
Plasma Creatinine	59 - 104	µmol/l	312	81 (71-90)	21	77 (73-89)	0.93
Plasma uric acid	202 - 416	µmol/l	287	363±89	19	337±63	0.10
Blood pH	7.35 - 7.45	—	292	7.387±0.029	19	7.391±0.042	0.68
Plasma Bicarbonate	18.0 - 29.0	mmol/l	275	26±2.1	18	26.3±2.1	0.51
Plasma anion gap	8 - 16	mmol/l	198	11.5±1.8	10	11.8±1.7	0.63
Urinary volume	—	ml	453	2200 (1620-2840)	30	1903 (1405-2558)	0.17
Urine pH	—	—	392	6.005 (5.63-6.4)	24	6.555 (5.958-6.875)	0.0065
Urine anion gap	—	mmol/l	295	30 (22.2-39.2)	21	30.1 (24.4-47.6)	0.31
Urine Na	40 - 220	mmol/ 24 hr	452	92.5 (62-129)	30	90 (47.5-118.5)	0.16
Urine K	25 - 125	mmol/ 24 hr	450	54 (39-70.8)	30	43 (27.8-60.8)	0.15
Urine Cl	110 - 250	mmol/ 24 hr	419	80 (55-119)	26	67 (41.8-101.3)	0.097
Urine Ca	2.50 - 7.50	mmol/ 24 hr	452	4.04 (2.485-6.113)	30	3.56 (2.603-5.643)	0.79
Urine P	13.0 - 42.0	mmol/ 24 hr	451	26.1 (19.9-32.5)	30	21 (18.2-28.6)	0.086
Urine Mg	2.50 - 8.50	mmol/ 24 hr	445	3.87 (2.96-4.75)	30	3.32 (2.828-4.09)	0.14
Urine uric acid	< 5900	µmol/ 24 hr	451	3403 (2641-4260)	29	3011 (2396-3886)	0.042
Urine urea	96 - 556	mmol/ 24 hr	314	355.2 (266.8-445.8)	21	345.4 (192.1-418)	0.44
Urine creatinine	8600 - 19400	µmol/ 24hr	451	14248 (10841-17092)	30	13678 (10208-16163)	0.29
Urine citrate	1.65 - 6.6	mmol/ 24 hr	442	2.653 (1.668-3.784)	27	2.372 (1.623-3.299)	0.32
Urine citrate/K	—	(mmol/24hr)/ (mmol/24hr)	441	0.048 (0.034-0.068)	27	0.056 (0.033-0.071)	0.56
Urine K-P	—	mmol/ 24 hr	450	26.7 (13.1-44.2)	30	19.3 (5.3-38.9)	0.86
Urine citrate/K-P	—	(mmol/24hr)/ (mmol/24hr)	440	0.092 (0.057-0.153)	27	0.099 (0.043-0.135)	0.66
NGIA	—	mmol/ 24 hr	413	25.6 (10.1-40.9)	26	24.7 (6.4-45.5)	0.51
Urine SO <sub>4</sub>	—	mmol/ 24 hr	443	18.3 (13-23.3)	25	19.5 (11.5-21.3)	0.96
Urine oxalate	<500	µmol/ 24 hr	448	418 (311-605)	26	395 (290-638)	1.00
Urine glycolate	150 - 600	µmol/ 24 hr	310	320 (168-467)	18	329 (154-458)	0.83
Urine NH <sub>4</sub>	10 - 107	mmol/ 24 hr	80	37.4 (28.8-47.5)	3	35.7 (27.5-48.7)	0.91
NAE	—	mmol/ 24 hr	80	85.9 (67.8-108.1)	3	70.4 (65.9-83.8)	0.37
Urine NH <sub>4</sub> /SO <sub>4</sub>	—	(mmol/24hr)/ (mmol/24hr)	80	1.895 (1.631-2.515)	3	2.38 (1.904-5.04)	0.50
Urine NH <sub>4</sub> /NAE	—	(mmol/24hr)/ (mmol/24hr)	80	0.438 (0.378-0.487)	3	0.507 (0.41-0.57)	0.71

The number of stone formers is indicated for each characteristic stratified by the genotype. Continuous variables are further described by their mean ± standard deviation or median (25<sup>th</sup>-75<sup>th</sup> percentiles) as appropriate. Between group differences are determined by Welch's t-test or Mann-Whitney U test as appropriate and the corresponding p-value is indicated. Abbreviations: NGIA, net gastrointestinal alkali intake; NH<sub>4</sub>, ammonium; SO<sub>4</sub>, sulfate; NAE, net acid excretion; p, p-value.

**Table 4: Associations between the E161K polymorphism and plasma and urinary acid-base parameters estimated by linear regression models and mixed effects linear regression models.**

Response variable	E161K unadjusted			E161K adjusted		
	$\beta$	95% CI	<i>p</i>	$\beta$	95% CI	<i>p</i>
<b>Under random diet</b>						
Plasma bicarbonate (mmol/l) (n=439)	-0.6633	-1.629;0.302	0.18	-0.697	-1.608;0.2138	0.13
Urinary pH (n=442)	0.3075	0.03415;0.5809	<0.05	0.2219	-0.0331;0.4769	0.0879
Urinary 24 hr citrate (mmol/24h) <sup>b</sup> (n=465)	-0.2229	-0.4369;-0.0088	<0.05	-0.1359	-0.3287;0.0569	0.17
Urinary 24 hr ammonium (mmol/24h) <sup>c</sup> (n=83)	-5.823	-23.88;12.23	0.52	2.083	-9.245;13.41	0.71
<b>Under low calcium and sodium diet</b>						
Plasma bicarbonate (mmol/l) <sup>a</sup> (n=281)	0.4404	-0.7242;1.605	0.46	0.5893	-0.5062;1.684	0.29
Urinary pH (n=346)	0.5047	0.2098;0.7996	<0.001	0.3805	0.1177;0.6433	<0.005
Urinary 24 hr citrate (mmol/24h) <sup>b</sup> (n=433)	-0.0983	-0.3204;0.1239	0.39	-0.0665	-0.2783;0.1453	0.54
Urinary 24 hr ammonium (mmol/24h) <sup>c</sup> (n=64)	-3.288	-25.89;19.31	0.77	8.240	-6.990;23.47	0.28

The estimate E161K has the wildtype as reference group. For plasma bicarbonate, urinary pH and urinary 24 hr citrate the between-center variability was taken into account as a random effect for all models. Adjusted models were created by backward selection from a full additive model containing sex, age, BMI, 24 hr urinary sulfate excretion (as a marker for dietary acid intake) and net gastrointestinal alkali absorption (as a marker for dietary alkali intake), including non-linear terms and interactions where appropriate, as described in the statistical part.

<sup>a</sup>variables available in the center Bern only <sup>b</sup>square-root-transformation were applied <sup>c</sup>variables available in the center Dallas only.

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Table 5: Associations between the E161K polymorphism and kidney stones estimated by mixed effects logistic regression models.

p.E161.K adjusted for	unadjusted		sex		age		BMI		sex & age		sex & BMI		age & BMI	
	OR 95% CI	p	OR 95% CI	p	OR 95% CI	p	OR 95% CI	p	OR 95% CI	p	OR 95% CI	p	OR 95% CI	p
Kidney stones														
Calcium oxalat (present)	0.57 0.15;3.74	0.47	0.62 0.16;4.10	0.54	1.06 0.22;21.69	0.88	1.32 0.25;2.45	0.79	1.21 0.23;22.46	0.86	1.34 0.25;2.49	0.78	1.3 0.24;2.45	0.80
Calcium phosphate (present)	3.71 1.45;10.69	<0.01	3.60 1.37;10.57	<0.05	2.89 1.10;8.45	<0.05	3.28 1.23;9.69	<0.05	2.90 1.08;8.66	<0.05	3.32 1.21;10.04	<0.05	2.78 1.03;8.28	<0.05
Uric acid (present)	<0.001	0.86	<0.001	0.87	<0.001	0.99	<0.001	0.96	<0.001	0.95	<0.001	0.31	<0.001	0.97

The estimate of E161K has the wildtype as reference group. The between-center variability was taken into account as a random effect in all models. Complete data of 365-388 kidney stone formers was available for inclusion in the regression models.

**Table 6. Characteristics of patients that underwent ammonium chloride loading according to E161K heterozygosity (N=69)**

Characteristic	Wild-type		Heterozygote		<i>p</i>
Center Bern	37	77.1%	13	61.9%	0.19
Center Dallas	11	22.9%	8	38.1%	
Age (yr)	48	41.84 (34.5-50.6)	21	39.5 (25.4-44.8)	0.17
Male sex	33	68.8%	15	71.4%	0.82

The number of stone formers is indicated for each characteristic stratified by the genotype. Categorical variables are further described by % and continuous variables by their median (25<sup>th</sup>-75<sup>th</sup> percentile). Between group differences are determined by Mann-Whitney U or chi-square test where appropriate and the corresponding *p*-value is indicated.

Table 7. Blood and urine parameters of non-stone formers according to E161K heterozygosity on random outpatient diet.

Characteristic	Normal range	Unit	N	Wild-type	N	Heterozygote	p
Plasma Na	132 - 142	mmol/l	98	138(137-139)	9	138(138-139)	0.57
Plasma K	3.5 - 4.7	mmol/l	98	4.3(4.1-4.5)	9	4.2(4.1-4.4)	0.69
Plasma Cl	97 - 108	mmol/l	98	106(105-108)	9	106(104-106)	0.50
Plasma Ca total	2.10 - 2.55	mmol/l	98	2.4(2.3-2.4)	9	2.3(2.3-2.4)	0.89
Plasma P	0.84 - 1.45	mmol/l	96	1.1(0.9-1.2)	9	1.1(1-1.3)	0.33
Plasma Mg	0.75 - 1.00	mmol/l	87	0.9(0.8-0.9)	7	0.9(0.7-0.9)	0.24
Plasma Creatinine	45 - 84	μmol/l	88	80(62-88)	8	80(77-88)	0.66
eGFR CKD-EPI	>90	ml/min per 1.73 m <sup>2</sup>	86	96(80-104)	8	88(84-92)	0.43
Plasma uric acid	202 - 416	μmol/l	79	298(238-354)	8	283(171-341)	0.40
Plasma random glucose	3.33 - 5.55	mmol/l	83	5(4.6-5.3)	8	4.8(4.6-4.9)	0.15
Plasma Bicarbonate	18.0 - 29.0	mmol/l	95	27(25-28)	9	24(24-28)	0.15
Serum PTH	15 - 65	pg/ml	61	30(24-39)	2	17(15-20)	—
Urinary volume	—	ml	75	1715(1173-2558)	8	1700(1579-2356)	0.49
Urine pH	—	—	75	6.1(5.8-6.4)	8	6.2(6-6.3)	0.44
Urine Na	40 - 220	mmol/ 24 hr	75	131(90-188)	8	149(125-184)	0.50
Urine K	25 - 125	mmol/ 24 hr	74	49(35-63)	8	48(47-54)	0.64
Urine Cl	110 - 250	mmol/ 24 hr	71	130(80-176)	7	132(127-173)	0.38
Urine Ca	2.50 - 7.50	mmol/ 24 hr	75	3.4(1.9-4.9)	8	3.7(2.6-4.4)	0.58
Urine P	13.0 - 42.0	mmol/ 24 hr	74	26(17.8-33.1)	8	24.4(21.8-27.3)	0.90
Urine Mg	2.50 - 8.50	mmol/ 24 hr	75	3.7(2.8-4.4)	8	3.6(3.2-4)	0.78
Urine uric acid	< 5900	μmol/ 24 hr	68	3015(2353-3972)	4	2927(2183-3823)	0.92
Urine creatinine	6300 - 19400	μmol/ 24hr	75	11378(9101-15265)	8	12264(8890-13199)	0.99
Urine citrate	1.65 - 6.6	mmol/ 24 hr	72	3.2(2.29-4.28)	7	2.54(2.2-2.67)	0.20
Urine citrate/K	—	(mmol/24hr)/ (mmol/24hr)	72	0.065(0.045-0.085)	7	0.047(0.036-0.064)	0.21
Urine K-P	—	mmol/ 24 hr	74	23(12-35)	8	22(18-31)	0.68
Urine citrate/K-P	—	(mmol/24hr)/ (mmol/24hr)	72	0.118(0.06-0.198)	7	0.102(0.06-0.161)	0.69
NGIA	—	mmol/ 24 hr	71	18.9(7.4-36.4)	7	30.6(22.9-32.4)	0.22
Urine SO <sub>4</sub>	—	mmol/ 24 hr	68	17(13-24)	4	18(16-21)	0.71
Urine oxalate	<500	μmol/ 24 hr	68	284(193-359)	4	299(282-306)	0.79

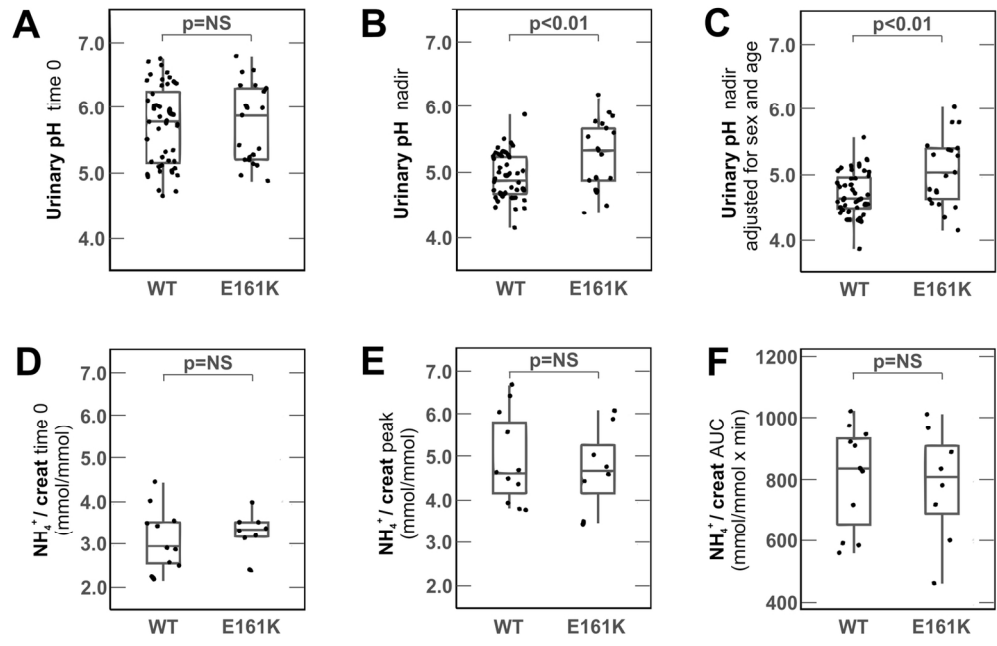
The number of participants is indicated for each characteristic stratified by the genotype. Continuous variables are further described by their median (25<sup>th</sup>-75<sup>th</sup> percentiles). Between group differences are determined by Mann-Whitney U test and the corresponding p-value is indicated. Abbreviations: PTH, parathormone; NGIA, net gastrointestinal alkali intake; p, p-value.

**Table 8. Blood and urine parameters of non-stone formers according to E161K heterozygosity on low Ca and Na diet.**

Characteristic	Normal range	Unit	N	Wild-type	N	Heterozygote	<i>p</i>
Urinary volume	—	ml	40	1985(1428-2590)	5	1900(1500-1925)	0.75
Urine pH	—	—	39	6.2(5.7-6.4)	5	6.7(6.3-6.7)	0.048
Urine Na	40 - 220	mmol/ 24 hr	39	120(94-174)	5	144(119-266)	0.15
Urine K	25 - 125	mmol/ 24 hr	39	47(37-66)	5	71(42-104)	0.29
Urine Cl	110 - 250	mmol/ 24 hr	31	111(95-165)	5	132(117-239)	0.30
Urine Ca	2.50 - 7.50	mmol/ 24 hr	39	3.1(2.1-4.2)	5	4.2(3.5-4.9)	0.24
Urine P	13.0 - 42.0	mmol/ 24 hr	39	24(18-28)	5	26(26-33)	0.29
Urine Mg	2.50 - 8.50	mmol/ 24 hr	39	3.6(2.6-4.6)	5	3.7(3.3-4)	0.85
Urine uric acid	< 5900	μmol/ 24 hr	33	3147(2594-3683)	5	3176(3052-3295)	0.61
Urine creatinine	6300 - 13400	μmol/ 24hr	39	12314(9233-14666)	5	9980(8847-17079)	0.97
Urine citrate	1.65 - 6.6	mmol/ 24 hr	35	3(2.1-4.9)	5	3(2.7-3.4)	0.84
Urine citrate/K	—	(mmol/24hr)/ (mmol/24hr)	34	0.067(0.05-0.091)	5	0.048(0.021-0.071)	0.24
Urine K-P	—	mmol/ 24 hr	39	26(18-37)	5	38(16-86)	0.52
Urine citrate/K-P	—	(mmol/24hr)/ (mmol/24hr)	34	0.123(0.081-0.2)	5	0.025(0.012-0.09)	0.035
NGIA	—	mmol/ 24 hr	30	14(-4-31)	5	45(8-91)	0.15
Urine SO <sub>4</sub>	—	mmol/ 24 hr	33	19(15-24)	5	21(18-23)	0.83
Urine oxalate	<500	μmol/ 24 hr	33	289(228-411)	5	304(200-322)	0.60

The number of stone formers is indicated for each characteristic stratified by the genotype. Continuous variables are further described by their median (25<sup>th</sup>-75<sup>th</sup> percentiles). Between group differences are determined by Mann-Whitney U test and the corresponding *p*-value is indicated. Abbreviations: NGIA, net gastrointestinal alkali intake; SO<sub>4</sub>, sulfate; *p*, *p*-value.

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