Mutations in SLC34A3/NPT2c Are Associated with Kidney Stones and Nephrocalcinosis

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ABSTRACT

Compound heterozygous and homozygous (comp/hom) mutations in solute carrier family 34, member 3 (SLC34A3), the gene encoding the sodium (Na⁺)-dependent phosphate cotransporter 2c (NPT2c), cause hereditary hypophosphatemic rickets with hypercalciuria (HRHR), a disorder characterized by renal phosphate wasting resulting in hypophosphatemia, correspondingly elevated 1,25(OH)₂ vitamin D levels, hypercalciuria, and rickets/osteomalacia. Similar, albeit less severe, biochemical changes are observed in heterozygous (het) carriers and indistinguishable from those changes encountered in idiopathic hypercalciuria (IH). Here, we report a review of clinical and laboratory records of 133 individuals from 27 kindreds, including 5 previously unreported HHRH kindreds and two cases with IH, in which known and novel SLC34A3 mutations (c.1357delTTC [p.F453del]; c.G1369A [p.G457S]; c.367delC) were identified. Individuals with mutations affecting both SLC34A3 alleles had a significantly increased risk of kidney stone formation or medullary nephrocalcinosis, namely 46% compared with 6% observed in healthy family members carrying only the wild-type SLC34A3 allele (P=0.005) or 5.64% in the general population (P<0.001). Renal calcifications were also more frequent in het carriers (16%; P=0.003 compared with the general population) and were more likely to occur in comp/hom and het individuals with decreased serum phosphate (odds ratio [OR], 0.75; 95% confidence interval [95% CI], 0.59 to 0.96; P=0.02), decreased tubular reabsorption of phosphate (OR, 0.41; 95% CI, 0.23 to 0.72; P=0.002), and increased serum 1,25(OH)₂ vitamin D (OR, 1.22; 95% CI, 1.05 to 1.41; P=0.003) (5.64%). Additional studies are needed to determine whether these biochemical parameters are independent of genotype and can guide therapy to prevent nephrocalcinosis, nephrolithiasis, and potentially, CKD.


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Inactivating mutations on both parental alleles of the solute carrier family 34, member 3 (SLC34A3), the gene encoding the sodium (Na⁺)-dependent phosphate cotransporter 2c (NPT2c), are the cause of hereditary hypophosphatemic rickets with hypercalciuria (HHRH; OMIM: 241530) — an autosomal recessive renal phosphate-wasting disorder that was originally described by Tieder et al. Individuals affected by HHRH who carry compound heterozygous or homozygous (comp/hom) SLC34A3/NPT2c mutations show increased urinary phosphate excretion leading to hypophosphatemic rickets, bowing, and short stature as well as appropriately elevated 1,25(OH)₂D levels. Elevated 1,25(OH)₂D levels, in turn, result in hypercalciuria because of enhanced intestinal calcium absorption and reduced parathyroid hormone (PTH) - dependent calcium reabsorption in the distal renal tubules. Even heterozygous SLC34A3/NPT2c mutations are frequently associated with hypercalciuria, but none of the carriers of SLC34A3/NPT2c mutations in the originally described HHRH patients were reported to have renal calcifications and kidney stones. Subsequent investigations, however, revealed that these complications affecting the kidneys were observed in numerous patients with comp/hom SLC34A3/NPT2c mutations. However, the small size of HHRH kindreds and the relatively high prevalence of renal calcifications in the general population (5.64%) have, thus far, prevented segregation-based statistical approaches to determine whether SLC34A3/NPT2c mutations do increase the risk of developing kidney stones or nephrocalcinosis. Likewise, it is unknown whether loss of NPT2c can lead to additional proximal tubular phenotypes such as Fanconi syndrome, which has been described in two patients with homozygous SLC34A3/NPT2a mutations who developed CKD later in life.

The presence of hypercalciuria, kidney stones, and nephrocalcinosis observed in HHRH kindreds is different from the findings in fibroblast growth factor 23 (FGF23)–dependent hypophosphatemic disorders, such as X-linked hypophosphatemia (XLH; mutant PHEX), autosomal dominant hypophosphatemic rickets (ADHR; mutant FGF23), or autosomal recessive hypophosphatemic rickets (ARHR; mutant DMP1, ENPP1, or FAM20C), in which affected individuals show, before treatment with oral phosphate and 1,25(OH)₂D, inappropriately normal or suppressed 1,25(OH)₂D levels despite significant hypophosphatemia and thus, no increase in urinary calcium excretion. Oral phosphate supplements combined with active vitamin D analogs are generally recommended for treatment of FGF23–dependent hypophosphatemic disorders. In contrast, HHRH is thought to require phosphate supplements alone, in part because endogenously elevated 1,25(OH)₂D levels are predicted to prevent an increase in PTH secretion triggered by intermittent elevations in serum phosphate. However, long-term studies are lacking that determine whether oral phosphate supplementation alone of HHRH patients is sufficient for prevention of renal calcifications and bone loss. It is likewise unknown how therapy should be monitored, whether secondary hyperparathyroidism can develop as observed in FGF23–dependent hypophosphatemic disorders, and whether phosphate requirements decrease with age, which has been reported for ADHR.

In the current study, we investigated five new HHRH kindreds and two new cases with idiopathic hypercalciuria (IH), in whom we discovered known and novel homozygous or compound heterozygous SLC34A3/NPT2c mutations. Review of the clinical and laboratory findings along with those findings reported for 22 previously published kindreds suggests that renal calcifications and/or kidney stones may be important, often unrecognized initial findings suggestive of comp/hom SLC34A3/NPT2c mutations. Importantly, heterozygous carriers also show an increased frequency of renal calcifications and biochemical profiles in plasma and urine that are intermediate to those profiles of individuals without SLC34A3/NPT2c mutations and comp/hom changes. Our data suggest that serum phosphate, tubular reabsorption of phosphate (TRP), and serum 1,25(OH)₂D levels predict the development of renal calcifications. However, additional studies are needed to determine whether these biochemical parameters are independent of genotype and can guide therapy to prevent renal calcifications and potentially, CKD.

**RESULTS**

**Novel Mutations in the SLC34A3/NPT2c Gene in Kindreds with HHRH and IH**

Five new kindreds were referred to us for genetic evaluation, with the index case presenting with classic HHRH during childhood. Metabolic bone disease was found to be associated with hypophosphatemia, and subsequent laboratory studies were consistent with FGF23–independent renal phosphate wasting. In addition, two unrelated children with IH presented to their pediatrician with renal stones and/or nephrocalcinosis on renal ultrasound and biochemical abnormalities that were consistent with those abnormalities seen in HHRH [i.e., hypercalciuria and elevated 1,25(OH)₂D levels]. Apparent bone disease was missing, and mild hypophosphatemia was only noted on more careful evaluation. Nucleotide sequence analysis of SLC34A3/NPT2c revealed known and novel mutations in kindreds A and B and case G (Supplemental Figure 1, A and B, Supplemental Tables 1 and 2). A novel homozygous nonsense mutation c.1369G>A (p.G457S) was detected in individuals A/IV-1, A/IV-2, A/IV-3, and A/IV-4, whereas both parents were heterozygous for this nucleotide change. The index case B/I-2 inherited the previously described intronic deletion c.560+27_561del (g.1440_1469del) from his mother (B/I-1) and the novel in-frame deletion c.1357delTTC (p. F453del) from his father (B/I-2); c.1369G>A (p.G457S) and c.1357delTTC (p. F453del) affect highly conserved amino acid residues (Supplemental Table 3). Case G was found to be compound heterozygous for a novel deletion c.367delC,
which introduces 14 novel amino acids followed by premature termination of the NPT2c protein after residue p.P48 (WTLPQLKDPWTLPSTP-Stop) and the known missense mutation c.575C>T(p.S192L).\(^2\) All three novel mutations are absent from the 1000 Genome,\(^23\) dbSNP,\(^24\) and the National Heart, Lung, and Blood Institute Gene Ontology Exome Sequencing Project (http://evs.gs.washington.edu/EVS/) databases, predicted by Polyphen to reduce protein function,\(^25\) and therefore, likely disease-causing. Detailed clinical and genotyping information on kindreds A–G is in Supplemental Material, Supplemental Figure 1, A and B, Supplemental Tables 1 and 2.

**Meta-Analysis of Clinical Information, Including Previously Reported Kindreds with HHRH**

Seven of eleven com/hom carriers of the identified SLC34A3/NPT2c mutations and one of ten heterozygous carriers presented initially with renal calcifications or developed these changes subsequently. To better understand whether this high prevalence of renal calcifications in these not previously reported kindreds can be generalized to all carriers of SLC34A3/NPT2c mutations and to identify possible predictors of renal calcifications, we performed a meta-analysis of the clinical information from 13 reports\(^1\),\(^3\),\(^6\),\(^9\),\(^11\),\(^22\),\(^26\)–\(^29\) and two unpublished families (Simm P, Briody J, Reyes M, Gibbons P, Alexander S, Rauch F, Jüppner H, Bergwitz C, Munns C, manuscript in preparation). Two reports of heterozygous mutations in kindreds with IH and HHRH\(^10\),\(^30\) were excluded from this analysis, because no mutations on the second allele had been identified; thus, segregation with the renal phenotype was not previously thought to be part of metabolic bone disease.\(^31\) Several of the above biochemical parameters have been implicated as predictors of renal calcifications\(^31\) and metabolic bone disease.\(^32\) To identify predictors in our HHRH kindreds, we first performed a univariate logistic regression analysis in individuals with all genotypes \(n=133\) (Table 2). As expected, TRP remained significantly associated with bone involvement, even after adjusting for genotype in a multivariate model; 1,25(OH)\(_2\)D, and ALP continued to be significantly associated with bone involvement in both carriers of SLC34A3/NPT2c mutations and one heterozygous and two comp/hom carriers of SLC34A3/NPT2c mutations at initial presentation (Figure 2C).

Prevalence of Renal Calcifications in Carriers of SLC34A3/NPT2c Mutations Is Increased

Renal calcifications were not previously thought to be part of the clinical presentation of HHRH.\(^4\),\(^5\) More recently, however, an increased frequency of medullary nephrocalcinosis and nephrolithiasis has been recognized\(^3\),\(^6\),\(^7\),\(^9\),\(^11\) in comp/hom carriers. These observations are further substantiated in the current report, because the index cases in three of five new kindreds showed renal calcifications on initial presentation. Furthermore,
it may be underestimated, because most asymptomatic individuals have not had imaging studies. A particularly important strength of our study is the analysis of heterozygous carriers of only those mutations that are clearly disease-causing when combined with another mutation on the second allele or homozygously present. Systematic evaluation of these heterozygous individuals will be an important aspect of future investigations.

Several genes that cause rare monogenic disorders have been associated with hypercalciuric nephrolithiasis (i.e., CLCN5, CASR, CLDN16, CLDN19, ADCY10, SLC34A1, SLC9A3R1, GLUT2, HSPG2, and FN1).\(^{31,33}\) whereas variants of uromodulin and fetuin seem to be protective.\(^{34}\)

Some of these genes affect tubular handling of calcium and phosphate in a way that is similar to what is observed in our patients with SLC34A3/NPT2c mutations. More recently, FAM20A mutations in enamel renal syndrome were found to be associated with renal calcifications, but these individuals do not seem to fit the biochemical profile of IH\(^{20}\); thus, it is more likely that loss of function of the extracellular kinase encoded by FAM20A affects local tissue factors that contribute to the development of renal calcifications. Furthermore, mutations in CYP24A1, the gene encoding the 24-hydroxylase, which is the key enzyme leading to inactivation of 1,25(OH)\(_2\)D, have been reported as a cause of hypercalciuric nephrolithiasis.\(^{35}\) It should be noted that, thus far, genome-wide association studies for uric acid nephrolithiasis,\(^{36}\) serum phosphate levels,\(^{37}\) and CKD\(^{34}\) have not supported an association of hypercalciuric stone disease with the SLC34A3/NPT2c locus. However, our meta-analysis clearly suggests that SLC34A3/NPT2c should be added to the above list of

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**Table 1. Frequency of renal calcifications is increased in carriers of SLC34A3/NPT2c mutations**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Normal (n=16)</th>
<th>Heterozygous (n=61)</th>
<th>Comp/Hom (n=43)</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>P Value</td>
<td>N</td>
<td>P Value</td>
</tr>
<tr>
<td>Renal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic</td>
<td>1</td>
<td>1.00</td>
<td>10</td>
<td>0.44/0.003(^{a})</td>
</tr>
<tr>
<td>Nephrolithiasis</td>
<td>1</td>
<td>1.00</td>
<td>10</td>
<td>0.44/0.003(^{a})</td>
</tr>
<tr>
<td>Nephrocalcinosis</td>
<td>0</td>
<td>1.00</td>
<td>3</td>
<td>1.00</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>15</td>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic</td>
<td>0</td>
<td>8</td>
<td>33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rickets/osteoalacacia</td>
<td>0</td>
<td>8</td>
<td>32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adult osteopenia/osteoporosis</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1.00</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>16</td>
<td>53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number (N) of individuals with renal phenotype who presented with nephrolithiasis or nephrocalcinosis and number of individuals (N) with bone phenotype who presented with rickets/osteoalacacia or osteopenia/osteoporosis. \(P\) values (Fisher exact t test) are shown compared with normal individuals.

\(^{a}\)Assuming average global prevalence of stones of 5.64% according to the work by Romero et al.\(^{12}\)

\(^{b}\)\(P\) values compared with heterozygous individuals.

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**Figure 1. Frequency of renal calcifications is increased in carriers of SLC34A3/NPT2c mutations.** Shown here is the prevalence of renal calcifications (i.e., nephrolithiasis and/or nephrocalcinosis) and bone disease (i.e., rickets/osteoalacacia or osteopenia/osteoporosis) among individuals with two normal alleles as well as heterozygous and comp/hom carriers of SLC34A3/NPT2c mutations. Calc., calcification; dis., disease.

Evaluation of 27 available kindreds indicates that approximately one half of all comp/hom carriers of SLC34A3/NPT2c mutations present with renal calcifications. Renal calcifications, furthermore, are the only presenting sign in 16%, whereas bone disease is (apparently) absent. Even heterozygous carriers of SLC34A3/NPT2c mutations show an approximately 3-fold higher incidence of renal calcifications, which may be related to their intermediate biochemical profile (Figures 2 and 3). Therefore, all affected individuals and their first-degree relatives should be examined for renal calcifications. The biochemical profile may help identify, in addition to genetic analysis of SLC34A3/NPT2c, those first-degree members in HHRH families who are at risk for developing renal complications as further discussed below.

Although prevalence of renal calcifications in heterozygous carriers in this initial survey is only significant compared with the prevalence reported in large cohorts of healthy controls,\(^{12,13}\) the biochemical profile of individuals with two normal alleles as well as heterozygous and comp/hom carriers of SLC34A3/NPT2c mutations. The biochemical profile may help identify, in addition to genetic analysis of SLC34A3/NPT2c, those first-degree members in HHRH families who are at risk for developing renal complications as further discussed below.

Although prevalence of renal calcifications in heterozygous carriers in this initial survey is only significant compared with the prevalence reported in large cohorts of healthy controls,\(^{12,13}\)...
hypercalciuric stone disease genes. Our finding may have important implications for the general population, because heterozygous nucleotide changes that alter the amino acid sequence or introduce deletions/insertions are relatively frequent in NPT2c, but it is currently unknown whether they are of biologic significance. Improved clinical characterization may provide a rationale for genetic analysis of SLC34A3/NPT2c in a subpopulation of hypercalciuric stone patients with clinical and biochemical profiles that resemble the profiles of HHRH.

To increase sample size, we decided to combine nephrocalcinosis and nephrolithiasis for statistical analysis, because these clinical findings are both associated with the increased urinary calcium excretion observed in HHRH. However, it should be noted that 13 of 43 (30%) comp/hom individuals showed nephrocalcinosis on ultrasound, which reaches statistical significance, compared with normal and heterozygous family members (Table 1), and 7 of these individuals did not have evidence for renal stones. Future studies may be able to evaluate the possibility that nephrocalcinosis is a unique feature of the hypercalciuria and hyperphosphaturia caused by loss of NPT2c in the proximal tubule, which raises interesting pathophysiological questions.

Figure 2. Heterozygous carriers of SLC34A3/NPT2c mutations have intermediate biochemical findings. Summary of biochemical values of the presented and published kindreds analyzed with respect to genotype. Individual values with mean ± 95% confidence interval are shown for (A) normalized serum calcium, (B) normalized serum phosphorus, (C) normalized serum creatinine, and (D) serum 1,25(OH)2 vitamin D. het, heterozygous; n/a, not applicable; wt, wild-type.

Serum 1,25(OH)2D, Phosphate, and TRP May Be Predictors of Renal Calciifications in Carriers of SLC34A3/NPT2c Mutations

It is unclear at the moment which genetic and biochemical criteria best predict the risk for renal calciifications in our HHRH kindreds. Based on the current understanding of the pathophysiology of HHRH, the defective sodium–phosphate cotransporter causes renal phosphate wasting, which triggers renal 1α-hydroxylase (CYP27B1) enzyme activity; this activity causes an increase in circulating 1,25(OH)2D levels, which in turn, increases intestinal Ca absorption, leading to an increased renal tubular Ca load and hypercalciuria. Thus, hypercalciuria, hyperphosphaturia, and possibly, tissue-specific effects of 1,25(OH)2D levels may lead to renal calciifications and stones. Hypercalciuria is the most common risk factor of kidney stones, which is present in 40%–50% of adults with recurrent calcium stones and 75%–80% of children with kidney stones. This disease is often referred to as IH because of increased intestinal absorption of calcium, and it can be associated with a mild renal phosphate leak, despite the lack of parathyroid hyperactivity.38 Association of increased 1,25(OH)2D levels with renal calciifications was observed in mouse models that lack Fgf2339 or Klotho40 or individuals with CYP24A1 mutations,35 even in the setting of low or normal urinary phosphate excretion. Finally, excessive phosphaturia, even with normal or low urinary calcium, can cause nephrocalcinosis in humans as is seen in XLH41 or in phosphate enema–induced nephrocalcinosis.42 Consistent with the pathophysiology of HHRH, we found that increased serum 1,25(OH)2D, low serum P, and decreased TRP may be positive predictors of renal calciifications. Only TRP remained associated with renal calciifications after controlling for genotype; however, serum P, 1,25(OH)2D, TRP, and ALP continued to be significant predictors of bone involvement. This initial evaluation suggests that decreased serum P levels and increased excretion of phosphate and serum 1,25(OH)2D merit additional evaluation as possible nongenetic predictors of renal calciifications.

We wondered whether specific SLC34A3/NPT2c mutations are associated with an increased serum 1,25(OH)2D, low serum P, decreased TRP, and renal calciifications (Supplemental Figure 2). However, not all individuals with a specific mutation have developed stones, and the numbers are small. It is, therefore, not possible to be certain about a genotype–phenotype effect at the present time.
Our study is limited by its relatively small sample size, because HHRH is a rare condition affecting, thus far, less than 50 individuals worldwide. Furthermore, clinical and biochemical data are often only available for the initial presentation, and family members who are heterozygous for a \( \text{SLC34A3/NPT2c} \) mutation or carry no mutation have been studied less well. In addition to a systematic evaluation of these individuals, long-term studies are required that determine whether oral phosphate supplementation alone of HHRH patients is sufficient for the prevention of renal calcifications and bone loss. It is likewise unknown how therapy should be monitored, whether secondary hyperparathyroidism can develop as observed in FGF23-dependent hypophosphatemic disorders,\(^\text{15}\) and whether phosphate requirements decrease with age, which has been reported for ADHR.\(^\text{16}\) It will also be important to determine whether lack of the NPT2c transporter leads to additional symptoms, such as Fanconi syndrome or CKD, which have been shown for individuals with \( \text{SLC34A1/NPT2a} \) mutations.\(^\text{14}\) Using either animal models of HHRH or

Figure 3. Heterozygous carriers of \( \text{SLC34A3/NPT2c} \) mutations have intermediate biochemical findings. Summary of biochemical values of presented kindreds and published kindreds analyzed with respect to genotype. Individual values with mean±95% confidence interval are shown for (A) serum PTH levels, (B) normalized ALP, (C) uCa/uCrea, and (D) TRP. %UL, percent upper limit of normal; het, heterozygous; wt, wild-type.

Table 2. Univariate and multivariate regression model identifies association of genotype and biochemical variables with renal calcifications

<table>
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<tr>
<th>Variable</th>
<th>Unit</th>
<th>Renal Calcifications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted, All</td>
<td>Adjusted, All (for Genotype or Sex/Age(^*))</td>
</tr>
<tr>
<td></td>
<td>N, OR, 95% CI</td>
<td>N, Adjusted OR, 95% CI</td>
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<tr>
<td>NPT2c mutation</td>
<td>Alleles</td>
<td>122, 3.90, 1.31–11.63</td>
</tr>
<tr>
<td>Women</td>
<td>Yes</td>
<td>133, 0.83, 0.41–1.69</td>
</tr>
<tr>
<td>Age</td>
<td>Year</td>
<td>94, 1.00, 0.97–1.03</td>
</tr>
<tr>
<td>Serum 25(OH)D</td>
<td>50 nmol/L</td>
<td>71, 1.46, 0.52–4.12</td>
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<tr>
<td>Serum calcium</td>
<td>1 SD</td>
<td>79, 0.91, 0.60–1.37</td>
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<tr>
<td>Serum phosphate</td>
<td>1 SD</td>
<td>100, 0.75, 0.59–0.96</td>
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<tr>
<td>Serum intact PTH</td>
<td>10% UL</td>
<td>78, 0.80, 0.59–1.07</td>
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<tr>
<td>Serum 1,25(OH)(_2)D 50 pmol/L</td>
<td>86, 1.22, 1.05–1.41</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>uCa/uCrea</td>
<td>50% UL</td>
<td>88, 1.39, 0.94–2.05</td>
</tr>
<tr>
<td>TRP (%)</td>
<td>10%</td>
<td>68, 0.41, 0.23–0.72</td>
</tr>
<tr>
<td>Serum ALP</td>
<td>1 SD</td>
<td>73, 1.04, 0.95–1.15</td>
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<tr>
<td>Bone disease</td>
<td>Yes</td>
<td>133, 2.90, 1.24–6.77</td>
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A univariate logistic regression model (GENMOD; SAS Institute, Inc.) was applied to estimate the unadjusted odds ratios (ORs) and those ORs were further adjusted for genotype (adjusted OR) as well as sex and age using a multivariate logistic regression model. An OR of one indicates that renal or bone involvement is equally likely to occur in individuals with a given variable. An OR greater than one indicates that renal or bone involvement is more likely to occur in individuals with high measurements of a given variable at the indicated units. An OR less than one indicates that renal or bone involvement is less likely to occur with high measurements of a given variable at the indicated units. 95% CI, 95% confidence interval.

\(^{\text{*}}\)Odds ratios were further adjusted for sex and age by using a multivariate logistic regression model.
Table 3. Univariate and multivariate regression model identifies association of genotype and biochemical variables with bone disease

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>Unadjusted</th>
<th>95% CI</th>
<th>P Value</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>P Value</th>
<th>Unadjusted, Comp/Hom Only</th>
<th>95% CI</th>
<th>P Value</th>
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<tr>
<td>NPT2c mutation</td>
<td>Alleles</td>
<td>122.08</td>
<td>7.84</td>
<td>55.09</td>
<td>&lt;0.001</td>
<td>33.9^*</td>
<td>9.52</td>
<td>120.9</td>
<td>&lt;0.001</td>
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<td>Age</td>
<td>Year</td>
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<td>9.92</td>
<td>99.92</td>
<td>0.20</td>
<td>41.01</td>
<td>1.02</td>
<td>9.72</td>
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<td>Serum calcium 1 SD</td>
<td>SD</td>
<td>79.00</td>
<td>1.32</td>
<td>80.00</td>
<td>0.28</td>
<td>77.00</td>
<td>1.41</td>
<td>0.87</td>
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<td>Serum phosphate 1 SD</td>
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<td>0.39</td>
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<td>0.63</td>
<td>&lt;0.001</td>
<td>98.00</td>
<td>0.50</td>
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<td>Serum PTH 10% UL</td>
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<td>0.73</td>
<td>0.62</td>
<td>0.87</td>
<td>&lt;0.001</td>
<td>76.00</td>
<td>0.94</td>
<td>0.78</td>
<td>1.14</td>
</tr>
<tr>
<td>Serum 25(OH)D 50 nmol/L</td>
<td>UL</td>
<td>65.00</td>
<td>0.21</td>
<td>0.08</td>
<td>0.56</td>
<td>0.004</td>
<td>29.00</td>
<td>1.45</td>
<td>1.04</td>
<td>2.02</td>
</tr>
<tr>
<td>Serum calcium 50% UL</td>
<td>%</td>
<td>88.00</td>
<td>2.62</td>
<td>1.36</td>
<td>5.05</td>
<td>0.004</td>
<td>85.00</td>
<td>1.45</td>
<td>0.66</td>
<td>3.21</td>
</tr>
<tr>
<td>Serum TRP(%)</td>
<td>%</td>
<td>66.00</td>
<td>0.23</td>
<td>0.13</td>
<td>0.40</td>
<td>&lt;0.001</td>
<td>66.00</td>
<td>0.21</td>
<td>0.08</td>
<td>0.56</td>
</tr>
<tr>
<td>Serum ALP 1 SD</td>
<td>SD</td>
<td>73.00</td>
<td>2.46</td>
<td>1.73</td>
<td>3.50</td>
<td>&lt;0.001</td>
<td>72.00</td>
<td>2.00</td>
<td>1.24</td>
<td>3.20</td>
</tr>
<tr>
<td>Renal calcifications</td>
<td>Yes</td>
<td>133.00</td>
<td>2.90</td>
<td>1.24</td>
<td>6.77</td>
<td>0.01</td>
<td>122.00</td>
<td>1.39</td>
<td>0.36</td>
<td>5.33</td>
</tr>
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</table>

A univariate logistic regression model (GENMOD; SAS Institute, Inc.) was applied to estimate the unadjusted odds ratios (ORs) and those ORs were further adjusted for genotype (adjusted OR) as well as sex and age using a multivariate logistic regression model. An OR of one indicates that renal or bone involvement is equally likely to occur in individuals with a given variable. An OR greater than one indicates that renal or bone involvement is more likely to occur in individuals with high measurements of a given variable at the indicated units. An OR less than one indicates that renal or bone involvement is less likely to occur with high measurements of a given variable at the indicated units. 95% CI, 95% confidence interval.

*Odds ratios were further adjusted for sex and age using a multivariate logistic regression model.

large HHRH kindreds, such as those kindreds described by Tieder et al.4,5

In summary, we here present five previously unreported HHRH kindreds and two individuals with IH in whom SLC34A3/NPT2c nucleotide sequence analysis identified known or novel mutations. Review of the clinical presentation of these kindreds and previously published HHRH kindreds suggests that renal calcifications and/or renal stones may be an important, often unrecognized initial symptom in carriers of SLC34A3/NPT2c mutations. Even heterozygous carriers can be affected by nephrocalcinosis and nephrolithiasis, which is consistent with their intermediate biochemical profile. Serum 1,25(OH)2D, phosphate, and TRP may be predictors of renal calcifications, and future studies will help determine whether these biochemical parameters are independent of genotype and can guide therapy to prevent nephrocalcinosis, nephrolithiasis, and potentially CKD.

CONCISE METHODS

Laboratory Assays

With the exception of genetic analyses, all laboratory studies were performed at laboratories used by the different investigators (normal ranges are provided in parentheses after each value). 25-Hydroxy vitamin D levels were measured by liquid chromatography with tandem mass spectrometry or chemiluminescence immunoassay, and 1,25(OH)2D levels were measured by radioimmunoassay or ELISA. Serum intact PTH levels were determined by electrochemiluminescence immunoassay, and FGF23 levels were measured by an ELISA that detects the intact hormone and C-terminal fragments thereof (Immutopics) as well as intact FGF23 (Kainos). The renal TRP (in percentage) was calculated using the following equation: 100 × (1 − (urine phosphorus × serum creatinine)/(serum phosphorus × urine creatinine)); when serum phosphorus is below the reference range for age, TRP should be above 90. Tubular maximum phosphate reabsorption per GFR (Tmp/GFR) was estimated using the Walton and Bijuvoet nomogram.6,7

SLC34A3 Genetic Analyses

Mutational and haplotype analysis of SLC34A3 was performed after informed written consent was obtained using forms approved by the Institutional Review Board of the Massachusetts General Hospital (MGH). The entire SLC34A3 gene, including approximately 800 bp 5′ of the transcriptional start site, all intervening sequences, and approximately 200 bp of the 3′-untranslated region, was amplified by PCR from genomic DNA of the index cases followed by nucleotide sequence analysis at the MGH DNA Sequencing Core Facility or Genewiz, Inc. as described.1 PCR assays to confirm the findings in index cases and analyze family members and controls were performed as described using Qiagen reagents or the Expand Long Template PCR System (Roche) at standard PCR cycling conditions followed by restriction enzymatic digest or nucleotide sequence analysis. Primer sequences are listed in Supplemental Table 4, and conditions for amplification and detection of the mutations are given in Supplemental Table 1. Searches of the National Center for Biotechnology Information–dbSNP24 and the 1000 Genomes Project25 were negative for the identified novel mutations, with the exception of Het. c.413C>T[p.S138F] (HGMD: CM060480), which was identified as a rare single nucleotide polymorphism rs141734934, Het: 0.001 based on nucleotide sequence analysis of 4352 chromosomes (1000 Genomes). GenBank accession numbers for SLC34A3 are as follows:
genomic contig, NT024000.15; cDNA, NM080877.1; protein, NP543153.1.

Statistical and Data Analyses
For our meta-analysis, we used clinical and laboratory findings (LFs) obtained at presentation before therapy was initiated. For statistical evaluation, we determined the number of individuals affected by (1) nephrolithiasis who had history of renal colic or based on imaging or stone analysis, (2) nephrocalcinosis, in whom renal mineral deposits were found on imaging studies (i.e., ultrasound or computed tomography), (3) rickets/osteomalacia based on history of bowing, corrective surgery, stress fractures, bone pain, or imaging studies (i.e., skeletal radiographic survey and $^{99m}$Tc diphosphonate bone scan), and (4) osteopenia/osteoporosis based on World Health Organization bone densitometry criteria. Asymptomatic individuals were negative for the above renal and bone phenotypes either by imaging or based on absence of history of symptoms suggesting the presence of renal calcifications or bone disease. Age-related reference values (RVs; i.e., 95% confidence intervals) were obtained from http://www.mayomedicallaboratories.com/, http://ccnlprod.cc.nih.gov/dlm/testguide.nsf/Index/1D336E0232533D3285256890C0059ECEA, and respective clinical laboratories; age-related RVs for TmP/GFR are in refs. 44–46, and age-related RVs for uCa/uCrea are from ref. 47. Assuming normal distribution, LFs can be transformed into z scores using means of age-appropriate RVs (RVmean) and SDs (where SD=RV/4) according to the formula: $z_{ref} = \frac{47. Assuming normal distribution, LFs can be transformed into $z$ values (RVs; imaging or based on absence of history of symptoms suggesting the presence of renal calcifications or bone disease. Age-related reference values (RVs; i.e., 95% confidence intervals) were obtained from http://www.mayomedicallaboratories.com/, http://ccnlprod.cc.nih.gov/dlm/testguide.nsf/Index/1D336E0232533D3285256890C0059ECEA, and respective clinical laboratories; age-related RVs for TmP/GFR are in refs. 44–46, and age-related RVs for uCa/uCrea are from ref. 47. Assuming normal distribution, LFs can be transformed into z scores using means of age-appropriate RVs (RVmean) and SDs (where SD=RV/4) according to the formula: $z_{ref} = \frac{L_{ref} - RVmean}{SD}$. $z$ Scores were used for all statistical analyses with the following exceptions: absolute values are presented for 25-hydroxy vitamin D, 1,25(OH)$_2$D, TRP, and TmP/GFR measurements. The data for PTH and age-specific uCa/uCrea are presented as percentage of the upper limit of normal RV. These exceptions are important, because for these assays, upper and lower RVs have not been established in sufficiently large number of normal age-specific controls. We used Prism for OSX, version 5.0d (GraphPad Software Inc.), to perform t tests for unpaired comparisons, one-way ANOVAs for multiple comparisons, and linear regression analyses. For univariate and multivariate logistic regression models, we used SAS (GENMOD; SAS Institute, Inc.) to estimate the odds ratios. To increase sample size, we treated all renal phenotypes (nephrolithiasis and nephrocalcinosis) or bone phenotypes (rickets, osteomalacia, and osteopenia/osteoporosis) collectively. Data are expressed as means±95% confidence intervals.

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DISCLOSURES
None.

REFERENCES


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