Evaluation of the calcium-sensing receptor gene in idiopathic hypercalciuria and calcium nephrolithiasis

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Background. Calcium urolithiasis is in part genetically determined and associated with idiopathic hypercalciuria. The calcium-sensing receptor (CaR) is a candidate gene for idiopathic hypercalciuria and calcium urolithiasis. The CaR is expressed in parathyroid glands and kidneys and regulates parathyroid hormone (PTH) secretion and renal tubular calcium reabsorption according to extracellular calcium concentration [7]. In the parathyroid gland, the CaR induces inhibition of PTH secretion upon binding of calcium. In the kidney, the CaR is highly expressed in multiple nephron segments, including medullary and cortical thick ascending limbs, proximal and distal convoluted tubules, and inner medullary collecting duct [8], where it regulates renal tubular calcium reabsorption. In addition, the CaR is involved in urine concentration and dilution, as it inhibits the action of vasopressin to balance the need for excreting calcium and conserving water [9]. Genetic variation at the CaR locus is linked to urine calcium excretion in rare monogenic traits, including familial benign hypercalcemia, neonatal severe hyperparathyroidism, and an autosomal dominant form of hypocalcemia [10-13]. We present the results of a sib-pair linkage analysis of the CaR locus in a large group of French Canadian sibships. We tested this locus for linkage to calcium stone formation and quantitative phenotypes relevant to calcium metabolism.

METHODS

Pedigrees and phenotyping

All families in the study were of French Canadian descent and were recruited from several specialized stone clinics and lithotripsy units as described [14]. All subjects gave informed consent and were interviewed by a member of the study. A single 24-hour urine collection under a free diet and a calcium-loading test were performed. Standard biochemical analyses on urine and se-
Table 1. Number of calcium stone-forming families and sibships

<table>
<thead>
<tr>
<th>N affected siblings per sibship</th>
<th>At least 1 stone passage</th>
<th>At least 2 stone passages</th>
<th>Trait T75* N sibships</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>28</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Total N sibships (families)</td>
<td>64 (55)</td>
<td>43 (41)</td>
<td>17 (17)</td>
</tr>
</tbody>
</table>

*Affected siblings for the trait T75 had at least one stone passage and calciuria above the 75th percentile in our database.

Molecular and statistical genetics

We physically mapped the CaR gene and three flanking highly polymorphic markers from Genethon (D3S1278, D3S3515, and D3S1551) by radiation hybrid mapping using a commercially available panel (GeneBridge 4 panel; Research Genetics, Huntsville, AL, USA) and polymerase chain reaction (PCR) methods. The RHMAP program was used to order all sequence tag sites [16].

Genotyping was performed on genomic DNA extracted from peripheral leukocytes by a salting-out procedure [17]. Amplifications of three multiallelic markers spanning the CaR gene locus and two intragenic markers of the CaR above the 75th percentile in our database were performed by polymerase chain reaction/electrophoresis methods. The intragenic markers consisted in two biallelic polymorphisms, A986S and G990R [18], amplified in a single fragment using primers CaR3343S (5'-CAGCCCATATGCAAGCAGAA-3') and CaR3500R (5'-TGGTGTCCGGTGTCAGCAT-3'). We used single-strand conformation polymorphism (SSCP) to resolve genotypes. The construction of genetic maps was carried out with the CRIMAP and MULTIMAP programs [19, 20].

Genetic and quantitative trait linkage (QTL) analyses on intermediate phenotypes relevant to calcium homeostasis were performed with the MAPMAKER/SIBS program, version 2.1 [21] with the “all pairs” option weighted by sibship size. The aim was to determine whether common genetic variations at the CaR locus specify particular quantitative phenotypes.

RESULTS

Mapping

A sequence tag of the CaR gene was assigned to chromosome 3q13.3-q21, confirming previous results [22]. The D3S3515 marker was closest to the CaR gene. The distance between both sequence tag sites was 0.105 rays (approximately 3.15 cM) with a lod score >17. The genetic map constructed spanned an 11.8 cM region with the following order: D3S1278-D3S3515-(A986S-G990R)-D3S1551 at distances of 5.4, 3.5, and 2.9 cM, respectively. The support for this order (lod score) was 2.99 log above the next best order.

Linkage analysis

Multipoint sib-pair linkage analyses did not show evidence for linkage between the CaR gene and calcium stone passage (at least 1 stone passage and at least 2 stone passages) in the total group. Furthermore, affected pairs of siblings concordant for calciuria above the 75th percentile (traits T75) did not show evidence for linkage. The option “estimate” of MAPMAKERS/SIBS was used to determine allele sharing in affected sib pairs. The proportion of affected sib pairs sharing 0, 1, and 2 alleles at the CaR loci was identical to theoretical values under rum samples were performed, as described [14]. Each affected proband had at least one calcium stone episode verified by crystallographic (pure calcium oxalate or combined calcium oxalate-calcium phosphate) or biochemical analysis when available. Overall, crystallographic analysis was available for 56% of probands (31 out of 55), biochemical analysis for 25% (14 out of 55). In 10 of the 55 patients (18%), no stone analysis was available, so calcium stone episodes were verified by intravenous pyelogram (radiodense stone). Available hospital records (abdominal ultrasound studies and x-ray film) were reviewed to confirm unaffected status for healthy relatives. Individuals with a history of urinary tract infection or with any secondary condition that might predispose to kidney stones (that is, inflammatory bowel disease, hyperparathyroidism) were attributed an undetermined affection status.

The cohort consisted of 55 pedigrees (64 sibships) comprising 359 pairs of affected siblings with at least one stone episode (Table 1). We defined “more severe” (at least 2 stone passages) and semiquantitative (at least 1 stone passage with calciuria above the 75th percentile, trait “T75”) affection statuses, yielding 196 and 52 pairs of affected siblings, respectively (Table 1). For the second affection status (at least 2 stone passages), subjects with only one stone episode were assigned an undetermined affection status. We used “more severe” phenotypes to enhance the putative genetic susceptibility in affected pairs of siblings. For the trait T75, every affected sib in a sib pair had at least one stone episode and a 24-hour calciuria above 0.102 mmol/kg/d (urine collections with 24-h creatinine excretion within 20% of that predicted by the Cockcroft-Gault formula) [15]. When 24-hour collections were inadequately performed, fasting urine calcium/creatinine ratios were used (T75 = value above 0.513 mmol/mmol). Table 2 shows clinical characteristics of affected and unaffected siblings for all three traits.
Table 2. Clinical characteristics of siblings in pedigrees with calcium and/or radiopaque nephrolithiasis

<table>
<thead>
<tr>
<th>Status</th>
<th>Serum calcium mmol/L</th>
<th>Serum PTH pmol/L</th>
<th>Calciuriaa mmol/kg/d</th>
<th>Fasting Ca/Cr mmol/mmol</th>
<th>Δ Ca/Cr mmol/mmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected</td>
<td>2.33 ± 0.10</td>
<td>3.12 ± 1.62</td>
<td>0.067 ± 0.034</td>
<td>0.38 ± 0.250</td>
<td>0.500 ± 0.359</td>
</tr>
<tr>
<td>Affected</td>
<td>2.31 ± 0.15</td>
<td>3.15 ± 1.48</td>
<td>0.089 ± 0.046</td>
<td>0.434 ± 0.323</td>
<td>0.596 ± 0.631</td>
</tr>
<tr>
<td>At least 1 stone passage</td>
<td>0.13</td>
<td>0.83</td>
<td>0.00002</td>
<td>0.05</td>
<td>0.12</td>
</tr>
<tr>
<td>At least 2 stone passages</td>
<td>0.13</td>
<td>0.46</td>
<td>8.75E-06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Affected</td>
<td>2.31 ± 0.13</td>
<td>3.24 ± 1.62</td>
<td>0.091 ± 0.043</td>
<td>0.440 ± 0.333</td>
<td>0.631 ± 0.698</td>
</tr>
<tr>
<td>Affected</td>
<td>0.28</td>
<td>0.46</td>
<td>1.60E-25</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Trait T75</td>
<td>2.34 ± 0.10</td>
<td>2.86 ± 1.304</td>
<td>0.135 ± 0.033</td>
<td>0.621 ± 0.263</td>
<td>0.674 ± 0.369</td>
</tr>
<tr>
<td>P value</td>
<td>0.41</td>
<td>0.21</td>
<td>0.58</td>
<td>0.77</td>
<td>0.003</td>
</tr>
</tbody>
</table>

All values are mean ± SD (and number of individuals indicated below); Δ Ca/Cr, difference between post-calcium load ratio and fasting ratio.

aAffected siblings for the trait T75 had at least 1 stone passage and calciuria above the 75th percentile in our database.

b24-Hour urine collections with variation in measured creatinine content within 20% of predicted (Cockcroft-Gault estimation [15]).

Fig. 1. Exclusion mapping of the calcium sensing receptor (CaR) loci. Lod scores were obtained with the “exclude” option of MAPMAKER/SIBS at specific relative risk (λs) values for which the maximum lod score did not exceed −2.0 over the interval.

Discussion

We have used a candidate gene approach to determine whether the CaR gene on chromosome 3q is linked to urinary calcium excretion phenotypes and/or calcium stone formation in a group of French Canadian pedigrees. We analyzed the genetic data with the affected sib-pair method, as it is well suited to situations in which inheritance is complex or unknown. The results suggest...

Quantitative trait locus mapping

To determine whether genetic variation at the CaR locus might modulate intermediate phenotypes related to calcium metabolism, we performed QTL analyses on serum calcium, parathyroid hormone (PTH) and 1,25(OH)2D3, fasting and postloading calciuria (before and after the ingestion of 1 g of elemental calcium), and 24-hour urine calcium excretion. Table 3 shows the results of QTL analyses in our families. No significant linkage was detected for any of the quantitative traits considered.

Discussion

We have used a candidate gene approach to determine whether the CaR gene on chromosome 3q is linked to urinary calcium excretion phenotypes and/or calcium stone formation in a group of French Canadian pedigrees. We analyzed the genetic data with the affected sib-pair method, as it is well suited to situations in which inheritance is complex or unknown. The results suggest...
that genetic variation at or near the CaR locus is not associated with hypercalciuric urolithiasis, using the entire group of stone-forming sibs, or a subgroup with more severe IH. In addition, the CaR locus is not associated with intermediate phenotypes of calcium homeostasis, including serum calcium, urine calcium excretion, PTH, and 1,25(OH)2D3 levels.

These results provide evidence that the CaR locus can be excluded as a major gene for nephrolithiasis at the α ratios (defined as the ratio of the prevalence in siblings of affected individuals and the population prevalence) [23] of 1.50 and 1.68, respectively, for ≥1 and ≥2 stone episodes, and 2.60 for stone-forming sib pairs concordant for hypercalciumia. Such results apply to the families studied here, and do not completely rule out the possibility that genetic variations of the CaR gene have major effects on kidney stone formation or on intestinal and/or renal calcium handling in other populations. Also, we cannot exclude that rare mutations of the CaR gene are associated with stone formation [13]. However, hypothetical variations of this gene predisposing to kidney stone formation must be uncommon in our stone-forming population.

In the present study, we have also found that urinary calcium excretion is increased in stone-forming versus non–stone-forming sibs. Affected sibs have increased 24-hour and fasting urine calcium excretion, as well as increased urine calcium following a calcium load. This is the first study to show statistically that hypercalciumia segregates with stone formation using family-based controls. Previous studies analyzing in vivo and in vitro biochemical markers in stone formers have used a case-control design, which is subject to bias from unrecognized stratification of populations.

Although there has been progress in identifying monogenic disorders associated with hypercalciumia [24–26], as well as delineating the “environmental” factors associated with stone formation [27], the genetic basis of IH is still obscure. We have previously excluded CYP1a1 (α-hydroxylase) as a major gene for calcium stone formation [14], but have recently suggested that a gene at or near the vitamin D receptor on chromosome 12 might be linked to this trait [28]. As the list of relevant candidate genes decreases, further studies should involve a more comprehensive “genome wide” approach.

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REFERENCES


