Hypercalcemia, Hypercalciuria, and Elevated Calcitriol Concentrations with Autosomal Dominant Transmission Due to CYP24A1 Mutations: Effects of Ketoconazole Therapy

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Background: Mutations of the CYP24A1 gene, which encodes the 1,25-dihydroxyvitamin D-24-hydroxylase cytochrome P450, Cyp24A1, are predicted to result in elevated 1,25-dihydroxyvitamin D concentrations, hypercalcemia, hypercalciuria, nephrolithiasis, and bone disease. Treatment of hypercalcemia associated with CYP24A1 gene mutations has not been described.

Methods: The genetic basis of a syndrome in a 44-yr-old Caucasian male characterized by intermittent hypercalcemia, hypercalciuria, elevated serum 1,25-dihydroxyvitamin D, undetectable serum 24,25-dihydroxyvitamin D, metabolically active nephrolithiasis, and reduced bone mineral density of the lumbar spine was examined. Sequencing of the CYP24A1 gene and biochemical and genetic analysis of seven family members in three generations was carried out. Because of hypercalcemia, hypercalciuria, and metabolically active nephrolithiasis, the patient was treated with a cytochrome 3A inhibitor, ketoconazole, 200 mg orally every 8 h, for 2 months.

Results: The sequence of the CYP24A1 gene showed two canonical splice junction mutations in the proband. Analysis of family members showed a phenotype associated one or both mutations, suggesting autosomal dominant transmission with partial penetrance of the trait. After therapy with ketoconazole, statistically significant reductions in previously elevated urinary calcium into the normal range were noted. Previously elevated serum 1,25-dihydroxyvitamin D and calcium concentrations decreased, and previously decreased PTH concentrations increased into the normal range, but the differences were not statistically significant.

Conclusions: In a syndrome characterized by intermittent hypercalcemia, hypercalciuria, elevated 1,25-dihydroxyvitamin D, undetectable 24,25-dihydroxyvitamin D concentrations, splice junction mutations of the CYP24A1 gene, and autosomal dominant transmission of the trait, treatment with ketoconazole is useful in reducing urinary calcium. (J Clin Endocrinol Metab 97: E423–E427, 2012)
Based on the failure to degrade 1,25(OH)\(_2\)D3 in cells, it is degraded by a cytochrome P450, the 1,25(OH)\(_2\)D3-24-hydroxylase (3), encoded by the CYP24A1 gene (4–6), and is degraded by a cytochrome P450, the 1,25(OH)\(_2\)D3-24-hydroxylase (7, 8), encoded by the CYP24A1 gene (9). Circulating 1,25(OH)\(_2\)D3 is regulated by the activity of the 25(OH)D3-1α-hydroxylase, which is responsible for production of 1,25(OH)\(_2\)D3 from 25(OH)D3, and its degradation by the 1,25(OH)\(_2\)D3-24-hydroxylase. 25(OH)D3-1α-hydroxylase activity is stimulated by PTH, low phosphate concentrations, IGF, and low calcium concentrations directly; its activity is inhibited by low PTH and high phosphate concentration and fibroblast growth factor-23 (10–14). 1,25(OH)\(_2\)D3-24-hydroxylase activity is increased by 1,25(OH)\(_2\)D3 (7). Gene deletion experiments have demonstrated the pivotal role of the 25(OH)D3-1α-hydroxylase (Cyp27b1) in the formation of 1,25(OH)\(_2\)D3 (6). Mice with germline deletion of the CYP24A1 gene, surprisingly, have normal 1,25(OH)\(_2\)D3 concentrations but fail to normally degrade exogenously administered 1,25(OH)\(_2\)D3 (15). Recently a syndrome characterized by hypercalcemia, hypercalciuria, and recessive mutations of the CYP24A1 gene was described (16). Based on the failure to degrade 1,25(OH)\(_2\)D3 in cells, it was inferred that the patients had a nonfunctional 1,25(OH)\(_2\)D3-24-hydroxylase. However, 24,25-dihydroxyvitamin D\(_3\) [24,25(OH)\(_2\)D\(_3\)] concentrations were not measured, and measures to treat the syndrome were not described. We now describe a patient with hypercalcemia, elevated serum 1,25(OH)\(_2\)D concentrations, undetectable serum 24,25(OH)\(_2\)D and splice junction mutations in the CYP24A1 gene. Sequencing of DNA and analysis of the phenotype in members of three generations shows autosomal dominant transmission with partial penetrance of the trait. We show that the patient’s abnormal metabolic profile could be treated effectively with ketoconazole.

### Materials and Methods

Studies were approved by the Mayo Clinic Institutional Review Board. Written consent was obtained from the proband, family members, or in the case of children, from their parents. Serum and urine chemistries were measured using standard tests at Mayo Clinic, Duke University, or the University of North Carolina. Serum 25-hydroxyvitamin D [25(OH)D] and 1,25(OH)\(_2\)D were measured using mass spectrometry (17). 24,25(OH)\(_2\)D was measured with a competitive binding assay (18, 19). CYP24A1 gene oligonucleotide primers were used to sequence exons and intron-exon boundaries (Supplemental Methods, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org). CYP27B1 gene sequencing was performed commercially. Bone mineral density (BMD) was measured by dual-energy x-ray absorptiometry. Computed tomography (CT) and ultrasonography were performed using standard protocols.

The patient was treated with ketoconazole, 200 mg orally every 8 h for 2 months. Laboratory values were repeated at monthly intervals.

Statistical analysis was performed using the JMP 9.0.1 program (SAS Institute, Cary, NC). Homogeneity of variances was assessed by the Levene test. A nonpaired t test assuming unequal variances was used to compare values of analytes before and after ketoconazole therapy. Values of analytes before therapy were assessed for trends by regression analysis.

### Results

The genotype and chemical phenotypes of three generations of the family are reported in Table 1 and Supplemental Fig. 1. The proband (II 3), a 44-yr-old Caucasian male, had intermittent hypercalcemia (10.4, 10.0, 10.1, and 11.1 mg/dl), hypercalciuria (316, 374, 367, and 505 mg per 24 h), elevated serum 1,25(OH)\(_2\)D (123, 62, 44, and 11.1 mg/dl), hypercalciuria (316, 374, 367, and 505 mg per 24 h), elevated serum 1,25(OH)\(_2\)D (123, 62, 44, and 90 pg/ml), undetectable serum 24,25(OH)\(_2\)D (<0.2 ng/ml), normal serum 25(OH)D (50, 36, 48, and 60 ng/ml), reduced serum PTH (8.1, 9.6, 6.3, and 6.0 pg/ml), normal total and bone alkaline phosphatase and collagen I β-collapsecollapsecollapse ors, metabolically active nephrolithiasis, and reduced BMD.

### Table 1. Genotype and chemical phenotype of three generations of family

<table>
<thead>
<tr>
<th>Proband and relatives</th>
<th>Mutation 1</th>
<th>Mutation 2</th>
<th>Ca (mg/dl) (8.9–10.1)</th>
<th>Pi (mg/dl) (2.5–4.5)</th>
<th>PTH (pg/ml) (15–65)</th>
<th>25(OH)D (ng/ml) (25–80)</th>
<th>1,25(OH)(_2)D (pg/ml) (18–64)</th>
<th>24,25(OH)(_2)D (ng/ml) (1.2–2.6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband (II 3)</td>
<td>IVS5 + 1G&gt;A</td>
<td>IVS6–2A&gt;G</td>
<td>10.4</td>
<td>3.6</td>
<td>8.1</td>
<td>50</td>
<td>123</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>I 2</td>
<td>IVS5 + 1G&gt;A</td>
<td>IVS6–2A&gt;G</td>
<td>11.4</td>
<td>5.3</td>
<td>16.96</td>
<td>43</td>
<td>67</td>
<td>3.02</td>
</tr>
<tr>
<td>II 2</td>
<td>IVS5 + 1G&gt;A</td>
<td>IVS6–2A&gt;G</td>
<td>10.6</td>
<td>3.2</td>
<td>12.97</td>
<td>37</td>
<td>101</td>
<td>0.36</td>
</tr>
<tr>
<td>II 1</td>
<td>IVS5 + 1G&gt;A</td>
<td>IVS6–2A&gt;G</td>
<td>11.1</td>
<td>2.4</td>
<td>22.05</td>
<td>47</td>
<td>104</td>
<td>0.38</td>
</tr>
<tr>
<td>III 1</td>
<td>IVS6–2A&gt;G</td>
<td>nd</td>
<td>&lt;1.0</td>
<td>37</td>
<td>66</td>
<td>28</td>
<td>0.28</td>
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<tr>
<td>III 2</td>
<td>IVS6–2A&gt;G</td>
<td>9.5</td>
<td>5.5</td>
<td>11</td>
<td>29.7</td>
<td>81.4</td>
<td>&lt;0.20</td>
<td></td>
</tr>
<tr>
<td>III 3</td>
<td>IVS5 + 1G&gt;A</td>
<td>10.3</td>
<td>5.1</td>
<td>9.0</td>
<td>49</td>
<td>86</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>II 4</td>
<td>9.8</td>
<td>3.7</td>
<td>18.96</td>
<td>37</td>
<td>59</td>
<td>1.62</td>
<td></td>
<td></td>
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</table>

Not determined.
TABLE 2. Serum and urinary analytes before and after treatment with ketoconazole

<table>
<thead>
<tr>
<th></th>
<th>Urinary Ca (mg per 24 h, [25–300])</th>
<th>PTH (pg/ml, [15–65])</th>
<th>Total serum Ca (mg/dl, [8.9–10.1])</th>
<th>Serum inorganic P (mg/dl, [2.5–4.5])</th>
<th>1,25(OH)₂ D (pg/ml, [18–64])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>390.5 ± 80.59</td>
<td>7.5 ± 1.68</td>
<td>10.4 ± 0.49</td>
<td>4.1 ± 0.57</td>
<td>79.7 ± 34.49</td>
</tr>
<tr>
<td>After</td>
<td>149.5 ± 14.84</td>
<td>22.5 ± 6.36</td>
<td>9.8 ± 0.42</td>
<td>4.1 ± 0.42</td>
<td>59.5 ± 16.2</td>
</tr>
<tr>
<td>P value</td>
<td>0.007</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, Not significant.

Serum angiotensin-converting enzyme and serum and urine monoclonal proteins were normal or absent. Fungal serologies were nonreactive. Chest CT showed a calcified node. Abdominal CT showed small bilateral renal cysts suggestive of mild polycystic disease. A 6-mm stone was noted in the left kidney. The BMD showed the following: left hip: femur neck, 0.831 g/cm², T-score −1.8, Z-score −1.3; total hip: 0.789 g/cm², T-score −2.2, Z-score −1.8; right hip: femur neck, 0.858 g/cm², T-score −1.6, Z-score −1.1; total hip: 0.803 g/cm², T-score −2.1, Z-score −1.8; and total lumbar spine (L1-L4): 0.831 g/cm², T-score −3.3, Z-score −3.1. The sequence of the CYP27B1 gene was normal without duplication. The sequence of the CYP24A1 gene showed two canonical intron-exon splice junction mutations (IVS5 + 1G>A and IVS6–2A>G) that likely would result in absent 25(OH)D/1,25(OH)₂D-24-hydroxylase expression (Table 1 and Supplemental Fig. 2). However, chemical analysis of blood from family members in three generations showed other affected individuals and questioned straightforward recessive inheritance (Table 1 and Supplemental Fig. 1).

Family history revealed an asymptomatic mother (father deceased), an asymptomatic brother and sister, and two daughters and one son. A 5-yr-old daughter (III 1) and 1-yr-old daughter (III 3) exhibited similar phenotypic features. The 5-yr-old daughter presented at 7 months with failure to thrive, hypercalcemia (16 mg/dl), hypercalciuria, and nephrocalcinosis. Her clinical status, hypercalcemia, and hypercalciuria improved after change to a low-calcium formula (Calcilo). The 1,25(OH)₂D levels remained elevated (94–111 pg/ml) and PTH suppressed (4 pg/ml with a serum calcium of 10.1). When seen at age 3 yr on a low-calcium (275 mg/day) and restricted vitamin D diet, she had elevated serum 1,25(OH)₂D concentrations (86 pg/ml), hypercalciuria (urine calcium to creatinine ratio 0.30; 5.3 mg/kg·d), high-normal serum calcium (10.3 mg/dl), low PTH concentrations (9.0 pg/ml), nephrocalcinosis, and renal cysts. The BMD of her lumbar spine showed a Z-score of −1.4. The 1-yr-old daughter presented at age 11 months with similar symptoms and biochemical and ultrasound findings [serum calcium, 14.2 mg/dl; 25(OH)D, 37 ng/ml; 1,25(OH)₂D, 66 pg/ml; intact PTH <1.0 pg/ml; urinary calcium to creatinine ratio >0.48; medullary calcinosis]. The son, aged 3½ yr, was asymptomatic but exhibited a partial biochemical phenotype with elevated 1,25(OH)₂D concentrations and medullary nephrocalcinosis. The proband’s brother, sister, and mother were asymptomatic and had the biochemical features shown in Table 1.

Genetic studies showed that the brother (II 2) and sister (II 1) also had both splicing mutations, and the mother and the three children each had one of the splicing changes (Table 1 and Supplemental Fig. 1). Overall, the pattern suggests dominant inheritance with reduced penetrance in which environmental and other genetic factors may be playing a role.

In view of the intermittent hypercalcemia, persistent hypercalciuria, and reduced BMD, the proband (II 3) was treated with ketoconazole, 200 mg orally every 8 h for 2 months. Ketoconazole, an inhibitor of the hepatic and Cyp3a1 enzyme and ergosterol synthesis, has been used successfully to treat hypercalcemia in patients with sarcoidosis and elevated 1,25-dihydroxyvitamin D concentrations (20). Serum and urine chemistries were measured before starting ketoconazole and 1 and 2 months after the initiation of therapy. Liver function tests were measured periodically to assess ketoconazole toxicity. As seen in Table 2, therapy with ketoconazole resulted in statistically significant decreases in urinary calcium (P = 0.007) excretion. Although serum 1,25(OH)₂D and calcium concentrations decreased into the normal range, the differences before and after therapy were not statistically significant. Serum PTH increased into the normal range after therapy but the changes were not statistically significant.

Discussion

Elevated 1,25(OH)₂D and undetectable 24,25(OH)₂D concentrations in a patient with intermittent hypercalcemia, hypercalciuria, nephrolithiasis, and renal cysts were associated with splice-junction mutations of the 25(OH)D₃/1,25(OH)₂D₃-24-hydroxylase (CYP24A1) gene. Seven family members from three generations showed chemical and clinical phenotypes with one or two CYP24A1 mutations, suggesting autosomal dominant transmission with partial penetrance of the trait. The
data suggest that in humans the 1,25(OH)2D3-24-hydroxylase activity regulates 1,25(OH)2D concentrations, and CYP24A1 gene mutations result in a clinically important phenotype. Our data are consistent with a recent publication in which mutations of the CYP24A1 gene in humans were associated with hypercalcemia, hypercalciuria and elevated 1,25(OH)2D (16). In contrast to that publication, we show absent serum 24,25(OH)2D in the proband and other family members, thereby indicating that the mutations result in a nonfunctional Cyp24a1 enzyme. However, a biochemical and, in some cases, a clinical phenotype in patients with just one CYP24A1 mutation indicate that a dosage reduction of Cyp24a1 in the appropriate setting is also significant. Alternatively, the described splicing mutations could have dominant-negative or even gain-of-function modes of action. Patients with two mutant alleles had the highest 1,25(OH)2D concentrations, whereas heterozygotes had lower levels, which were elevated beyond the normal range. Also, three members of generation II (with two mutations) and three members of generation III (with one mutation) had very low levels of 24,25(OH)2D, whereas the mother, with just the IVS5 + 1G>A mutation, had values in the normal range. It is not clear whether this difference is due to variable penetrance associated with other genetic or environmental factors or whether a second CYP24A1 mutation has been missed in children in generation III. Of interest, in the study of Schlingmann et al. (16), the pattern of inheritance appeared to be recessive, although in the one family with a mutation disrupting a splice site, only a single mutation was found.

The elevated 1,25(OH)2D concentrations are likely to be the cause of the patient’s hypercalcemia, hypercalciuria, and hyperphosphaturia. Elevated 1,25(OH)2D concentrations would increase intestinal calcium absorption and result in hypercalcemia and hypercalciuria. This mechanism is further supported by the rapid improvement in the serum and urine calcium of his daughters after change to a low-calcium formula. Elevated 1,25(OH)2D concentrations also resulted in a suppression of serum PTH concentrations due to a combination 1,25(OH)2D-mediated hypercalcemia and, perhaps, direct effects on PTH gene transcription. The low PTH concentrations could have contributed to the patient’s hypercalciuria by increasing the fractional excretion of calcium or reducing the fractional reabsorption of calcium in the distal nephron.

Our data demonstrate that it is possible to inhibit increased urinary calcium excretion with ketoconazole therapy, thereby reducing the risk of nephrolithiasis and nephrocalcinosis. Although the serum 1,25(OH)2D and calcium concentrations decreased into the normal range, the decrease was not statistically significant. It is likely, however, that the changes in 1,25(OH)2D and calcium were contributing to the hypercalcemia observed in this patient. Ketoconazole has been used to inhibit 1,25(OH)2D synthesis in patients with granulomatous disease in whom extrarenal 25(OH)D-1α-hydroxylase enzyme is present. Of interest, the proband’s 25(OH)D concentrations were in the upper range of normal, despite normal dietary vitamin D intake and normal sunshine exposure. The high-normal 25(OH)D concentrations are likely due to the lack of conversion of 25(OH)D to 24,25(OH)2D by the Cyp24a1 enzyme. The effect of ketoconazole should be interpreted with caution because only a single patient was studied. An underlying trend with respect to changes in urinary calcium is unlikely because pretreatment values did not show a statistically significant downward trend on regression analysis.

The reduced BMD in this patient is likely due to elevated 1,25(OH)2D concentrations and an increase in bone resorption. We cannot, however, eliminate the possibility that absent 24,25(OH)2D concentrations are contributing to the bone loss. We do not know whether ketoconazole therapy will result in an improvement in BMD.

In conclusion, we describe a novel syndrome of hypercalcemia, hypercalciuria, nephrolithiasis, and low BMD due to splice junction abnormalities of the CYP24A1 gene evident in patients with one and two mutations. In adults, the hypercalciuria and hyperphosphaturia in this syndrome are reduced with ketoconazole treatment.

Acknowledgments

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