



Calcium in rare disease

EXCEPTIONAL CASE

Successful treatment of hypercalcaemia associated with a CYP24A1 mutation with fluconazole

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Abstract

Mutations in CYP24A1, encoding the vitamin D 24-hydroxylase enzyme, are known to cause a range of clinical phenotypes and presentations including idiopathic infantile hypercalcaemia and adult-onset nephrocalcinosis and nephrolithiasis. In the context of raised or borderline high serum calcium levels, suppressed PTH and persistently elevated 1,25 dihydroxy vitamin D levels, this rare condition should be considered. We present a case where this biochemical pattern was seen and mutations in CYP24A1 were confirmed. We were able to successfully control serum calcium levels and reduce urinary calcium excretion by treatment with low-dose fluconazole, which inhibits vitamin D-synthesizing enzymes (including 25-hydroxylases and 1- α -hydroxylase) thereby reducing levels of 1,25-dihydroxy vitamin D.

Key words: CYP24A1, fluconazole, hypercalcaemia, hypercalciuria, ketoconazole, vitamin D

Background

Since the identification of CYP24A1 mutations in children with idiopathic infantile hypercalcaemia [1], the spectrum of phenotypes attributable to CYP24A1 variants has been expanded, with CYP24A1 mutations identified in cases of idiopathic calcium stone formation and nephrocalcinosis [1–4].

The biochemical hallmark of CYP24A1 mutations is a persistently increased 1,25-dihydroxy vitamin D3 level. CYP24A1 encodes the vitamin D 24-hydroxylase enzyme, which catalyses the hydroxylation of the biologically active form of vitamin D (1,25-dihydroxy vitamin D3), and its precursor (25-hydroxy vitamin D3), to inactive forms for excretion. Mutations in CYP24A1 inhibit these functions, resulting in increased levels of active vitamin D, and subsequent persistent hypercalcaemia.

Mutations in CYP24A1 may also lead to increased levels of 25-hydroxy vitamin D. This led investigators to trial treatment with ketoconazole, a P450 enzyme inhibitor which also inhibits vitamin D-synthesizing enzymes (including 25-hydroxylases and 1- α -hydroxylase). In patients with mutations in CYP24A1, ketoconazole may be a useful therapy to prevent hypercalcaemia and hypercalciuria and their systemic complications.

Tebben *et al.* report an adult male with a splice-site mutation in CYP24A1 in whom they demonstrated, after 2 months of treatment with ketoconazole (200 mg tds), a significant reduction in urinary calcium excretion and a modest reduction in serum calcium [2]. Nesterova *et al.* also describe the treatment of a renal stone former with a CYP24A1 mutation with ketoconazole (up to 800 mg/day), showing a reduction in 1,25 dihydroxy vitamin

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D, serum calcium and urine calcium levels [3]. Although these reports are encouraging, the long-term use of ketoconazole, especially at high doses, in subjects with CYP24A1 mutations is not advisable due to toxicity of the drug (<https://www.gov.uk/drug-safety-update/oral-ketoconazole-do-not-prescribe-or-use-for-fungal-infections-risk-of-liver-injury-outweighs-benefits>), with more recent MHRA warnings in respect of its use as medical therapy of endogenous Cushing's syndrome.

Case report

We report a male English patient who had originally presented with renal colic at 10 years of age and had a history of recurrent renal stones. He subsequently re-presented with symptomatic hypercalcaemia [with suppressed PTH (14 ng/L) and hypercalciuria (8.8 mmol/24 h), aged 45 years]. There was no history of vitamin D supplementation, although he occasionally uses tanning beds. He was initially investigated for occult malignancy and sarcoidosis. Body imaging, serum electrophoresis, tumour makers and serum angiotensin-converting enzyme were all normal. He was treated empirically with an aggressive IV fluid regime and oral prednisolone, resulting in partial correction of serum calcium likely ascribable largely to the IV fluids (Figure 1). No malignancy was found, but renal imaging revealed bilateral nephrocalcinosis and an inherited cause of hypercalciuric renal stones was therefore postulated. Levels of 25-hydroxy vitamin D were raised at 168 nmol/L, and magnesium levels were normal

(0.83 mmol/L); following recovery of a presumed hypercalcaemia-induced acute kidney injury, he had persistent evidence of chronic kidney disease (CKD) with eGFR of ~33 mL/min/1.73 m² (CKD stage G3bA1). Using PCR and Sanger sequencing, we confirmed a known pathogenic homozygous p.E143del mutation, segregating from each parent, in CYP24A1 (Figure 1A). The parents were not known to be consanguineous. This mutation has been previously described by Schlingmann et al. [1] and others and has an allele frequency of 0.06% reported in the Exome Aggregation Consortium (ExAC) database (<http://exac.broadinstitute.org/>) generated from whole exome sequencing data of >60 000 individuals.

While fluconazole is not the most potent inhibitor of 1 α -hydroxylase (and of adrenal 11,-beta- and 17,- hydroxylases), it is far less toxic and more widely available than ketoconazole. Indeed, it is now being used for the medical management of surgically intractable Cushing's syndrome [5, 6]. We therefore hypothesized that it would likely be a clinically useful treatment for this patient. Low-dose fluconazole (50 mg orally daily) was commenced, allowing reduction and then complete discontinuation of oral prednisolone. While on this therapy, 1,25 dihydroxy vitamin D levels fell from inappropriately high levels of 118 to 69 pmol/L (over a period of 98 days), 25-hydroxy vitamin D levels remained elevated following treatment (175 nmol/L), confounded in part by tanning bed use by the patient. Prior to steroid treatment, serum calcium levels (adjusted for albumin) were 3.27 mmol/L and fell to 2.61–2.71 mmol/L following fluid

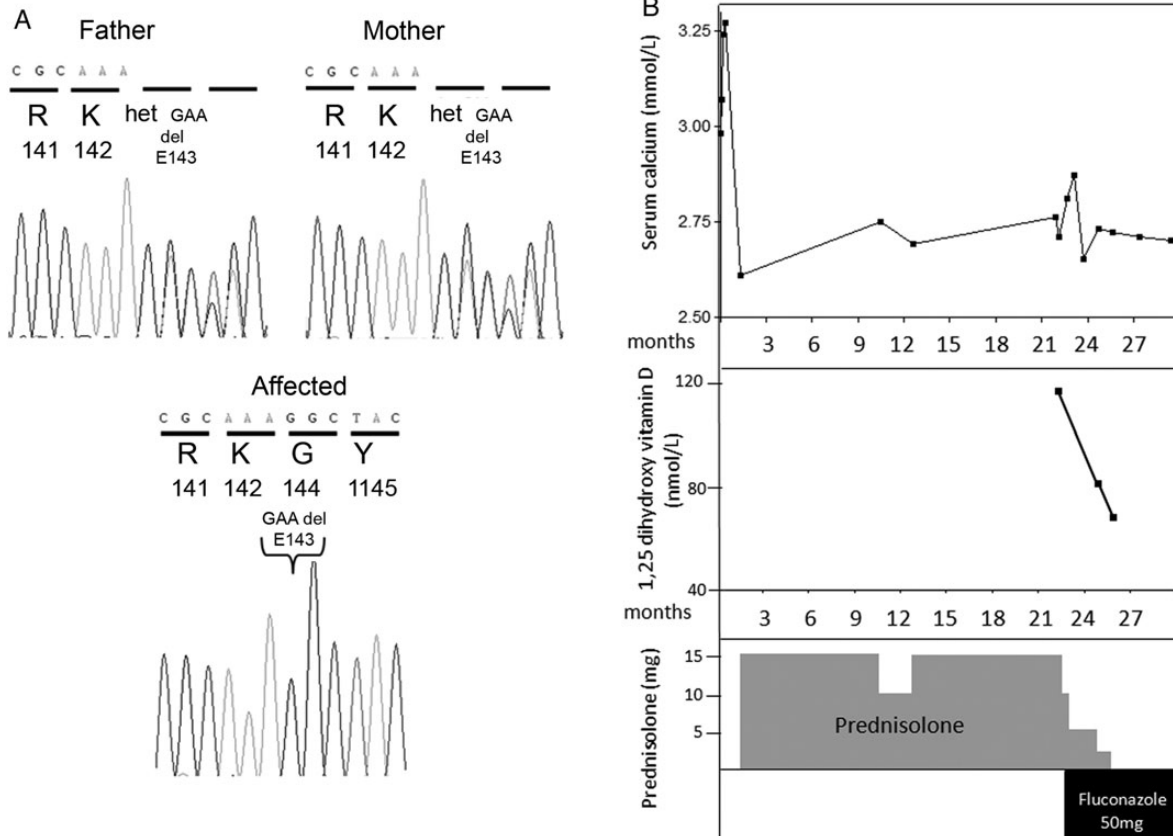


Fig. 1. Genetic investigations and clinical course of patient. (A) Sequence chromatograms are shown for index case and parents, identifying p.E143del mutation and confirming segregation. (B) Clinical course of patient and response of serum calcium (adjusted for albumin, reference range 2.2–2.6 mmol/L) and 1,25 dihydroxy vitamin D levels (using a validated immunoassay (<http://www.idsplc.com/en-gb/products/ids-isys-125-dihydroxy-vitamin-d-is-2400>), reference range 20–120 pmol/L) to therapy with variable prednisolone dose (mg) and fluconazole 50 mg od.

resuscitation (\pm prednisolone effect). Introduction of fluconazole allowed calcium levels to stabilize, and prednisolone treatment was discontinued (Figure 1B) with a fall in urine calcium levels to 5.2 mmol/24 h. There have been no noted side effects of the drug, in particular no liver toxicity, and we propose to maintain the patient on this therapy, with appropriate monitoring of liver function tests. We have instructed the patient to avoid tanning beds and to avoid inadvertent vitamin D supplementation from dietary supplements and drinks.

Discussion

There is growing evidence that CYP24A1 mutations, although rare, can underlie adult presentations of renal stone disease/nephrocalcinosis, and consideration of CYP24A1 mutations as the underlying cause should be given. A recent genetic screen of 256 renal stone patients identified just 1 patient (0.4%) with a homozygous CYP24A1 mutation [4]. Once identified, a therapy to prevent the associated hypercalcaemia, which may be complicated by hypercalciuria, renal stones and nephrocalcinosis should be considered. Long-term steroids are not a targeted treatment of this condition, and indeed in some patients with CYP24A1 mutations, the serum calcium may be resistant to lowering by steroids [7]. In addition, seasonal sunlight exposure [8] (and tanning beds) may exacerbate serum calcium levels. We propose that drugs known to inhibit vitamin D hydroxylation should be considered.

Azole agents are cytochrome inhibitors primarily used as antifungal agents. They are heterocyclic ring compounds and are generally classified as either imidazoles (e.g. ketoconazole) or triazoles (e.g. fluconazole), containing two or three nitrogen atoms, respectively, in the azole ring. They exhibit their antifungal action through inhibition of lanosterol 14- α demethylase, a cytochrome P450 enzyme important for the synthesis of a fungal plasma membrane constituent. Recognition that these agents could inhibit other cytochrome P450-dependent enzyme systems, such as those involved in vitamin D hydroxylation, led to the use of ketoconazole as a treatment for hypercalcaemia. Administration of ketoconazole has been successfully used for the treatment of hypercalcaemia associated with hyperparathyroidism and sarcoidosis. In patients with CYP24A1 mutations, successful short-term ketoconazole treatment has been described [2, 3]. Although these reports are encouraging, the use of ketoconazole in subjects with CYP24A1 mutations could be potentially problematic due to potentially serious adverse effects including hepatotoxicity.

The risk of hepatotoxicity is considerably less with newer triazole agents, such as fluconazole. Schuster *et al.* have screened various azole compounds for selective inhibition of vitamin D hydroxylases [9]. While fluconazole was not the most potent 1 α -hydroxylase inhibitor, due to its favourable side-effect profile, we hypothesized that it would be a clinically useful treatment for patients with mutations in CYP24A1. We closely monitored our patient for efficacy of the drug and potential toxic effects and conclude that fluconazole is well tolerated and is effectively controlling the hypercalcaemia, without the use of steroids, by directly reducing 1,25 dihydroxy vitamin D levels. We cannot

completely exclude confounding environmental effects that may have caused a fall in 1,25 dihydroxy vitamin D and serum calcium, but we are not aware of any significant changes in the patient's behaviour; indeed, the patient persisted with tanning bed use. Another important caveat is that we cannot predict the long-term outcome or whether intolerance to the drug will occur or whether there might be long-term cumulative hepatotoxicity.

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Conflicts of interest statement

None declared. The results presented in this paper have not been published previously in whole or part, except in abstract format.

(See related article by Dusso *et al.* The hypercalcaemia of CYP24A1 inactivation: new ways to improve diagnosis and treatment. *Clin Kidney J* (2015) 8: 456–458.)

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