CASE REPORT

A novel *CASR* gene mutation in an octogenarian with asymptomatic hypercalcaemia

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An increasing number of patients are diagnosed with primary hyperparathyroidism after having hypercalcaemia detected incidentally during routine biochemical screening. Many are asymptomatic at the time of diagnosis. An 80-year-old woman was found to have asymptomatic hypercalcaemia. Initial investigations suggested a diagnosis of primary hyperparathyroidism. Subsequent investigations revealed that, in fact, she had familial hypocalciuric hypercalcaemia. Direct DNA sequencing of the calcium-sensing receptor (*CASR*) gene confirmed that the patient was heterozygous for c.2501delC, a novel frame shift mutation predicted to cause loss of function of the *CASR* gene. Several other family members were subsequently found to carry the same mutation. Suspected cases of hypocalciuric hypercalcaemia should be confirmed by detection of mutations within the *CASR* gene. Establishing the correct diagnosis will enable the patient and family members to avoid unnecessary investigations or operations.

Introduction

Primary hyperparathyroidism (PHPT) is a common cause of hypercalcaemia. Other common causes of hypercalcaemia in an elderly patient include drug causes such as thiazide diuretics and lithium, humoral hypercalcaemia of malignancy, multiple myeloma, and tertiary hyperparathyroidism associated with end-stage renal failure. In this report, we describe an 80-year-old woman who had asymptomatic hypercalcaemia and was diagnosed with PHPT. Subsequent investigations led to the unexpected diagnosis of a genetic condition that had escaped detection for many years.

Case report

An 80-year-old woman was noted to have hypercalcaemia in 2003 during routine blood tests. Apart from having known type 2 diabetes and hypertension, she was otherwise well and had no family history of note. The patient denied taking any calcium or vitamin supplements. Her medications included sustained-release nifedipine, metformin, valsartan, isophane, and neutral human insulin (total, 26 units/day). She had no symptoms suggestive of hypercalcaemia, nor any history of fractures. The physical examination was unremarkable. Her serum calcium concentration was elevated on repeated testing, the adjusted calcium being 2.64 to 2.76 mmol/L (reference range, 2.15-2.55 mmol/L). Her serum phosphate and alkaline phosphatase concentrations were normal. Despite her hypercalcaemia, the concomitant parathormone (PTH) level was not suppressed (6.1 pmol/L; reference range, 1.6-6.9 pmol/L). It was suspected that she had PHPT. A plain radiograph revealed clustered calcifications over the lower pole of the left kidney. An ultrasound of her neck showed a multi-nodular goitre but no evidence of a parathyroid adenoma. In view of the negative imaging, she was referred for further evaluation. Urine collection revealed a low urinary calcium excretion of 1.6 mmol/day (reference range, 2.0-7.4 mmol/day). Urinary calcium was 0.3 mmol/L with a concomitant urinary creatinine of 1.3 mmol/L. Her serum calcium and creatinine were 2.7 mmol/L and 68 μmol/L, respectively, giving a low fractional calcium excretion of 0.58%. It was therefore suspected that she might have familial hypocalciuric hypercalcaemia (FHH). Direct DNA sequencing of the calcium-sensing receptor (*CASR*) gene confirmed the patient was heterozygous for a c.2501delC mutation (Fig 1). This mutation is a novel deletion mutation, deleting the cytosine (C) in codon 834 (TCT), creating a premature stop codon downstream of the mutation because of shifting of the reading frame. This results in a truncated protein and loss of function of the CASR protein.

Following the patient's genetic diagnosis, it transpired that she had a daughter who had been told earlier that she had elevated calcium levels. We invited the daughter to attend for review and her medical records revealed she had an incidental finding of hypercalcaemia with a non-suppressed PTH, but extensive investigations, including a neck ultrasound had failed to localise the source of the 'excess' PTH. She was found to have a fractional calcium excretion of 0.62%, consistent with a diagnosis of FHH. A subsequent

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genetic analysis confirmed that the daughter carried the same *CASR* mutation as her mother. Several other family members were subsequently tested and found to have the condition (Fig 2).

Discussion

Primary hyperparathyroidism is classically associated with bone disease and renal calculi, but an increasing number of often asymptomatic patients are being diagnosed when hypercalcaemia is noted on a routine biochemical screen.[1] The biochemical diagnosis of PHPT is established by the presence of hypercalcaemia, in association with a non-suppressed PTH level. In approximately 10% of patients with PHPT, hypercalcaemia occurs in association with 'normal' serum PTH concentrations.

Familial hypocalciuric hypercalcaemia is a benign condition characterised by autosomal dominant inheritance with high penetrance. Affected patients usually present in childhood with incidental finding of hypercalcaemia, hypocalciuria, and mild-to-moderate hypermagnesaemia.[2] Calcium binds to an extracellular CASR, which is expressed abundantly in parathyroid tissues and kidneys, where its activation inhibits PTH secretion and promotes urinary calcium excretion. Inactivating mutations of the *CASR*, as seen in our patient, cause a right shift in the set point for calcium inhibition of PTH secretion and stimulation of urinary calcium excretion, leading to hypercalcaemia and hypocalciuria, and has recently been found to be the molecular basis underlying FHH.[3] Individuals who inherit two inactive gene copies are more severely affected, presenting with neonatal severe hyperparathyroidism, characterised by marked hypercalcaemia, skeletal demineralisation, and parathyroid hyperplasia. If a parathyroidectomy is not performed, it can be fatal. Activating mutations of the *CASR*, on the other hand, causes a left shift in the calcium set point, leading to hypocalcaemia and hypercalciuria. To date, 213 different *CASR* gene mutations have been described.[4] These consist of 188 missense mutations, 17 nonsense mutations, four insertion mutations, one deletion/insertion mutation, one deletion mutation, one silent and one splice mutation. A large number of these mutations occur within exon 7, as in our case.[4]

A diagnosis of FHH may be suggested by a family history of hypercalcaemia but because patients are often asymptomatic, affected family members may not be aware of their hypercalcaemia. Our patient is unique in having escaped the diagnosis for so many years. Familial hypocalciuric hypercalcaemia is sometimes diagnosed after patients have had unsuccessful surgery for presumed PHPT.[5] The absence of osteopaenia, osteitis fibrosa, nephroliathiasis, or symptoms of hypercalcaemia traditionally favours a diagnosis of FHH over that of

一名患有高鈣血症的80歲病人中的*CASR*基因新突變

在常規生化篩查中，診斷患有高鈣血症同時發現有甲狀膀腺功能亢進症的病人有上升趨勢。很多個案都沒有任何症狀。一名80歲病人有無症狀的高鈣血症，初步診斷為甲狀膀腺功能亢進症，其後的檢查顯示病人有家族性低鈣尿高鈣血症。*CASR*基因經DNA序列分析確定病人的c.2501delC出現雜合，即一種預測會引致*CASR*基因失去功能的新相移突變。病人的家人後來發現帶有相同的基因突變。對於低鈣尿高鈣血症個案，應檢查*CASR*基因是否有突變。正確診斷可以使病人及家屬避免不必要的檢查或手術。

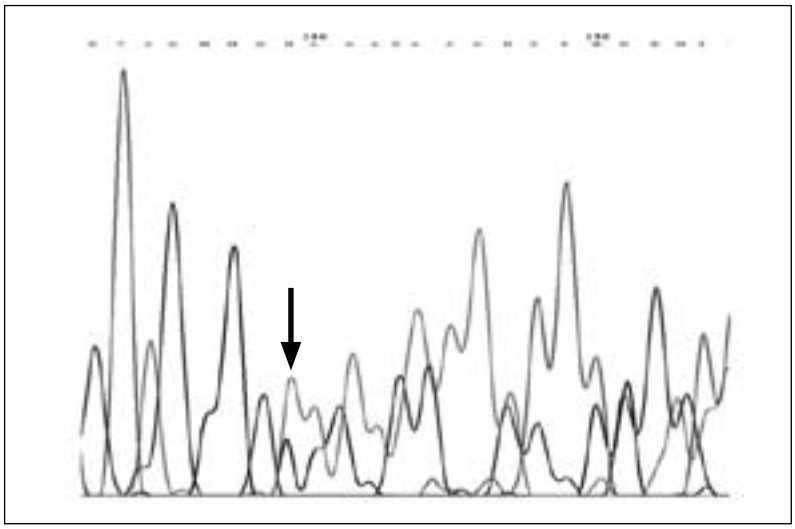

FIG 1. Partial sequence of exon 7 of the *CASR* gene of the patient
The mutant sequence shows an overlap with the normal sequence after the deletion of a cytosine at base position 2501. The first N in the chromatogram (arrow) marks the position of the deletion and the start of the overlapping pattern. The sequence is shown in the antisense direction

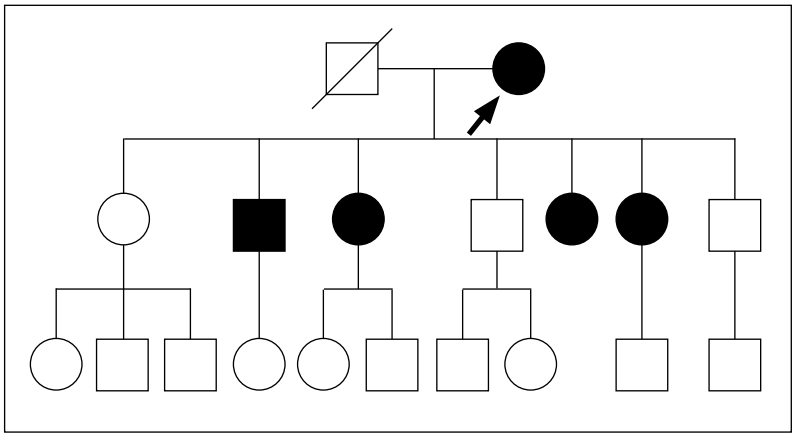

FIG 2. Family pedigree showing individuals with familial hypocalciuric hypercalcaemia
Individuals with hypercalcaemia who are confirmed carriers of the mutation are denoted by (■) and (●). Individuals with normocalcaemia who are negative for the mutation are denoted by (□) and (○). The proband is indicated by an arrow

PHPT,[5] though PHPT patients are often asymptomatic too. The diagnosis of FHH is suggested by the presence of reduced urinary calcium excretion in the presence of hypercalcaemia. The fractional excretion of calcium is typically less than 1% in patients with FHH. Nevertheless, the fractional excretion of calcium may also be low in PHPT; values may range between 1 and 5%.[5] Suspected cases of FHH should therefore be confirmed by detection of mutations within the *CASR* gene.[6] Subjects diagnosed with FHH should be reassured that their prognosis is excellent, though they may need future genetic counselling. Given the increasing number of patients who are evaluated for asymptomatic hypercalcaemia, it is important for clinicians to be alerted to the possibility of FHH. Establishing the correct diagnosis will enable the patient and other family members to avoid unnecessary investigations or operations.

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