

Think twice: a rare calcium sensing receptor mutation and a new diagnosis of familial hypocalciuric hypercalcaemia

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Summary

Distinguishing primary hyperparathyroidism (PHPT) from familial hypocalciuric hypercalcaemia (FHH) can be challenging. Currently, 24-h urinary calcium is used to differentiate between the two conditions in vitamin D replete patients, with urinary calcium creatinine clearance ratio (UCCR) <0.01 suggestive of FHH and >0.02 supportive of PHPT. A 26-year-old Caucasian gentleman presented with recurrent mild hypercalcaemia and inappropriately normal parathyroid hormone (PTH) following previous parathyroidectomy 3 years prior. He had symptoms of fatigue and light-headedness. He did not have any other symptoms of hypercalcaemia. His previous evaluation appeared to be consistent with PHPT as evidenced by hypercalcaemia with inappropriately normal PTH and UCCR of 0.0118 (borderline low using guidelines of >0.01 consistent with PHPT). He underwent parathyroidectomy and three parathyroid glands were removed. His calcium briefly normalised after surgery, but rose again to pre-surgery levels within 3 months. Subsequently, he presented to our centre and repeated investigations showed 24-h urinary calcium of 4.6 mmol/day and UCCR of 0.0081 which prompted assessment for FHH. His calcium-sensing receptor (*CASR*) gene was sequenced and a rare inactivating variant was detected. This variant was described once previously in the literature. His mother was also confirmed to have mild hypercalcaemia with hypocalciuria and, on further enquiry, had the same *CASR* variant. The *CASR* variant was classified as likely pathogenic and is consistent with the diagnosis of FHH. This case highlights the challenges in differentiating FHH from PHPT. Accurate diagnosis is vital to prevent unnecessary surgical intervention in the FHH population and is not always straightforward.

Learning points:

- Distinguishing FHH from PHPT with co-existing vitamin D deficiency is difficult as this can mimic FHH. Therefore, ensure patients are vitamin D replete prior to performing 24-h urinary calcium collection.
- Individuals with borderline UCCR could have either FHH or PHPT. Consider performing *CASR* gene sequencing for UCCR between 0.01 and 0.02.
- Parathyroid imaging is not required for making the diagnosis of PHPT. It is performed when surgery is considered after confirming the diagnosis of PHPT.

Background

FHH is typically a benign autosomal dominant disorder characterized by persistent mild hypercalcaemia, normal or mildly elevated PTH levels and low urinary calcium

excretion. Unlike PHPT, FHH does not cause end organ damage and there is no biochemical resolution after surgery. Therefore, it is crucial to differentiate PHPT from



FHH to avoid unnecessary parathyroidectomy, particularly in patients with atypical presentations or borderline biochemical findings. Low UCCR is currently used as the primary test that distinguishes FHH from PHPT. However, there may be overlap in urinary calcium excretion, and additional testing such as *CASR* gene testing is required to make an accurate diagnosis.

This case illustrates the diagnostic challenges in distinguishing the two conditions and demonstrates how this patient was exposed to unnecessary parathyroidectomy. This case also demonstrates the importance of genetic testing in confirming the diagnosis of FHH. The *CASR* gene testing in this patient detected a rare inactivating pathogenic variant that was only once described in the literature. This case now links two generations of FHH phenotype with this *CASR* mutation, confirming its pathogenicity. Current practices to differentiate PHPT from FHH are not uniform, and clinicians should exercise caution prior to recommending surgery to hypercalcemic patients.

Case presentation

A 26-year-old, previously fit and well Caucasian gentleman was referred for assessment and management of hypercalcaemia by his GP. He was symptomatic with a 1-month history of fatigue and light-headedness. He denied nausea, polyuria, polydipsia, myalgias, arthralgias, abdominal pain, constipation, flank pain and his mentation was intact. Dietary calcium and vitamin D intake was high including cheese, salmon, eggs, milk, beef and yoghurt. He denied taking calcium or vitamin D supplements and was not taking any medications that could cause hypercalcaemia. As a carpenter, he was physically very active with plenty of sun exposure, approximately 8 h a day.

He presented interstate 2 years prior when he was diagnosed with PHPT. Subsequently three parathyroid glands were removed. Otherwise he had no history of hypertension, renal calculi or fragility fractures. He never smoked and he drank alcohol each weekend. Significant family history included pancreatitis in his sister and bone malignancy in his cousin; however, there was no family history of hypercalcaemia.

On examination, he was alert, orientated and clinically euvolaemic. The abdomen was soft and non-tender. Other systemic examination was unremarkable and he was normotensive. There were no clinical features of endocrinopathy.

Investigation

Investigations in our centre 2 years after his parathyroidectomy confirmed ongoing mild hypercalcaemia of 2.72 mmol/L (2.15–2.55 mmol/L) with an inappropriately normal PTH at 2.7 pmol/L (1.6–6.9 pmol/L). Incidentally, he was noted to have hypervitaminosis D with a value of 213 nmol/L, (reference range: 50–150 nmol/L) which became within acceptable range 2 months later after modifying his diet to 140 nmol/L, (reference range: 50–150 nmol/L). Urea was 5.3 mmol/L (2.5–7.1 mmol/L), creatinine was 85 µmol/L (60–110 µmol/L) and eGFR was >90 mL/min (>90 mL/min). Abdominal imaging excluded nephrocalcinosis. 24-h urinary calcium showed calcium excretion of 4.6 mmol/day and UCCR of 0.0081. Given his low UCCR, *CASR* gene sequencing was performed and revealed a heterozygous likely pathogenic variant (Tier 2-ACMG classification): c.3235 T>C p.(*1079Gln>*)8).

CASR gene sequencing was also performed on the proband's parents. There was no pathogenic variant detected in his father. However, the same *CASR* variant seen in this patient was identified in his mother. Further investigation of his mother showed mild hypercalcaemia with relative hypocalciuria tested while vitamin D replete and inappropriately normal PTH. The calculated UCCR was 0.0053. These findings support the diagnosis of FHH in the mother of this patient and also indicated that the pathogenic *CASR* variant was maternally inherited.

Collateral history of his PHPT diagnosis was obtained. Two years prior at a different centre, he was mildly hypercalcaemic with inappropriately normal PTH and UCCR of 0.0118 (using current guidelines of >0.01 indicative of PHPT). He had one Sestamibi-avid parathyroid gland and proceeded to parathyroidectomy. However, three glands were found to be enlarged intra-operatively and hence were removed. Of those, two were adenomas and one was a normal parathyroid gland. His corrected calcium transiently normalised post-operatively; however, it became elevated again within 3 months of the surgery. His PTH remained inappropriately normal (see [Table 1](#) for collated biochemistry). He underwent genetic testing for Multiple Endocrine Neoplasia Type 1 syndrome (MEN1), which was negative.

Outcome and follow-up

Our patient and his family were advised of the diagnosis and the autosomal dominant nature of inheritance was briefly discussed. They were referred to another centre for



Table 1 Biochemistry performed 2015–2018. Parathyroidectomy performed 8 March 2016.

	25/8/15	27/8/15	23/3/16	14/6/16	3/4/18	18/4/18	19/6/18
Corrected calcium (2.15–2.55 mmol/L)	2.69		2.42	2.61	2.72	2.67	2.7
Vitamin D (50–150 nmol/L)	110				213		140
1,25-Dihydroxyvitamin D (60–208)							126
Phosphate (0.8–1.4 mmol/L)					1.26	1.13	0.87
Urea (2.5–7.1 mmol/L)							5.3
Creatinine (60–110 µmol/L)							85
eGFR (>90 mL/min)							>90
Parathyroid hormone (1.6–6.9 pmol/L)	3.7			3.9	2.7		2.8
24-h urinary calcium (2.5–7.5 mmol/day)		5.8				4.6	
Urinary Calcium: creatinine clearance		0.0118				0.0081	

in-depth genetic counselling. His incidental finding of hypervitaminosis D has been treated with reduced dietary vitamin D intake. No routine follow-up investigations have been recommended. The family is aware that parathyroid surgery is not indicated and will avoid this procedure in the future.

Discussion

FHH has three subtypes. FHH type 1 is an autosomal dominant genetic condition where heterozygous inactivating mutations in the *CASR* cause hypercalcaemia and hypocalciuria (1). It has a high penetrance. FHH2 is caused by defective Gα11 signalling protein (gene *GNA11*) downstream of *CASR* (1). AP2σ2 (gene *AP2S1*) is responsible for endocytosis of *CASR*, and loss-of-function mutation in this protein results in FHH type 3 (1, 2). FHH3 tends to have higher mean fasting plasma calcium compared with FHH1 (1). FHH1 accounts for 65% of FHH patients, while FHH3 accounts for 5% and the rarer FHH2 <1% (2). The remaining 30% are unclassified. Autoimmune hypercalcaemia is a possible differential diagnosis in this case. This is an acquired condition characterised by agonism or antagonism of the *CASR* which can mimic genetic causes of hyper or hypocalcaemia (3). It should be considered in cases where the *CASR* mutation screen is negative and there is a previous history of normocalcaemia with no family history of calcaemic disorder. Our patient does not have a personal or family history of autoimmune disease.

Our patient most likely has FHH1, given he had a calcium-sensing receptor inactivating mutation with the same mutation detected in his mildly affected mother. This rare stop-loss variant p.(*1079Glnext*8) in exon 7 of *CASR* gene was previously described once in the literature (4). It presents a single nucleotide substitution at codon

1079, which is the stop codon of the *CASR* gene. The reference nucleotide T (Thymine) was substituted for C (Cytosine) at position 3235 in exon 7 (The last exon of *CASR* gene). This nucleotide substitution is predicted to result in loss of the stop codon (TAA), which is replaced by a new codon (CAA) for Glutamine. A new stop codon is predicted to form after eight amino acids from the original stop codon. Consequently, the mRNA or the protein produced is predicted to be longer in size than that produced by a wild-type (WT) *CASR* gene.

At the time of reporting, this rare variant was initially classified as a variant of uncertain clinical significance using the 2015 ACMG Standards and guidelines for the interpretation of sequence variants (5). However, following an update of the ACMG/AMP guidelines from the ClinGen SVI working group (6), it was reclassified as likely pathogenic (PM2, PM4, PS3_Supporting, PS4_Supporting). This *CASR* variant is absent from population databases (EXAC, gnomAD) and is predicted to result in a change of protein length. Also, the same variant has been reported in two members (mother and daughter) from a single family with a phenotype consistent of FHH (4). The pathogenicity of this variant was confirmed by functional analysis of *CASR* maturation, cell surface expression, and signalling using transient transfection studies in HEK293 cells in the same case-control study (4).

The *in vitro* functional studies showed that *CASR* protein with (*1079Glnext*8) variant has reduced cell surface expression and markedly impaired phosphorylation signalling of the ERK1/2 MAPKs pathway when compared to WT *CASR*. This finding is consistent with loss-of-function (inactivating) mutation characterising FHH. As this variant was reported only once previously, familial segregation analysis was recommended to provide further evidence of pathogenicity. Parental genetic testing



confirmed that the *CASR* mutation detected was inherited from the proband's mildly affected mother, consistent with autosomal dominant inheritance.

It is important to differentiate FHH from PHPT, as the management of these two conditions differs: FHH is managed conservatively, while PHPT requires surgery for cure. There are also genetic concerns that need addressing in FHH given it is an autosomal dominant condition. Patients with PHPT tend to be older, females and have high PTH (1). PTH is more likely to be inappropriately normal with hypercalcaemia in patients with FHH.

Vitamin D should be replete prior to full assessment of calcium disorders. Vitamin D deficiency results in calcium resorption in the kidney and can result in hypocalciuria (7). Thus, PHPT with vitamin D deficiency can be misdiagnosed as FHH. Treatment of hypovitaminosis D is thought to be safe in PHPT and, in some cases, can reduce hypercalcaemia due to treatment of concomitant secondary hyperparathyroidism (7). Interestingly, our patient was incidentally diagnosed with hypervitaminosis D. Physiologic processes prevent vitamin D toxicity due to excessive sunlight; however, excessive vitamin D supplementation can lead to vitamin D toxicity (8). Vitamin D levels in our patient normalised with reduced dietary intake and symptoms of vitamin D toxicity were not present.

Urinary calcium is vital in workup for calcium disorders. Currently, UCCR <0.01 indicates hypocalciuria and is used to guide genetic screening of *CASR* (9). Studies have suggested using a higher cut-off (4, 10). In a series of 182 patients with hypercalcaemia secondary to FHH and PHPT, a shift of UCCR from <0.01 to <0.02 increased the sensitivity of detecting FHH from 65% to 95%. Alternatively, a Pro-FHH scoring system has been proposed that has shown good predictive power for PHPT, although this has only been tested in a small population of patients (11). It should also be noted that PHPT can occur in FHH (12) and certainly this could have been the case for our patient.

Lastly, it is important to recognise that positive parathyroid scintigraphy indicates increased mitochondrial activity in parathyroid tissue (13, 14). This can be consistent with a parathyroid adenoma or parathyroid hyperplasia, although tracer washout tends to be faster in the latter (13, 14, 15). Parathyroid hyperplasia is known to occur in FHH (14). Thus, sestamibi may not distinguish benign parathyroid hyperplasia in FHH from pathologic hyperplasia in PHPT. Imaging should be reserved for surgical targeting once PHPT has been confirmed with confidence.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Patient consent

Consent has been obtained from the patient, the patient's mother and the patient's father, given all three are mentioned in this case report.

Author contribution statement

All three authors have contributed to the writing and editing of this manuscript and the physician responsible for the patient is one of the authors.

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