



Parathyroid hormone-dependent familial hypercalcemia with low measured PTH levels and a presumptive novel pathogenic mutation in CaSR

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Abstract

Familial hypocalciuric hypercalcemia (FHH) is a benign autosomal dominant condition characterized by lifelong asymptomatic hypercalcemia. FHH is typically caused by a heterozygous inactivating mutation of the calcium-sensing receptor (CaSR) and characterized by moderate hypercalcemia, inappropriately normal or elevated serum parathyroid hormone (PTH), and relative hypocalciuria ($Fe_{Ca} < 2\%$) with histologically normal parathyroid glands. FHH should be distinguished from primary hyperparathyroidism so that unnecessary parathyroid surgery is avoided. We report a case that presented with asymptomatic, familial hypercalcemia but low PTH and normal (non-low) urinary calcium excretion found to be secondary to a novel pathogenic inactivating mutation of the CaSR gene. We present an asymptomatic 54-year-old Malaysian woman with incidentally discovered hypercalcemia, intermittent hypophosphatemia, and $Fe_{Ca} > 2\%$. PTH levels were repeatedly below the mean of the reference range (on two separate assays) and sometimes even below the lower reference limit. Two siblings, one niece, and her son had hypercalcemia without nephrolithiasis. Cinacalcet, used as a PTH-suppression test, normalized serum total and ionized calcium after 7 days of cinacalcet 30 mg BID, confirming her hypercalcemia was PTH-mediated. Given her family history, genetic testing was pursued and discovered a novel pathogenic mutation of the CaSR gene confirming the diagnosis of FHH type 1. Our case represents an atypical presentation of FHH1 with low PTH and $Fe_{Ca} > 2\%$. This contributes to the expanding clinical and biochemical spectrum of CaSR inactivating mutations and presents an innovative approach to evaluating biochemically uncertain familial hypercalcemia with cinacalcet before pursuing expensive genetic analysis.

Keywords Calcium-sensing receptor gene · Familial hypocalciuric hypercalcemia · Hypercalcemia · Parathyroid hormone

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Introduction

Familial hypocalciuric hypercalcemia (FHH) is a benign autosomal dominant condition that is characterized by lifelong, non-progressive, and asymptomatic hypercalcemia [1–3]. FHH type 1 is typically caused by a heterozygous inactivating mutation of the gene coding for the calcium-sensing receptor (CaSR), which regulates parathyroid hormone (PTH) secretion and renal calcium excretion [1, 3, 4]. At present, more than 139 mutations in the CaSR gene causing FHH1 have been reported [5]. Less commonly, FHH may be due to mutations of the G protein subunit alpha11 gene (GNA11, FHH type 2), or in the adaptor-related protein complex 2 gene (AP2S1, FHH type 3) [6]. The classic biochemical abnormalities found in FHH include moderate hypercalcemia,

inappropriately normal or elevated serum PTH, and relatively low urinary calcium with histologically normal parathyroid glands [1, 4, 7]. FHH should be distinguished from primary hyperparathyroidism (PHPT), so that unnecessary parathyroid surgery is avoided [8].

We report a case of asymptomatic, familial hypercalcemia but low to normal levels of measured serum PTH and normal (non-low) urinary calcium excretion. A logical and innovative approach was used in the evaluation of our patient's familial hypercalcemia and atypical biochemistry (i.e., suppressed PTH and normocalciuria) using a short course of cinacalcet as a diagnostic calcium-normalization test to demonstrate her hypercalcemia was PTH/CaSR mediated. Genetic sequencing discovered a presumptive novel pathogenic inactivating mutation of the CaSR gene, thereby confirming the diagnosis of FHH type 1.

Case presentation

A 54-year-old woman from Malaysia was assessed in a general endocrinology clinic for incidentally discovered, asymptomatic hypercalcemia. Informed written consent for publication was given. She had no history of nephrolithiasis, gastroesophageal reflux or constipation, osteoporosis, fractures, renal insufficiency, or any psychiatric complaints. Her past medical history was significant for post-traumatic chronic myofascial pain syndrome for which she used analgesics (i.e., cyclobenzaprine, ibuprofen, gabapentin) as needed. She denied supplemental calcium intake beyond a single daily multivitamin and the use of any medications that would influence calcium metabolism including hydrochlorothiazide or lithium. Her family history was reported to include unexplained hypercalcemia without nephrolithiasis in two siblings, one niece, and her son.

On examination, she appeared well and was non-obese (BMI 21.4 kg/m²). Head and neck examination revealed no band keratopathy. There was no evidence of ectopic or subcutaneous calcification. Her proximal muscle strength and reflexes were normal.

Her bloodwork over a 14-month period is shown in Tables 1 and 2 and Fig. 1 (supplementary material). She had calcium levels that ranged between 2.56 and 2.86 mmol/L

(reference 2.10–2.55 mmol/L) with normal albumin, borderline low phosphate between 0.71 and 0.95 mmol/L (reference 0.80–1.50 mmol/L), 24 h urine calcium 6.12 mmol/day (reference 2.50–7.50 mmol/day), and urinary fractional excretion of calcium (Fe_{Ca}) 2.13%. Serum PTH levels were repeatedly well below the mean of the reference range and occasionally even below the 5th percentile (mean 11 ng/L, range 6–17 ng/L, reference 7–37 ng/L). Remaining bloodwork including serum protein electrophoresis, calcitriol, calcidiol, estimated glomerular filtration rate, thyroid-stimulating hormone, parathyroid hormone-related peptide levels, and bone mineral density were normal. Table 2 shows representative biochemical data from 17 contemporaneously diagnosed patients at our center with primary hyperparathyroidism due to surgically proven adenoma, along with biochemical results from a contemporaneously diagnosed subject from a large and previously characterized FHH family.

Given her clear family history of hypercalcemia but unconvincing biochemistry for FHH, we used a short course of cinacalcet as a diagnostic tool, to distinguish whether her hypercalcemia was CaSR-mediated and judiciously direct further investigations. Baseline total and ionized calcium, phosphate, magnesium, PTH, and spot Fe_{Ca} levels were obtained before administering cinacalcet 30 mg PO twice daily for 7 days. On the seventh day of therapy, the above measures were repeated and demonstrated clear evidence of calcium normalization and further suppression of PTH, confirming her hypercalcemia was mediated by the CaSR (Table 3). Importantly, she reported no new clinical symptoms during this short treatment course.

Once her hypercalcemia was established to be CaSR-mediated, genetic sequencing was pursued and revealed a presumptive novel pathogenic mutation in her CaSR gene (heterozygous c.1377 + 1G > T variant), confirming the diagnosis of FHH type 1. This variant is predicted to abolish the splice donor site of exon 4 and has not been reported in ExAC or the literature. Most of the proband's reportedly affected family members live in Malaysia thus clinical and genetic assessment was not possible. However, the proband's 27-year-old son has recently been found to have mild hypercalcemia and subsequent testing revealed a similar biochemical profile (calcium 2.59 mmol/L (reference range 2.10–2.55 mmol/L), PTH 13 and 20 (reference range 17–37 ng/L), 24 h urine calcium 5.38 mmol/day, and Fe_{Ca} 1.99%) and the same novel CaSR gene mutation (heterozygous c.1377 + 1G > T variant).

Table 1 Proband's laboratory investigations

	Patient's results	Reference range
Calcium (mmol/L)	2.56 – 2.86	2.10–2.55
Phosphate (mmol/L)	0.71 – 0.95	0.80–1.50
Serum PTH (ng/L)	6 – 17	7–37
Fe _{Ca}	2.13%	
Magnesium (mmol/L)	1.03	0.65–1.05

Discussion

FHH is a benign form of PTH-mediated hypercalcemia caused by a heterozygous inactivating mutation of CaSR [1, 4] leading to compensatory hyperparathyroidism, hypercalcemia with relative hypocalciuria [3, 9]. CaSR is expressed in the

Table 2 Typical serum calcium and PTH values with Diasorin XL PTH assay for other FHH and PHPT cases at our center

	Calcium (mmol/L)	PTH (ng/L)	FeCa (%)
FHH1 patient	2.83	39	1.08
Primary hyperparathyroidism patients (<i>n</i> = 17), median, range	2.74 (2.55–2.92)	53 (32–94)	1.45 (0.8–4.2) [†]

The same PTH assay used for measurements in Tables 1 and 2

Hypercalcemic, hyperparathyroid cases seen since January 2015, excluding any familial causes known or suspected, lithium use, chronic kidney disease. All cases verified to have parathyroid adenoma at surgery with normal biochemical indices postoperatively

[†] Three cases did not have FeCa calculated due to presence of overt hypercalciuria

parathyroid glands and kidneys, where it maintains calcium homeostasis by regulating PTH secretion and renal tubular calcium reabsorption [3]. Inactivating germline CaSR mutations result in an altered calcium ion-dependent set point for PTH release from the parathyroid chief cells and a rightward shift of the PTH-serum calcium dose-response curve [8]. This altered set point explains the inappropriately normal or high serum PTH seen in FHH [10]. This same mutated CaSR in the renal tubular cells leads to increased tubular reabsorption of calcium and a relatively low renal calcium excretion (i.e., relative hypocalciuria $FeCa < 1\%$) [9].

The CaSR is a member of the G protein-coupled receptor family expressed uniformly in all tissues participating in systemic mineral metabolism [11, 12]. It is comprised of 1078 amino acids and contains a 612 amino acid extracellular domain (ECD) essential for co-translational processing, receptor dimerization, binding ligands, and transmitting activation signals through the seven transmembrane domains (TMD's), made up of residues 613–862, and the intracellular domain (ICD), made up of residues 863–1078 [3, 8]. The majority of FHH1-associated mutations are located at the ECD; however, they can occur at the TMD, ICD, or as a consequence of

defective trafficking to the plasma membrane resulting in reduced cell surface receptor expression [8].

Our findings confirm our patient's atypical biochemistry (suppressed PTH, lack of hypocalciuria) are still a result of a heterozygous CaSR mutation (c.1377 + 1G > T) predicted to abolish the splice site of exon 4, producing a novel genotype of FHH1. Abolishment of the exon 4 splice donor site would result in a truncated CaSR extracellular domain in the cysteine-rich domain region of the CaSR [13]. The deletion of a portion of this domain results in loss of cysteine at position 541 and 545 and is hypothesized to result in compromised function of the CaSR due to the role of the cysteine-rich region in signal transmission from the Venus flytrap (VFT) domain to the seven-transmembrane domain [14, 15]. The impact of alteration of the cysteine-rich domains on the CaSR function is variable and has been shown to tolerate a significant degree of amino acid substitution [14, 15] such that the full impact of exon 4 deletion is unclear. Potential explanations for her atypical biochemical presentation include a variable FHH1 phenotype caused by the underlying CaSR splice donor site mutation leading to loss of a portion of the cysteine-rich extracellular domain or an altered PTH gene resulting in suboptimal detection with some PTH assays [16].

Several authors have reported patients with FHH can exhibit variability in serum calcium and PTH values depending on the underlying CaSR mutation [3, 17, 18]. Variability in serum calcium levels amongst 15 individual families with FHH was described as far back as 1981 by Marx et al. However, CaSR gene sequencing was not performed in these cases to correlate the

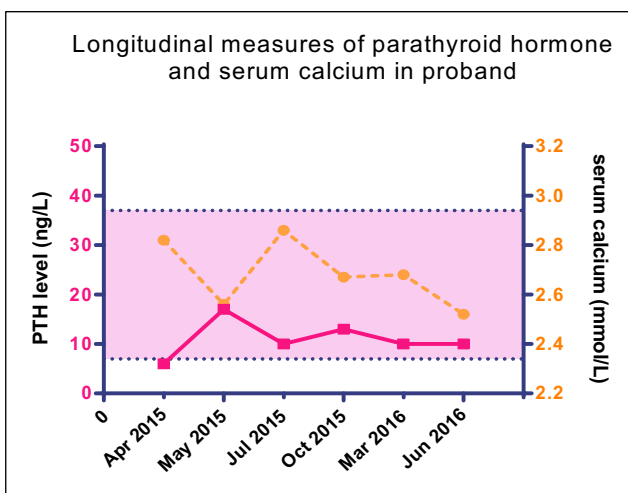


Fig. 1 Longitudinal biochemical observations in proband; shaded area represents the normal reference range on the PTH assay

Table 3 Cinacalcet suppression test investigations

	Pre-cinacalcet	Post-cinacalcet
Calcium (mmol/L)	2.74	2.28
PTH (ng/L)*	26	20
$FeCa$	2.13%	2.11%
Magnesium (mmol/L)	1.03	0.90
Phosphate (mmol/L)	0.89	0.95

*Note the Roche Cobas 8000 PTH assay was used during the cinacalcet test, see methods/supplemental data for further details

different genotypes with their biochemical presentation [19]. More recently, a Danish population study evaluated 66 FHH patients with 11 different CaSR mutations and demonstrated marked differences in the biochemical FHH phenotype, with significant inter-mutation variation in both plasma calcium and PTH values [12]. However, none of the reported cases had serum PTH levels below the reference range as seen with our case. Perhaps most strikingly, Aida et al. describe a Japanese woman who inherited two copies of a CaSR missense mutation from her related heterozygous parents resulting in a homozygous gene mutation, classically associated with severe neonatal hyperparathyroidism. However, her genotype only caused a modest defect in her CaSR function and resulted in a mild clinical and biochemical phenotype that was not detected until later in life [20].

Limitations to the interpretation of our patient's biochemistry include the impact dietary calcium may have on urinary calcium excretion, as well as the method of urinary calcium measurement. However, in healthy individuals, a higher intake of calcium is typically only associated with a small increase in urinary calcium [21]. Additionally, studies based on healthy volunteers ingesting varying degrees of calcium supplementation, have reported a "plateau effect" of calcium intake on urinary calcium excretion [22, 23]. We used the calcium creatinine clearance ratio, also known as Fe_{Ca} , and spot urine calcium measurements to quantify our patient's hypercalciuria. Fe_{Ca} , as an index of renal calcium excretion, has performed equally well when compared to 24-h renal calcium excretion and 24-h renal calcium/creatinine excretion ratio as a discriminative test for patients with FHH and PHPT [24]. In contrast, when searching for overt hypercalcemia, spot urine calcium measurements should not be used interchangeably with 24-h values due to their low sensitivity [25].

In cases of uncertainty regarding the role of PTH and the CaSR in hypercalcemic patients, the CaSR agonist cinacalcet may be of use as a diagnostic tool. Cinacalcet is a calcimimetic that increases the sensitivity of the CaSR in both the parathyroid gland and kidney to calcium. In patients with hyperparathyroidism or CaSR-mediated hypercalcemia, cinacalcet should lower PTH and increase renal calcium excretion thus lowering serum calcium [26]. In our case, we hypothesized the administration of cinacalcet would result in a decrease in serum calcium if our patient's hypercalcemia was indeed PTH/CaSR-mediated. Her biochemical response to this test (calcium normalization and further suppression of PTH) confirmed our theory. With the concomitant knowledge of the family history of hypercalcemia, it was then logical to pursue genetic testing for FHH.

The use of cinacalcet as a diagnostic tool for routine PHPT has been described previously by Cailleux et al. [26]. The authors found 60 mg of cinacalcet provided similar PTH lowering results as the potentially more dangerous calcium loading test [26]. Although their patient population did not include atypical or FHH patients, this study provides evidence that the

use of cinacalcet is safe, effective, and has many potential applications as a diagnostic test where PTH levels are equivocal in the face of hypercalcemia.

Conclusion

We report a case of FHH1 that presented with familial hypercalcemia, suppressed serum PTH, and normocalciuria caused by a novel inactivating CaSR mutation. The underlying mechanism of her atypical biochemistry reminds clinicians to maintain a high index of suspicion for FHH in cases of familial hypercalcemia. Our case contributes to the growing knowledge of the expanding clinical and biochemical spectrum of CaSR inactivating mutations and affords an innovative approach to evaluating biochemically uncertain familial hypercalcemia with cinacalcet before pursuing expensive genetic analysis.

Compliance with ethical standards

Ethics approval and consent to participate University of Calgary ethical approval was given for publication of biochemical data for a local control population.

The article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants (patient and her son) included in the study.

Conflicts of interest None.

Abbreviations FHH familial hypocalciuric hypercalcemia ; CaSR calcium-sensing receptor ; PTH parathyroid hormone ; PHPT primary hyperparathyroidism ; GNA11 G protein subunit alpha11 gene ; AP2S1 adaptor-related protein complex 2 gene ; BMI body mass index ; Fe_{Ca} fractional excretion of calcium ; ExAC exome aggregation consortium ; ECD extracellular domain ; TMD transmembrane domains ; ICD intracellular domain ; FHH1 familial hypocalciuric hypercalcemia type 1 ; VFT Venus flytrap

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