Clinical, Biochemical, and Genetic Profile of an Indian Kindred with Type 1 Familial Hypocalciuric Hypercalcemia

Sir,

Familial hypocalciuric hypercalcemia (FHH) is a rare genetic disorder with autosomal dominant inheritance and high penetrance that manifests with mild parathyroid hormone (PTH)-dependent hypercalcemia and hypocalciuria.[1,2] The disorder results from an inherited defect in calcium sensing mechanism at the level of parathyroid gland and the thick ascending limb of loop of Henle of kidney.[3] FHH is genetically heterogenous and three subtypes have been described, namely, FHH1, FHH2, and FHH3, related to inactivating mutations in the genes encoding calcium sensing receptor (CaSR), G-protein subunit alpha-11 (GNA11), and adaptor-related protein complex 2 sigma 1 subunit (AP2S1) proteins, respectively.[3] Most patients with FHH are asymptomatic.[4] The disorder runs a benign course in most patients; parathyroidectomy is neither required, nor it is curative.[5] Symptoms of neonatal hypocalcemia have been described in unaffected children of women affected with this rare disorder.[6] Here, we describe an Indian kindred with genetically confirmed type 1 FHH where the presentation in the index case was delayed till the age of 48 years, and confounded by secondary hyperparathyroidism related to vitamin D deficiency.

A 48-year-old female initially presented to an orthopedic specialist with complaints of generalized body ache for the past 2 years. Her initial work-up including complete blood count, liver and kidney function tests was unremarkable. She had mild hypercalcemia (serum calcium: 10.8 mg/dL, N: 8.5-10.5 mg/dL), mild hypophosphatemia (serum phosphate: 2.4 mg/dL, N: 2.5-4.5 mg/dL), raised serum PTH (164.7 pg/mL, N: 15-65 pg/mL), and low serum 25-hydroxyvitamin D levels (6.8 ng/mL, N: 20-50 ng/mL) [Table 1]. A further work-up of hypercalcemia was not done and the patient was administered calcium (1 gram per day elemental calcium) and injectable (cholecalciferol 600,000 IU intramuscular once a week for 2 weeks), followed by oral vitamin D (cholecalciferol 60,000 IU sachet once a week for 8 weeks) preparations. On a follow-up visit, serum PTH levels reduced to 102 pg/mL; however, hypercalcemia persisted [Table 1]. Thereafter, she was referred to our department for evaluation. On revisiting the history, she had no complaints other than the generalized body ache. She denied history of nausea, vomiting, fragility fractures, neuropsychiatric manifestations, and intake of lithium or any other drugs known to cause hypercalcemia. Her symptom of body ache improved significantly after vitamin D supplementation. She was premenopausal and had regular menses. She had a history of renal colic 12 years back when she was detected to have bilateral renal micro-concretions without any definite calculus and advised adequate fluid intake. No metabolic work-up was done at that time, and there was no recurrence of abdominal pain following the initial episode. She had two children, 14 and 20 years of age, who had normal birth and development history. There was no family history of hypercalcemia or failed parathyroid surgery in any

Table 1: Biochemical profile of the index patient and her children

Parameter	Index Patient			Daughter (20 years)		Son (14 years)		Reference Range
	Visit 1	Visit 2 (After 8 weeks)	Visit 3 (After 12 weeks)	Visit 1	Visit 2 (After 8 weeks)	Visit 1	Visit 2 (After 8 weeks)	
Urea (mg/dL)	16	14	14	16	16	12	13	10-40
Creatinine (mg/dL)	0.6	0.7	0.6	0.4	0.5	0.5	0.4	0.5-1.0
Serum calcium (mg/dL)	10.8	11.7	11.4	10.6	10.5	11.6	11.5	8.5-10.5
Serum phosphate (mg/dL)	2.4	3.4	3.0	2.4	3.1	4.3	4.9	2.5-4.5
Alkaline Phosphatase (IU/L)	83	111	98	270	92	426	234	80-240
Albumin (g/dL)	4.8	4.8	4.0	4.3	4.3	4.2	4.9	3.5-5.0
iPTH (pg/mL)	164.7	102	122.4	270	63.5	351	63.9	15-65
25(OH) D (ng/mL)	6.8	56.4	57.4	4.0	24.5	6.8	30.9	<12: Deficient 12-20: Insufficient >20: Sufficient
Urinary calcium, (mg/24 h)	NA	60	36	76	60	17.5	45	100-300 mg
Urinary creatinine, (mg/24 h)	NA	1080 (17.7 mg/kg)	854 (14 mg/kg)	852 (15.8 mg/kg)	1080 (20 mg/kg)	498	877	11-20 mg/kg/24 h
Urinary CCCR	NA	0.003	0.002	0.003	0.002	0.001	0.001	<0.01 suggests hypocalciuria

25 (OH) D: 25-hydroxyvitamin D; CCCR: Calcium Creatinine Clearance Ratio; iPTH: intact parathyroid hormone; NA- Not available

relative. Her height, weight, and body mass index (BMI) were 168 cm, 61 kg, and 21.6 kg/m^2, respectively. There were no features to suggest multiple endocrine neoplasia (MEN) syndrome. Rest of her general and systemic examination was unremarkable.

We repeated her biochemical investigations. Serum and urine calcium were measured using ortho-cresolphtalein complexone (o-CPC) method on Modular P800 Clinical Chemistry autoanalyser (Roche Diagnostics, Germany). Albumin-adjusted or "corrected" calcium is known to underestimate calcium status in patients with increased serum albumin[7]; therefore, we have not reported "corrected" serum calcium values in this paper. Serum and urine creatinine were measured using kinetic Jaffe method on the same autoanalyser. Serum PTH was measured using an electrochemiluminescent immunoassay on cobas e-411 autoanalyser (Roche Diagnostics, Germany) and serum 25(OH) D was measured using a chemiluminescent immunoassay on Liaison XL autoanalyser (DiaSorin Inc., USA). Urine calcium creatinine clearance ratio (CCCR) was calculated as: [24-hour urine calcium × serum creatinine] ÷ [24-hour urine creatinine × serum calcium]. A urine CCCR of <0.01 was taken as evidence of hypocalciuria.

Repeat biochemical profile suggested mild PTH-dependent hypercalcemia (serum calcium: 11.4 mg/dL, serum PTH: 122.4 pg/mL) with hypocalciuria (24-hour urine calcium: 36 mg, urine CCCR: 0.002) in the face of Vitamin D sufficiency (serum 25(OH) D: 57.4 ng/mL) [Table 1]. Work-up for end-organ manifestations of hyperparathyroidism including bone mineral density (BMD) (Discovery A, Hologic Inc., USA) [Table 2], and ultrasonography of kidney, ureter, and bladder (KUB) region was unremarkable. Given the biochemical picture of mild PTH-dependent hypercalcemia, hypocalciuria, and absence of any end-organ manifestations of PTH excess, we considered a possibility of FHH and requested for family biochemical screening. Her parents and two sisters had normal biochemical profile. However, both her children had a similar biochemical picture, comprising mild PTH-dependent hypercalcemia (offspring 1, serum calcium: 10.6 mg/dL, serum PTH: 270 pg/mL; offspring 2, serum calcium: 11.6 mg/dL, serum PTH: 351 pg/mL) and hypocalciuria (offspring 1 and 2, CCCR: 0.003 and 0.001, respectively) in the face of vitamin D deficiency (offspring 1 and 2, serum 25(OH) D: 4.0 and 6.8 ng/mL, respectively). Both of them received oral cholecalciferol supplementation (60,000 IU sachet weekly for 8 weeks), following which serum PTH levels reduced significantly to high-normal range (offspring 1 and 2, serum PTH: 63.5 and 63.9 pg/mL, respectively), while hypocalciuria (offspring 1 and 2, CCCR: 0.002 and 0.001, respectively) and hypercalcemia (offspring 1 and 2, serum calcium: 10.5 and 11.5 mg/dL, respectively) persisted [Table 1]. This raised the suspicion further for an inherited defect in calcium sensing mechanism, and we proceeded with genetic testing to confirm the diagnosis. The genetic findings were interpreted according to the recommendations of American College of Medical Genetics.[8]

Next-generation sequencing (NGS, Medgenome Laboratories, Bangalore, India) in the index case revealed a heterozygous missense pathogenic variation in exon 3 of the *CASR* gene (chr3; g.121976155C>T; c.413C>T; Depth 83x)) that resulted in amino acid substitution of methionine for threonine at codon 138 (p. Thr138Met). The CASR gene has multiple pseudogenes in the human genome. Therefore, further validation of NGS result was performed using Sanger sequencing (Medgenome Laboratories, Bangalore, India), which confirmed the variant in the index patient, and subsequently, both her children [Figure 1]. We searched for published variants in literature and this variant has been previously described.[9,10] A diagnosis of FHH type 1 was therefore confirmed and the family was counselled about the benign nature of this condition.

Estimation of serum PTH levels is an important initial step in broad subtyping of hypercalcemia into PTH-dependent and PTH-independent cause. Primary hyperparathyroidism (PHPT) is by far the most common cause of PTH-dependent hypercalcemia, with prevalence estimates of 1–4 cases per 1,000 adults.[11] On the other hand, FHH is a rare but important cause of PTH-dependent hypercalcemia, the prevalence of which has been reported at 1 in 78,000.[12] FHH is characterized by mild hypercalcemia (usually <12 mg/dL), which is present since infancy, normal or mildly elevated serum PTH levels, and usually no end-organ manifestations.[3]

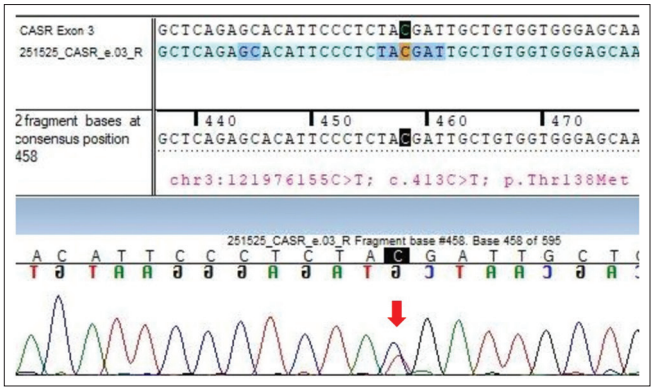

Figure 1: Sequence chromatogram and the reference sequence showing the variation in exon 3 of the CASR gene (chr3:121976155C>T; c.413C>T, p. Thr138Met) detected in heterozygous condition by Sanger sequencing in the index case and her children

Table 2: BMD of the index patient using DXA

Site	BMD (g/cm^2)	*T* Score	*Z* Score
Spine L1-L4	1.1	1.2	1.9
Neck Femur	0.8	-0.1	0.6
Distal 1/3 radius	1.5	-0.1	0.6

BMD: Bone mineral density; DXA: Dual-energy X-ray absorptiometry

In FHH, the mild biochemical defect due to impairment in calcium sensing mechanism, is present since infancy.[4] However, this defect was never detected in the index patient till she presented to us at the age of 48 years for evaluation of non-specific body aches. The presentation was also confounded by very high serum PTH levels in the index patient and her offspring, which were related to the co-existing vitamin D deficiency. The elevated PTH levels declined significantly following correction of vitamin D deficiency in the index patient and her children. A correction of vitamin D-deficient state in such cases is not only important to accurately interpret "hypocalciuria", but also to judge as to what extent PTH elevation is primary or secondary in nature. Diagnosis can be challenging especially in cases with associated vitamin D deficiency and borderline values of CCCR.[13] The mild hypercalcemia in index case was overlooked by the orthopedics specialist in the first instance, and mega doses of vitamin D and calcium were used to treat the deficiency state. This highlights the problem of frequent use of high doses of parental vitamin D; the consequence of this overzealous treatment, that is, "vitamin D toxicity" has been abundantly reported in the recent past from India.[14]

Akin to glucokinase (glucose sensor) defect in maturity onset diabetes of young type 2 (MODY2), where macrosomia and neonatal hypoglycemia have been reported, the presence of calcium sensing defect in a mother with FHH can lead to jitteriness related to neonatal hypocalcemia in her unaffected offspring.[6,15] In our kindred, both the children were affected, and were therefore spared of this possible complication in the neonatal period. Drawing further analogies to the glucokinase defect where hyperglycemia is mild, is not associated with vascular complications, and requires only close observation, patients with FHH have mild reset hypercalcemia, do not usually experience end-organ complications of PTH excess, and can be managed conservatively.[1,16]

In a patient with PTH-dependent hypercalcemia, a low urine CCCR despite vitamin D sufficiency is a good starting point to suspect FHH.[16] More than 80% of patients with FHH have urine CCCR of <0.01. Most patients with PHPT have urine CCCR of >0.02; however, about 20% patients with PHPT, especially those with co-existing vitamin D deficiency may manifest with a low urine CCCR of <0.01.[17] Similarly, serum PTH levels can be elevated in 15–20% of cases of FHH, while about 10% cases with PHPT may present with normal serum PTH levels (which are still inappropriate for the degree of hypercalcemia).[1] Thus, modest overlap remains between FHH and PHPT, and no biochemical investigation may be completely reliable to differentiate between the two conditions.[17] Genetic testing is confirmatory for FHH, and should include testing for *CASR, AP2S1,* and *GNA11* genes either sequentially (in the order described when using Sanger sequencing) or simultaneously (in case next generation sequencing is employed).

To conclude, we present a detailed clinical, biochemical, and genetic analysis of an Indian kindred with FHH. Our case highlights the need to follow a systematic approach in evaluation of a patient with PTH-dependent hypercalcemia.

Consent for publication

Written informed consent was obtained from the patient for this publication.

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Conflicts of interest

There are no conflicts of interest.

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