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Case Report

Case Reports of Patients Diagnosed with Familial Hypocalciuric Hypercalcemia, A Disorder That Should be Kept in Mind in Hypercalcemia Cases

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ABSTRACT

Familial hypocalciuric hypercalcemia (FHH) causes hypercalcemia by three genetic mechanisms: Inactivating mutations in the calcium-sensing receptor (*CaSR*), G-protein subunit α11 or adapter-associated protein complex 2, sigma 1 subunit. In other cases, hypercalcemia causes significant morbidity and mortality, while FHH usually follows a benign course. Failure to diagnose FHH may result in unwarranted treatment or surgery for a false diagnosis of primary hyperparathyroidism, given the significant overlap of biochemical features. Patients carrying a heterozygous loss-of-function mutation in the *CaSR* gene are typically referred to as FHH-type 1 (FHH1). Although FHH1 causes lifelong hypercalcemia, it is usually benign and asymptomatic. FHH is the most common syndrome of *CaSR* gene mutation; it may sometimes be associated with a hypercalciuric tendency depending on the variant. Although hypercalcemia is a frequently encountered condition in our clinical practice, FHH is a clinic that we do not often think of. This paper presents a family diagnosed with FHH, having heterozygous *CaSR* mutations in three generations.

Keywords: Familial, hypercalcemia, hypocalciuric

INTRODUCTION

Familial hypocalciuric hypercalcemia (FHH) is a group of autosomal dominant genetic diseases. It is characterized by persistent hypercalcemia, hypophosphatemia, hypermagnesemia, normal or mildly elevated serum parathyroid hormone (PTH) levels, and low urinary calcium excretion.[1] FHH, also called familial benign hypercalcemia, was initially defined as a variant of primary hyperparathyroidism (PHPT).[2] FHH is a life-long condition; it is usually caused by one of many heterozygous inactivating mutations in the calcium-sensing receptor *(CaSR)* gene, which could up-regulate the set point of parathyroid cells. When the *CaSR* receptor is inactivated, PTH is not suppressed despite relatively high calcium, which makes FHH similar to PHPT. In PHPT, although the renal reabsorption of calcium is higher than normal due to the high PTH level, hypercalciuria still occurs[3].

Patients carrying a heterozygous loss-of-function mutation in the *CaSR* gene are typically referred to as FHH-type 1 (FHH1)[4]. FHH1 causes lifelong hypercalcemia, but it is usually benign and asymptomatic. FHH is the most common syndrome of *CaSR* gene mutation; it may sometimes be associated with a hypercalciuric tendency depending on the variant.

Although hypercalcemia is a frequently encountered condition in our clinical practice, FHH is a clinic we do not often consider. This paper presents a family diagnosed with FHH, having heterozygous *CaSR* mutations in three generations.

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Patient Information and Clinical Findings

A 10-year-old, 6-month-old male patient was referred to our outpatient clinic after detecting high calcium in his routine blood tests. There was no previously detected high calcium level or loss of appetite, vomiting, constipation, polyuria, muscle weakness, anxiety, depression, or neurocognitive disorders (confusion, stupor, coma). On physical examination, the patient's height was 142.3 cm (50-75p), weight 41.9 kg (75-90p), and there was no consanguinity between the parents in the family history. His general condition was good, and system examinations were normal. Systolic and diastolic blood pressures were within normal limits for age. Laboratory results were as follows: Ca: 11.31 mg/dL, ALP: 306 U/L, Mg: 2.15 mg/dL, P: 3.65 mg/dL, PTH: 73.6 ng/L, 25(OH)D: 11.4 ng /mL, fT4: 1.13 pg/mL, thyroid stimulating hormone: 3.27 mU/L and urinary calcium/creatine: <0.01 mg/dL. Kidney ultrasonography was normal. 24-hour urine analysis and serum measurements of the patient were performed, and the calcium/creatinine clearance ratio was calculated below 0.01. During the patient's follow-up, persistent hypercalcemia, normal PTH levels, and low urinary calcium were detected, and the family was screened (Figure 1). Incidental hypercalcemia was found in her 4-month-old sister, mother, and grandmother; therefore, genetic screening was performed for FHH (Table 1).

Genetic Evaluation

After the informed consent was obtained, we extracted whole blood samples of the proband and his family members and sent them to the Genetic laboratory for DNA testing. NextSeq500 system was used for whole exon sequencing after obtaining the target gene. Sanger sequencing was used to investigate the heterozygous mutation of *CaSR*. The *CaSR* gene mutation study of the proband revealed a mutation; *CaSR* gene mutation of c.2532_2539delCAGCTTT (p.Ser845fs*133) was identified as heterozygous.

The clinical diagnosis was considered to be FHH1. The pedigree of the family for *CaSR* is shown in Figure 1.

Interventions and Follow-up

Until today, we encountered nephrolithiasis only in the sister of the patients who did not receive medical treatment other than hydration. There was no additional complaint or need for treatment. Other family members did not show adverse events such as nephrolithiasis, nephrocalcinosis, and renal dysfunction. Our patients are followed up with annual biochemistry tests (serum calcium, phosphate, creatinine), 24-hour urinary calcium measurement, and biennial renal ultrasonography. Our male patient was transferred to adult endocrinology when he turned 21, and his follow-up continues uneventfully. Genetic counseling was given to all affected individuals.

DISCUSSION

CaSR is mainly expressed in the parathyroid gland, kidney, and bone; acts as the primary regulator of calcium homeostasis.[5] The best-characterized role of *CaSR* is in the parathyroid gland, where it senses various factors that suppress the release and production of circulating ionized calcium (Ca2+), particularly PTH. Endogenous agonists of *CaSR* include Ca2+, Mg2+, other divalent ions that activate intracellular pathways, and polyamines such as spermine; Gq/11-mediated signals leading to the formation of inositol monophosphate (IP1) are the main pathway.[6,7] Loss-of-function mutations in *CaSR* reduce the capacity of the parathyroid gland and kidney to detect changes in serum Ca2+ concentrations.[4,8] Various mutations have been identified in *CaSR*, and functional effects depend on the affected site; these lead to varying degrees of *CaSR* inactivation.[9] Regarding FHH, more than 200 mutations identified in *CaSR* were detected; however, no strong correlation was found between genotype and phenotype in case-specific *CaSR* mutations.[10] The phenotype of

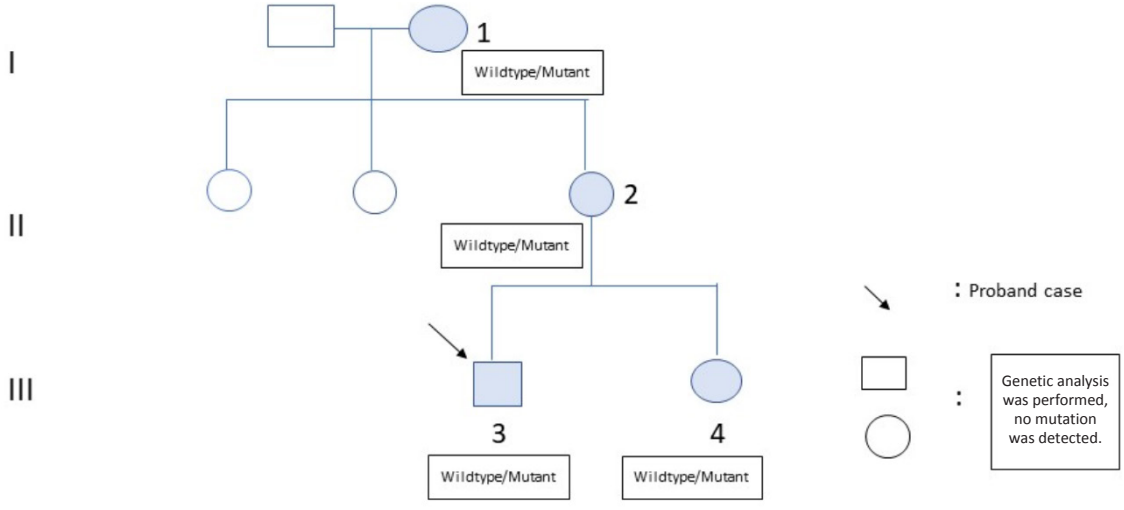

Figure 1. Pedigree. Blue filled symbols are family members with FHH, having *CaSR* mutation

Table 1. Genetic screening for FHH

	Pedigree no	Gender	Age	Ca (mg/dL)	P (mg/dL)	PTH (pg/mL)	Ca/Crea in spot urine (mg/dL)	Treatment	Clinic	*CaSR* gene analysis
Grandmother	I-1	Female	72	11.2	3.45	12.1	<0.01	Hydration	Asymtomatic	[c.2532_2539delCAGCTTT (P.sER845fs*133)]
Mother	II-2	Female	42	11.1	3.55	10.4	<0.01	Hydration	Asymtomatic	[c.2532_2539delCAGCTTT (P.sER845fs*133)]
Sister	III-3	Female	6	10.6	3.70	10.6	<0.01	Hydration	Asymtomatic	[c.2532_2539delCAGCTTT (P.sER845fs*133)]
Brother	III-4	Male	21	11.31	3.65	12.3	<0.01	Hydration	Asymtomatic	[c.2532_2539delCAGCTTT (P.sER845fs*133)]

FHH: Familial hypocalciuric hypercalcemia

most patients with *CaSR* mutation is normal, and most of them are asymptomatic. Many *CASR* mutations have been identified before, but the heterozygous mutation c.2532_2539delCAGCTTT (p.Ser845fs*133) has not been reported in the literature. FHH is usually inherited in an autosomal dominant manner, but recessive cases have also been reported.[9] The genetic inheritance in our patients was autosomal dominant, as in most reported cases. We described 3 generations of a family associated with a heterozygous *CaSR* mutation [c.2532_2539delCAGCTTT (p.Ser845fs*133)]. The co-existence of hypocalciuria, normal or high PTH, and hypercalcemia (in the absence of vitamin D deficiency) in carriers of c.2532_2539delCAGCTTT (p.Ser845fs*133) in the *CaSR* gene is a strong indicator of FHH1.[11-17] All heterozygous family members had elevated serum ionized calcium levels and normal PTH levels, which is fully consistent with hypocalciuria and the FHH1 phenotype. In 98% of the cases, the Ca/Creatinine ratio is below <0.001. In our cases, the Ca/Creatinine ratio was <0.001, which is consistent with the literature. In addition, the detected genotype of our case was clinically compatible with FHH and contributed to the literature in this respect. It is thought that 20% of the mutations implicated in FHH genetics have not been identified yet.[18] Lietman et al.[19] and Szczawinska et al.[20] reported two different families carrying the *CaSR* Q459R mutation. All heterozygous carriers of the mutation had normal biochemical values, including serum calcium levels that were within or rarely above the reference range. Likewise, there was no accompanying biochemical disorder in our cases. In the study of Kobayashi et al.[21], it was suggested that the hypercalcemia observed in heterozygous carriers was masked by the low calcium intake generally found in the Japanese population. No studies have been conducted on low calcium intake in our country, but we know that our patients follow a diet rich in calcium. In plasma, calcium may bond to proteins (less than 50%) or may form complex bonds with anions such as phosphate, citrate, or sulfate (10-15%) or may be present as ionized calcium (about 50%). Among those, ionized calcium is critical because it is the biologically active part.[22] Ong et al.[23] showed that 45% of cases with hypercalcemia would be missed by measuring only total calcium in patients with high total calcium and/or ionized calcium. Patients with primary HPT and FHH usually have a similar phenotype, but FHH rarely requires treatment, while primary HPT often requires parathyroid gland surgery. A recent study suggested that the Pro-FHH equation is better than 24-hour CaCl/CrCl to differentiate Primary HPT and FHH patients.[24] Hypercalcemia has many causes; differential diagnosis covers hypocalciuric hypercalcemic syndrome types 2 and 3, HPT (especially familial HPT), vitamin D metabolism disorders, and low glomerular filtration rate. Hypocalciuric hypercalcemia syndrome type 2 is associated with mutations of the *GNA11* gene located on chromosome 19p13.3, encoding one of the G-protein subunits (G-α11). This form constitutes 10% of familial benign hypercalcemia cases.[25] Type 3, on the other hand, consists of mutations in the *AP2S1* gene located on chromosome 19q13.3, usually at the arginine level at position 15. AP2S1 mutations are responsible for approximately 20% of FHH cases.

This form is associated with a more severe variant of FHH. FHH1 has a heterozygous inactivating mutation on the 3rd chromosome, on the *CaSR* gene. This syndrome is also called Marx-Auerbach syndrome or Familial benign hypercalcemia. The prognosis of this type is quite good, and it has been reported that there is no significant decrease in life expectancy. It may be because most of the cases, who are asymptomatic, cannot be detected clinically.[26] Hypercalcemia incidentally detected in our patients also supports the literature. The absence of hypermagnesemia in our cases ruled out FHH2 in diagnosis. FHH3 has mutations in the adapter-related protein complex-2 and sigma-1 subunits encoded by the *AP2S1* gene.[10,18,26-29] Some publications indicate that learning difficulties and neuropsychiatric findings may also occur in types 2 and 3.[30] In our cases, the absence of these features does not support the possible diagnosis of type 2 and 3 FHH. Regarding the cases with FHH reported in the literature, recurrent pancreatitis attack that may develop due to hypercalcemia is another issue to consider. For this reason, abdominal pain, laboratory evaluation, and amylase follow-up are important in symptomatic cases.[26] It can lead to symptomatic hypercalcemia with hypophosphatemia, a PTH increase with age, low bone mineral density, and cognitive dysfunction.[31,32] Approximately 20% of hypocalciuric hypercalcemia cases are unrelated to the defined genes, suggesting that other yet unknown genes play a role.[33]

Except for gene mutations that play a role in type 2 and 3 FHH, FHH syndromes associated with *CaSR* gene mutation should be differentiated from HPT with normal PTH.[34]

In hypocalciuric hypercalcemia syndromes, the familial character of hypercalcemia and the calcium/creatinine clearance ratio <0.01 support familial benign hypercalcemia rather than HPT despite the gray area.

There are other autosomal dominant genetic forms of familial hypercalcemia; they are associated with tumors, especially HPT associated with tumor suppressor gene mutations.[35]

Along with confirmatory genetic testing, appropriate screening of family members diagnosed with FHH1 and providing genetic counseling are crucial to avoid unnecessary treatment. Symptomatic individuals should be treated to prevent exacerbation of hypercalciuria and accompanying complications (nephrocalcinosis, nephrolithiasis, and impaired renal function). In symptomatic patients, treatment aims to relieve symptoms with the lowest possible dose of calcium and active vitamin D analog rather than to achieve normocalcemia. Thiazide diuretics can be combined with calcitriol to reduce renal calcium excretion. We did not need to use thiazide diuretics in our patients. Treatment of hypercalcemia should target the etiology and serum calcium level. Treatment was not started because our patient was asymptomatic. It has been reported that cinacalcet therapy may be considered if the patient is symptomatic, or serum Ca is >11.2 mg/dL, or 1 mg/dL higher than the Ca upper limit. In deciding to continue the drug, the disappearance of symptoms and returning serum calcium levels to normal values in an average of 8-12 weeks were considered an adequate response to the treatment.[23] However, it should be noted that the safety and efficacy of cinacalcet use are not known in cases under 18, as there is no FDA approval.

CONCLUSION

In this study, we examined a family with FHH and showed that the possible pathogenic cause behind FHH is a mutation in the *CaSR* gene (c.2532_2539delCAGCTTT (p.Ser845fs*133). The history of hypercalcemia in the family should prompt physicians to consider rare familial causes in patients with hypoparathyroidism. These diagnoses have critical implications regarding the management, screening, and genetic counseling of affected individuals.

Ethics

Informed Consent: The informed consent was obtained.

Peer-reviewed: Externally peer-reviewed.

Authorship Contributions

Concept design: A.D.B., Y.Y., A.C.C., Data Collection or Processing: A.D.B., Y.Y., A.C.C., Analysis or Interpretation: A.D.B., Y.Y., A.C.C., Literature Search: A.D.B., Y.Y., A.C.C., Writing: A.D.B., Y.Y., A.C.C.

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