

## Case Report

# A Case Report of Familial Hypocalciuric Hypercalcaemia with a Heterozygous Mutation of the Calcium Sensing Receptor Gene in a Chinese Paediatric Patient

JWK BAO, KL NG, TKC TSUI

**Abstract** The differentiation of familial hypocalciuric hypercalcaemia from the more common primary hyperparathyroidism harbours clinical significance, as unnecessary investigations and treatment could be avoided. We report a 22-month-old asymptomatic Chinese patient with an incidental finding of hypercalcaemia with biochemical features suggestive of familial hypocalciuric hypercalcaemia. The diagnosis was confirmed by mutation analysis of the *CASR* gene. Genetic analysis of the family showed the inheritance of familial hypocalciuric hypercalcaemia in an autosomal dominant manner.

**Key words** Calcium sensing receptor; *CASR*; Familial hypocalciuric hypercalcaemia

### Introduction

There are three types of familial hypocalciuric hypercalcaemia (FHH). FHH Type 1 is the commonest caused by loss-of-function mutation of the *CASR* gene while type 2 and 3 are caused by the *GNA11* and *AP2S1* mutations respectively. The biochemical profile of FHH includes hypercalcaemia, elevated or inappropriately normal parathyroid hormone level and hypocalciuria. It poses clinical significance to differentiate FHH from primary hyperparathyroidism as this could avoid unnecessary investigations and procedures.

Department of Paediatrics and Adolescent Medicine, United Christian Hospital, 130 Hip Wo Street, Kwun Tong, Kowloon, Hong Kong, China

JWK BAO (鮑詠芹) MBChB, MRCPCH  
KL NG (吳國樑) FRCP, FRCPC

Department of Chemical Pathology, Prince of Wales Hospital, 30-32 Ngan Shing Street, Shatin, N.T., Hong Kong, China

TKC TSUI (徐滄芝) MRCP(UK), FRCPA, FHKCPath

Correspondence to: Dr. JWK BAO  
Email: bwk184@ha.org.hk

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### Case Report

#### Case Presentation

The proband is a 22-month-old girl who first presented in August 2015 to the outpatient clinic with a complaint of poor growth. Her birth history and perinatal history was unremarkable. She enjoyed good past health. Upon reviewing the family history, patient's mother reported incidental finding of hypercalcaemia during antenatal checkup. Otherwise, both parents and elder sister enjoyed good past health. Developmental milestones were met. Growth charts were reviewed showing a decreasing centile of body weight since introduction of solid food, and was at the 3rd centile on the day of the first consultation. Examination was unremarkable except presence of a soft ejection systolic murmur compatible with a flow murmur. An impression of inadequate caloric intake was made at the end of the consultation.

#### Investigations

There was incidental finding of hypercalcaemia upon the workup for failure to thrive. Serum total calcium level was 3.03 mmol/L (Reference range: 2.25-2.75 mmol/L). Rechecked level was 3.13 mmol/L. Blood ionized calcium was 1.67 mmol/L (Reference range: 1.13-1.32 mmol/L). Serum phosphate level was 1.57 mmol/L (Reference range: 0.97-2.10 mmol/L). Serum magnesium level was

1.02 mmol/L (Reference range: 0.70-0.99 mmol/L). Plasma intact parathyroid hormone level was 3.0 pmol/L (Reference range: 1.6-6.9 pmol/L) which was inappropriately normal. Serum total 25-hydroxyvitamin D level was 52 nmol/L, which was sufficient. 24 Hour urine was collected with a urine volume of 846 ml, urine calcium level of <0.15 mmol/L and urine creatinine level of 1.2 mmol/L. The concurrent serum total calcium and serum creatinine levels were 3.18 mmol/L and 33  $\mu$ mol/L respectively. The calcium to creatinine clearance ratio was <0.001, which confirmed hypocalciuria using a cut-off of <0.01. Ultrasound kidney, X-ray for long bone and echocardiogram were normal.

### Genetic Analysis

In view of hypercalcaemia with low urinary calcium excretion, the diagnosis of FHH was suspected. Mutational analysis of the *CASR* gene (OMIM#601199) was performed on the peripheral blood leukocytes of the patient with informed consent from parents. Genomic DNA was extracted from leukocytes using a standard procedure. Polymerase chain reaction (PCR) amplification of all the coding exons (exon 2 to exon 7) and flanking introns of the *CASR* gene was performed. The PCR products were purified and sequenced in both forward and reverse directions using the BigDye cycle sequencing kit and analyzed with ABI-3100 automated sequencer. The patient was heterozygous for a missense mutation c.652T>C (p.Tyr218His) in exon 4 of *CASR* gene (GenBank Reference NM\_000388.3) (Figure 1). The missense mutation changes the codon from TAT to CAT, leading to the change of amino acid residue from tyrosine to histidine at codon 218, which has been reported as a pathogenic mutation in patient with FHH.<sup>1</sup> The mutant residue is located in and disrupts one of the five calcium-

binding sites within the extracellular domain of the calcium ( $\text{Ca}^{2+}$ ) sensing receptor (CaSR) and affects the ability of the CaSR to bind  $\text{Ca}^{2+}$  in a cooperative fashion.<sup>1,2</sup>

### Family Study

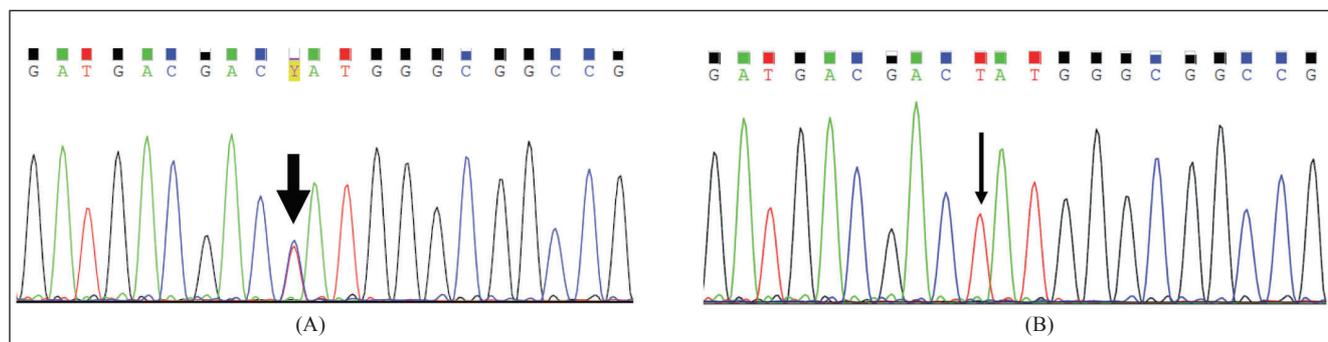
The patient's family, and subsequently the extended family on the maternal side were referred for cascade screening. Both the proband's mother and maternal grandfather showed the same heterozygous *CASR* mutation. The inheritance of FHH in an autosomal dominant manner is shown in the pedigree in Figure 2.

### Treatment and Follow Up

The patient remained asymptomatic upon subsequent follow up. She was referred to our dietitian. Her body weight caught up to 25th centile. No pharmacological treatment for hypercalcaemia was required.

### Discussion

The *CASR* is a G protein-coupled receptor highly expressed on the renal tubules and parathyroid gland. It has three domains, namely the intracellular, transmembrane and extracellular domain.<sup>1</sup> The mutation of such causes an abnormal set point of calcium dependent PTH secretion. Higher calcium level is required to suppress the PTH secretion. Renal tubular reabsorption of calcium is also affected. Patient with activating mutation of *CASR* would result in autosomal dominant hypocalcaemia, causing severe hypocalcaemia.<sup>3</sup> Inactivating mutation would cause either severe neonatal hyperparathyroidism (NSHPT) or FHH, depending on whether it is a heterozygous (FHH) or homozygous/compound heterozygous (NSHPT) mutation.<sup>1</sup>



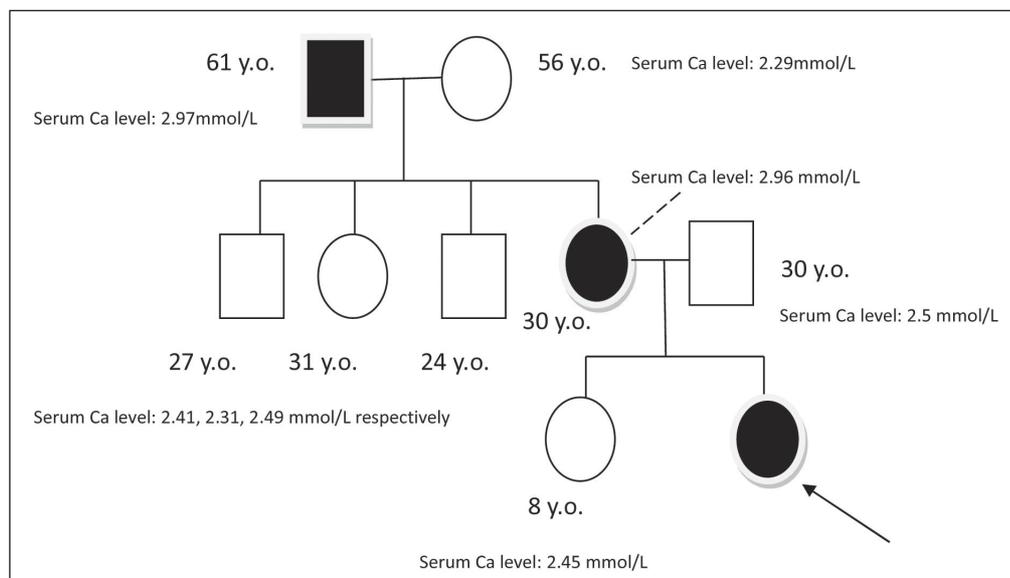
**Figure 1** (A) Electropherogram of the heterozygous missense mutation c.652 T>C (p.Tyr218His) in exon 4 of the *CASR* gene detected in the proband. The nucleotide c.652T>C is marked by an arrow. (B) The corresponding wild-type sequence detected in a normal control.

The degree of hypercalcaemia in FHH and NSHPT reflects a gene dosage effect. However, the phenotypes of *CASR* mutation may not always correspond to the genotypes.<sup>4</sup> Some *CASR* mutations may have a dominant negative effect causing a higher degree of hypercalcaemia that is usually seen in heterozygotes. However, some mutations may have mild effect on calcium homeostasis. Thus some patients with heterozygous mutation may be normocalcemic.

FHH is a benign condition. Suspicion should arise if a young asymptomatic patient was found to have hypercalcaemia. FHH does not require any treatment. In particular, standard subtotal parathyroidectomy is unindicated, as it would not result in lowering of serum calcium level, opposed to that in primary hyperparathyroidism. Hence, the diagnosis of FHH has clinical importance as its differentiation from primary hyperparathyroidism could avoid unnecessary investigations and treatment. The main differences between FHH and primary hyperparathyroidism would be the presence of symptomatic hypercalcaemia, decreased bone density and previous normocalcaemia in patients with primary hyperparathyroidism. The major differentiation tool in clinical practice is the calcium to creatinine clearance ratio. A ratio of less than 0.01 is suggestive of FHH, while a ratio of higher than 0.02 suggests primary hyperparathyroidism.<sup>5</sup> However, the definitive diagnosis of FHH depends on mutation analysis. There are three genetic types of FHH. FHH type 1 accounts for 65% of cases caused

by inactivating mutations in the *CASR* gene. The other 35% have either a mutation in the *GNA11* gene in FHH type 2 or *AP2S1* gene in FHH type 3.<sup>6</sup> Analysis of the *CASR* gene can be considered first in patients suspected of FHH. A negative *CASR* analysis by sequencing cannot exclude the diagnosis FHH. It has been postulated that *AP2S1* missense mutations affecting Arg15 residue represented a mutational hotspot in FHH Type 3 and might account for >20% individuals with definite clinical and biochemical diagnosis of FHH without *CASR* mutations.<sup>6</sup> It remains unclear whether clinical relevant phenotypic differences are present in patients with different types of FHH. However, a recent study suggested that *AP2S1* mutations affect calcium homeostasis more severely than *CASR* mutations evidenced by higher plasma calcium concentrations in patients with FHH type 3 than patients with FHH type 1, despite having similar PTH concentrations and urinary calcium excretion.<sup>7</sup> Although genetic analysis is definitive and superior, biochemical screening tests (e.g. serum total calcium and 24 hour urine calcium to creatinine clearance ratio) are still useful as first line investigations. It is recommended to include calcium to creatinine clearance ratio as an essential part upon the workup for hypercalcaemia to differentiate between FHH and primary hyperparathyroidism.<sup>8</sup> Once the disease causing mutation of FHH is identified for the proband, subsequent family screening by target mutation testing could be performed.

Although FHH is believed to run a benign course, there



**Figure 2** Pedigree of the studied family. Squares represent male and circles represent female. Hatched symbols represent affected individuals with *CASR* gene mutation. Arrow indicates the proband.

are rare reported cases of FHH associated with pancreatitis, nephrolithiasis, gallstones, articular chondrocalcinosis and premature vascular calcification.<sup>9,10</sup> Some of the reported complications were observed during middle age. Before more evidence is available, it is recommended to follow up FHH paediatric patients till adulthood.

### Declaration of Interest

There is no conflict of interest to declare.

### References

- Hannan F, Nesbit M, Zhang C, et al. Identification of 70 calcium-sensing receptor mutations in hyper- and hypo-calcaemic patients: evidence for clustering of extracellular domain mutations at calcium-binding sites. *Hum Mol Genet* 2012;21: 2768-78.
- Magno A, Ward B, Ratajczak T. The Calcium-Sensing Receptor: A Molecular Perspective. *Endocr Rev* 2011;32:3-30.
- Wong WC, Lam CW, Tong SF, Tong CT. Persistent hypocalcaemia in a Chinese girl due to de-novo activating mutation of the calcium-sensing receptor gene. *Hong Kong Med J* 2011; 17:157-60.
- Taki K, Kogai T, Sakumoto J, Namatame T, Hishinuma A. Familial hypocalciuric hypercalcemia with a de novo heterozygous mutation of calcium-sensing receptor. *Endocrinol Diabetes Metab Case Rep* 2015;2015:150016.
- Christensen S, Nissen P, Vestergaard P, Heickendorff L, Brixen K, Mosekilde L. Discriminative power of three indices of renal calcium excretion for the distinction between familial hypocalciuric hypercalcaemia and primary hyperparathyroidism: a follow-up study on methods. *Clin Endocrinol (Oxf)* 2008;69: 713-20.
- Nesbit MA, Hannan FM, Howles SA, et al. Mutations in AP2S1 cause familial hypocalciuric hypercalcemia type 3. *Nat Genet.* 2013;45:93-7.
- Vargas-Poussou R, Mansour-Hendili L, Baron S, et al. Familial Hypocalciuric Hypercalcemia Types 1 and 3 and Primary Hyperparathyroidism: Similarities and Differences. *J Clin Endocrinol Metab* 2016;101:2185-95.
- Shinall M, Dahir K, Broome J. Differentiating familial hypocalciuric hypercalcemia from primary hyperparathyroidism. *Endocr Pract* 2013;19:697-702.
- Marx S, Attie M, Levine M, et al. The hypocalciuric or benign variant of familial hypercalcemia: clinical and biochemical features in fifteen kindreds. *Medicine (Baltimore)* 1981;60:397-412.
- Gunn I, Gaffney D. Clinical and laboratory features of calcium-sensing receptor disorders: a systematic review. *Ann Clin Biochem* 2004;41:441-58.