Persistent hypocalcaemia in a Chinese girl due to a novel de-novo activating mutation of the calcium-sensing receptor gene

WC Wong 黃偉進
CW Lam 林青雲
SF Tong 湯瑞芬
CT Tong 唐志德

A significant proportion of patients formerly diagnosed with idiopathic hypoparathyroidism actually have activating mutation of the calcium-sensing receptor (CaSR) gene. Awareness of the possibility of activating mutation of CaSR gene in patients with sporadic idiopathic hypoparathyroidism is important because of its relevance to clinical management. This report is of a novel activating mutation of the CaSR gene identified in a 10-year-old Chinese girl who was initially diagnosed as having idiopathic hypoparathyroidism at 6 years of age after presenting with seizures. Her serum calcium level was difficult to maintain near the lower limit of normal despite treatment with high-dose calcitriol. Treatment with calcitriol produced significantly elevated urinary calcium-to-creatinine ratio. Direct sequencing of the CaSR gene showed a novel heterozygous mutation (p.Q735P (c.2204A>C)). Molecular genetic analysis of her parents demonstrated that both parents did not harbour the child’s mutation, indicating that her mutation had arisen de novo.

Introduction

The calcium-sensing receptor (CaSR) gene plays an important role in calcium metabolism. Activating mutations of the CaSR gene are responsible for autosomal dominant hypocalcaemia, which is a rare disease characterised by hypocalcaemia, hypoparathyroidism, and hypercalciuria. A positive family history is clear for most patients, but de-novo mutations should be suspected for those patients without autosomal dominant inheritance.

Case report

A 6-year-old girl first presented in November 2005 with a generalised tonic-clonic seizure (for a few minutes) associated with upper respiratory tract infection. Her birth history was uneventful, and she lived in China until the age of 6 years. Her medical history revealed that she had several episodes of febrile convulsions with admissions to hospitals in China since the age of 1 year. The seizures were brief and no further investigations were performed. Apart from the episodes of febrile convulsions, she had no relevant history. At physical examination, she had normal growth parameters and there were no obvious congenital anomalies. Neurological examination was normal.

Laboratory evaluation showed hypocalcaemia level of 1.56 mmol/L (reference range, 2.10-2.65 mmol/L) and hyperphosphataemia level of 3.12 mmol/L (0.82-1.40 mmol/L). Her serum sodium and potassium levels were normal. Her serum parathyroid hormone (PTH) level was suppressed to 1.2 pmol/L (reference range, 1.6-6.9 pmol/L), magnesium level was mildly decreased to 0.62 mmol/L (0.66-0.95 mmol/L), and 25-hydroxyvitamin D level was normal. Urinary calcium-to-creatinine ratio (UCa/Cr) was 0.41 mmol/mmol. Computed tomographic scan of the brain revealed calcification at the basal ganglia (Fig). Ultrasound scan of the kidney showed no nephrocalcinosis. Electrocardiogram and echocardiogram were normal. 22q11 microdeletion was excluded by fluorescence in-situ hybridisation analysis. There was no history of hypocalcaemia in either side of the family, and both parents had normal serum calcium levels.

She was initially diagnosed as having idiopathic hypoparathyroidism and mild hypomagnesaemia. Calcitriol, calcium, and magnesium supplements were started at the age of 6 years. The medications were titrated according to the serum calcium level and were aimed at keeping the serum calcium at the lower limit of normal. In the following few years, the serum magnesium level was normalised with low-dose magnesium supplements. However, the serum calcium was difficult to maintain near the target level despite treatment with high-dose calcitriol (1 µg/day) and calcium supplements (elemental...
that her mutation had arisen de novo.

Based on the diagnosis of activating mutation of the CaSR gene and the high risk of ongoing nephrocalcinosis, calcitriol was tapered off. Treatment with the thiazide diuretic hydrochlorothiazide was initiated at the age of 10 years. Her UCa/Cr decreased to 0.3-0.5 mmol/mmol. Although the patient is still hypocalcaemic, with serum calcium level ranging from 1.62 to 1.75 mmol/L, she is currently asymptomatic, which may reflect a chronic clinical adaptation to the low serum calcium level.

Discussion

Historically, hypoparathyroidism of unknown aetiology has been called idiopathic hypoparathyroidism. However, a significant proportion of patients, perhaps as many as one-third, previously diagnosed with idiopathic hypoparathyroidism may have activating mutations in the CaSR gene.2

CaSR is a member of the heptahelical G protein–coupled receptors that is highly expressed in the parathyroid glands and kidneys. Cloning of the CaSR gene is located on chromosome 3p13 and it plays an important role in calcium homeostasis. Activating mutations lower the set point for maintenance of serum calcium concentration by inhibition of PTH secretion, as well as enhancing renal calcium and magnesium excretion, leading to hypoparathyroidism, hypocalcaemia, and hypomagnesaemia. Pollak et al3 first reported the activating mutation E127A in patients with familial hypoparathyroidism in 1994. To date, more than 80 activating mutations in the CaSR gene have been reported. Although patients with activating mutations of the CaSR gene usually have a familial occurrence of the disease, sporadic forms have been reported.4

In patients with activating mutation of CaSR, the age of onset of symptoms ranges from infancy to adulthood. Although there is considerable heterogeneity in the severity of the hypocalcaemia caused by activating mutation of CaSR, most patients with activating mutation of CaSR are asymptomatic; some patients however, especially children during febrile episodes, can exhibit neuromuscular irritability and seizures.2,5

Distinguishing activating mutation of CaSR from idiopathic hypoparathyroidism can be challenging in the clinical setting. One important feature of this disorder is the presence of an inappropriately high rate of urinary calcium excretion, regardless of the presence of hypocalcaemia. Yamamoto et al6 have shown that the pretreatment mean UCa/Cr was significantly higher in patients with activating mutation of CaSR when compared with sporadic idiopathic hypoparathyroidism. Inhibition of PTH secretion in activating mutation of CaSR is the first

FIG. Computed tomographic scan of the brain showing calcification at the bilateral basal ganglia in a 6-year-old girl
mechanism. The second mechanism is that the activating mutation of CaSR, expressed at the thick ascending limb of the loop of Henle in the kidney, inhibits calcium reabsorption in addition to the diminished PTH secretion from the parathyroid gland. Hypomagnesaemia is another characteristic finding in activating mutation of CaSR. In a meta-analysis of 38 patients with activating mutation of CaSR, 71% had serum magnesium levels of lower than 0.7 mmol/L. Gunn and Gaffney suggested that a serum magnesium level of lower than 0.7 mmol/L, a PTH level between 1.0 and 3.5 pmol/L, and a pretreatment UCa/Cr of higher than 0.30 mmol/mmol are all suggestive of a CaSR gene mutation.

However, the only way to accurately make the diagnosis is by genetic analysis, especially in the absence of a positive family history. The differentiation of activating mutation of the CaSR gene and sporadic idiopathic hypoparathyroidism is important because patients with the former are at greater risk of nephrolithiasis and nephrocalcinosis, especially after therapy with 1,25-dihydroxyvitamin D3. Rates of nephrocalcinosis in patients with activating mutation of CaSR have been reported to be between 38% and 57%.2,8

Given that patients with hypoparathyroidism are normally treated with calcium and calcitriol, it is important to note that it is not easy to normalise the serum calcium level in activating mutation of CaSR even with high-dose 1,25-dihydroxyvitamin D3. On the other hand, severe renal calcium wasting can worsen with calcitriol treatment. Furthermore, one study even showed that the expression of the CaSR gene may be upregulated by 1,25-dihydroxyvitamin D in the parathyroid glands and renal tubules.9 Hence, treatment with calcitriol and calcium may stimulate excessive calcium excretion because of increased expression of activated CaSR in the nephrons, potentially further increasing the risk of nephrocalcinosis. Thus, care should be exercised to avoid overtreatment of these patients with 1,25-dihydroxyvitamin D3 or calcium supplements. Asymptomatic patients with activating mutation of CaSR should not be routinely treated with calcitriol, which should be reserved for symptomatic patients. The goal should be to increase the serum calcium concentration only to a level sufficient to render the patient asymptomatic and not necessarily to a normal level.3

Thiazide diuretics, which can lower urinary calcium excretion at any given level of serum calcium, should be considered for the treatment of patients with activating mutation of CaSR. The hypocalciuric action of thiazide diuretics has been successfully proven for the treatment of idiopathic hypercalciuria in children.30 Sato et al11 showed that thiazide diuretics have been successfully used to reduce urinary calcium excretion and enable reduction of the dose of 1,25-dihydroxyvitamin D3 in children with activating mutation of CaSR.

Parathyroid hormone replacement is a logical treatment for this disorder because PTH plays a central role in calcium regulation. Mittelman et al12 demonstrated that treatment with recombinant human PTH can maintain a serum calcium level close to the lower end of normal while lowering urinary calcium excretion in a child with activating mutation of CaSR. However, there is still little experience of the use of recombinant human PTH in children. Further studies evaluating the long-term safety and efficacy of PTH in children with activating mutation of CaSR are warranted.

**Conclusion**

This report illustrated that de-novo activating mutation of the CaSR gene should be suspected in children with sporadic idiopathic hypoparathyroidism. Genetic analysis should be considered for patients who have hypocalcaemia, hypoparathyroidism, and hypercalciuria. Due to the high risk of nephrocalcinosis, asymptomatic patients with CaSR mutation should not be routinely treated with calcitriol. Thiazide diuretics will reduce urinary calcium excretion and may allow serum calcium to be maintained at reasonable levels without incurring undue hypercalciuria.

**References**

6. Yamamoto M, Akatsu T, Nagase T, Ogata E. Comparison of hypocalcemic hypercalciuria between patients with idiopathic hypoparathyroidism and those with gain-of-
function mutations in the calcium-sensing receptor: is it possible to differentiate the two disorders? J Clin Endocrinol Metab 2000;85:4583-91.


