CASE REPORT

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

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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

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一名華籍女孩因新型原發性鈣離子感應接受器基因變異而出現持續性低鈣血症

以往被診斷為特發性副甲狀腺機能減退症的病人中，部分其實是由於鈣離子感應接受器（*CaSR*）的基因產生活化性突變而引起。由於兩者的治療方向及目標有所不同，及早發現*CaSR*的基因活化性變異便顯得十分重要。本文報告一名10歲華籍女孩在4年前被診斷特發性副甲狀腺機能減退症，儘管她已被處方高劑量活性維他命D3及鈣補充劑，其血清鈣濃度仍一直無法維持接近正常下限的水平。另一方面，尿鈣過高的水平卻逐漸提高。基因序列分析發現*CaSR*基因第735號密碼子的第2204個核苷酸出現新型錯義突變。病童的父母並沒有出現相應的基因變異，所以斷定這病童的基因變異是原發的。

calcium 40 mg/kg/day), and this treatment produced a considerably elevated UCa/Cr. When her serum calcium levels were between 1.5 and 1.7 mmol/L, the UCa/Cr was 1.2 to 3.2 mmol/mmol.

The persistently high urinary calcium excretion despite the coexistence of hypocalcaemia led to suspicion of an activating mutation of *CaSR*. Informed consent was obtained from her parents, and genetic analysis was performed with genomic DNA extracted from peripheral whole blood. DNA fragments from the *CaSR* gene were amplified by polymerase chain reaction and the products were sequenced by direct DNA sequencing.[1] The results showed that the patient was heterozygous for a novel mutation (p.Q735P (c.2204A>C)) in the *CaSR* gene. This was a missense mutation from A to C in the nucleotide 2204 of codon 735, leading to gain-of-function of the *CaSR*. Molecular genetic analysis demonstrated that neither of her parents harboured this mutation, indicating that her mutation had arisen de novo.

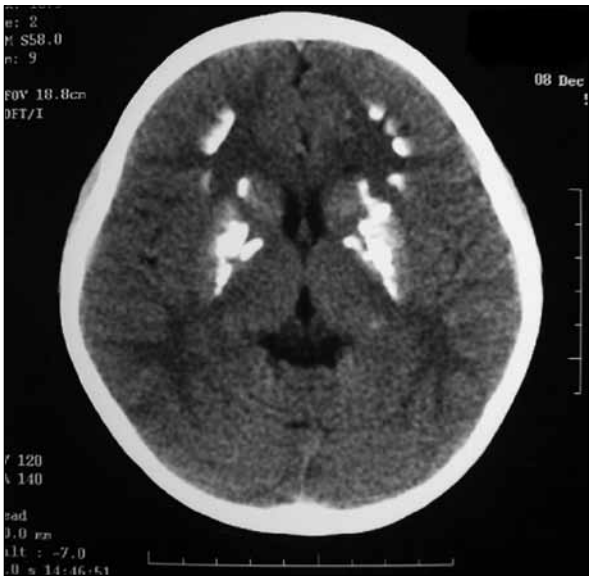

FIG. Computed tomographic scan of the brain showing calcification at the bilateral basal ganglia in a 6-year-old girl

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 al[3] 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 al[6] 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

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.[7] Gunn and Gaffney[7] 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.[5]

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.[10] Sato et al[11] 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 al[12] 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.

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