CASE REPORT

# A new mutation in the calcium-sensing receptor gene causing hypocalcaemia: case report of a father and two sons

**M.K. Schoutteten[1]*, B. Bravenboer[2], S. Seneca[3], K. Stouffs[3], B. Velkeniers[2]**

[1]Department of Internal Medicine, Maastricht University Medical Centre, the Netherlands, [2]Department of Endocrinology, University Hospital Brussels, Belgium, [3]Centre for Medical Genetics, University Hospital Brussels, Research Unit Genetics and Fertility, Vrije Universiteit Brussel, Brussel, Belgium, *corresponding author: tel.: +31 (0)43-3875007, fax: +31 (0)43-3875006, email: melanie.schoutteten@hotmail.com

## ABSTRACT

Background: Regulation of calcium is mediated by parathyroid hormone (PTH) and 1.25-dihydroxyvitamine D3. The calcium-sensing receptor (CaSR) regulates PTH release by a negative feedback system. Gain-of-function mutations in the *CaSR* gene reset the calcium-PTH axis, leading to hypocalcaemia.

Patients and methods: We analysed a family with hypocalcaemia. The proband was a 47-year-old man (index, patient $I_1$), who presented with paraesthesias in both limbs. He has two sons (patient $II_1$ and $II_2$). The probands' lab results showed: serum calcium of 1.95 mmol/l, albumin 41 g/l, phosphate 0.81 mmol/l and PTH 6.6 ng/l (normal 15-65 ng/l). Based on this analysis, we suspected a hereditary form of hypocalcaemia and performed genetic testing by polymerase chain reaction and Sanger sequencing of the coding regions and intron boundaries of the *CaSR* gene. Genetic analysis revealed a new heterozygous mutation: c.2195A>G, p.(Asn732Ser) in exon 7. The lab results of patient II1 showed: serum calcium of 1.93 mmol/l, phosphate 1.31 mmol/l, albumin 41 g/l, and PTH 24.3 ng/l. His genotype revealed the same activating mutation and, like his father, he also lost his scalp hair at an early adolescent age. Patient $II_2$ is asymptomatic, and has neither biochemical abnormalities, nor the familial *CaSR* gene mutation. He still has all his scalp hair.

Conclusions: 1) The c.2195A>G, p.(Asn732Ser) mutation in exon 7 of the *CaSR* gene leads to hypocalcaemia, and has not been reported before in the medical literature. 2) Possibly, this mutation is linked to premature baldness.

**What was known on this topic?**
Autosomal dominant hypocalcaemia is a syndrome causing hypocalcaemia by activating the calcium-sensing receptor (CaSR). Several genes have been identified.

**What does this add?**
This publication adds the knowledge of a newly discovered activating mutation in the *CaSR* gene of a father and one son. Moreover, we report the remarkable presentation of alopecia in both patients.

## KEYWORDS

Autosomal dominant hypocalcemia, calcium-sensing receptor, hypocalcemia

## INTRODUCTION

Chronic hypocalcaemia is a frequent problem and can be life-threatening. Most patients report paraesthesia, but hypocalcaemia can also cause muscle cramps, seizures and cardiac arrhythmias. Long-term complications of chronic hypocalcaemia include tissue calcifications in the brain and kidneys, cataract and osteoporosis. Parathyroid hormone (PTH) is responsible for minute-to-minute regulation of the plasma calcium.[1,2] Hence, causes of hypocalcaemia are classified according to PTH concentration *(table 1)*.

**Table 1.** *Functional classification of hypocalcaemia*

| |
|---|
| PTH absent |
| Hereditary hypoparathyroidism<br>Acquired hypoparathyroidism (surgical or radiation induced )<br>Hypomagnesaemia |
| PTH ineffective |
| Chronic renal failure<br>Vitamin D deficiency (inadequate diet or sunlight, defective vitamin D metabolism due to medication, malabsorption)<br>Pseudohypoparathyroidism |
| PTH overwhelmed |
| Severe, acute hyperphosphatemia (tumour lysis, acute renal failure, rhabdomyolysis)<br>Osteitis fibrosa after parathyroidectomy |

The parathyroid cells express calcium-sensing receptor (CaSR), a G-protein-coupled receptor providing a negative feedback system to the calcium-PTH axis.[3-5] CaSR is mainly located in parathyroid glands and the renal tubule. By sensing ionised calcium, the CaSR regulates PTH release and PTH-independent calciuresis. Gain-of-function mutations in the *CaSR* gene shift the calcium-PTH axis to a lower set point. The mutant CaSR becomes activated by a low ionised serum calcium concentration and subsequently inhibits PTH release. Autosomal dominant hypocalcaemia (ADH) is a syndrome characterised by an inappropriately low PTH according to a symptomatic hypocalcaemia and a relative hypercalciuria.[1,4,5] A small subgroup of ADH patients develop additional renal loss of sodium, chloride and magnesium, resulting in hyperreninaemia, hyperaldosteronism, hypokalaemia and metabolic alkalosis. Most of them present during childhood, which is diagnosed as Bartter's syndrome type 5.[4,6]

We present a family of a father and his sons diagnosed with ADH caused by a new mutation in the *CaSR* gene.

## CASE REPORT

A 47-year-old bald man (index, patient $I_1$) was seen with complaints of paraesthesia in both limbs. No Chvostek sign could be provoked. His past medical history included autoimmune hypothyroidism. Blood analysis showed a serum calcium of 1.95 mmol/l at presentation. His phosphate was 0.81 mmol/l and PTH was low 6.6 ng/l *(table 2)*. The calciuresis was high at 7.6 mmol/24 h. Patient $I_1$ was initially diagnosed with idiopathic hypoparathyroidism and treated with calcitriol. We performed genetic testing by polymerase chain reaction (PCR) and Sanger sequencing of the coding regions and intron boundaries of the *CaSR* gene. Genetic analysis by PCR amplification and DNA sequencing revealed a new mutation in the gene coding for CaSR: c.2195A>G, p.(Asn732Ser) in exon 7. Since patient $I_1$ is a heterozygous carrier, genetic counselling was recommended with his two sons.

One son (patient $II_1$) was analysed at the age of 25. He reported a tingling sensation in both hands. He smoked 25 cigarettes a day. Physical examination revealed no Chvostek sign, hypertension 140/70 mmHg, and a remarkable baldness. PCR of the *CaSR* gene exposed the same mutation as patient $I_1$ had. Lab results of patient $II_1$ showed hypocalcaemia 1.92 mmol/l with a normal concentration of albumin, phosphate and PTH. Calciuresis was also high-normal at 6.3 mmol/24 h *(table 2)*.

**Table 2.** *Biochemical analysis of blood and urine at presentation*

| | Reference | Patient $I_1$ | Patient $II_2$ | Patient $II_3$ |
|---|---|---|---|---|
| Calcium mmol/l | 2.1-2.55 | 1.95 | 1.93 | 2.19 |
| Albumin g/l | 32.0-47.0 | 41 | 41 | 40 |
| Phosphate mmol/l | 0.90-1.50 | 0.81 | 1.39 | 1.07 |
| Magnesium mmol/l | 0.70-1.00 | 0.53 | 0.82 | / |
| Intact PTH ng/l | 1.3-6.8 | 6.6 | 24.3 | 33.7 |
| 25-hydroxy vitamin D nmol/l | > 90 | / | 98 | 34.4 |
| 1.25-Dihydrox vitamin D pmol/l | 50-110 | 60 | / | / |
| TSH mIU/l | 0.4-6.2 | 2.360 | 1.160 | 1.130 |
| **24 h urine collection** | | **Patient $I_1$** | **Patient $II_2$** | **Patient $II_3$** |
| Volume ml | | 1950 | 2800 | / |
| Calcium mmol/l | | 3.92 | 2.25 | / |

PTH = parathyroid hormone; TSH = thyroid stimulating hormone.

The youngest son (patient $II_2$) is asymptomatic and still has all his scalp hair. No mutation in the calcium-sensing receptor could be found.

## DISCUSSION

Activating mutations of the *CaSR* gene can cause symptomatic hypocalcaemia. ADH has a wide clinical spectrum.[5,7] As in our cases, most patients report paraesthesia in the limbs, but chronic severe hypocalcaemia might be life-threatening.[1,4,5,7] The phenotype is determined by the calcium level. Signe et al. found that the severity of clinical neurological symptoms is inversely related to serum calcium levels.[7]

The *CaSR* gene is located on chromosome 3q13.5,8. The first activating mutation was described by Pollak et al. in 1994.[9] The CaSR mutation database (http://www.casrdb.mcgill.ca) contains more than 40 known activating mutations in *CaSR* gene.[5] Also several recent case reports have demonstrated new activating mutations.[8,10] Genetic analysis in patient $I_1$ and patient $II_1$ revealed another new activating mutation: c.2195A>G, p.(Asn732Ser) in exon 7. Because this mutation has not been described before, it is classified as 'variant of uncertain significance'. In silico analysis of the mutation shows that substitution of asparagine by serine is most probably pathogenic. Most ADH patients are heterozygous. One family is known with a homozygote mutation, but this is not associated with a more severe phenotype.[5]

Remarkably, our affected patients are completely bald. Alopecia is a known symptom occurring in polyglandular autoimmune syndrome type I (PGA I).[1,5] Although patient $I_1$ has an autoimmune hypothyroidism, no Addison's disease, mucocutaneous candidiasis, keratopathy, vitiligo, parietal cell atrophy, insulin-dependent diabetes mellitus or autoimmune hepatitis is known in this family. Therefore, it is unlikely that the alopecia in our patients fits into an autoimmune syndrome. Moreover, PGA I is inherited as an autosomal recessive trait and has a childhood onset.[1,5] Another hereditary syndrome is an autosomal recessive mutation in the *hairless* gene located on chromosome 8p12.[11]

However, neither a biopsy, nor genetic counselling towards the *hairless* gene was performed. Most probably, a mutant gene coding for alopecia is located close to the *CaSR* gene and was simultaneously inherited.

Treatment of hypocalcaemia is symptomatic. Hypocalcaemia is treated by calcium supplementation. Calcitriol or alphacalcidol is added to stimulate intestinal calcium absorption. Normalisation of hypocalcaemia can cause a rise in hypercalciuria resulting in kidney stone formation. Therefore, treatment with active vitamin D and calcium supplementation should be performed carefully to prevent symptoms and arrhythmias, but also to prevent possible long-term complications of this treatment. Each patient requires regular monitoring of serum calcium, calciuria and renal function. Adding hydrochlorothiazide can limit calciuria.[4]

Ideally, the activated CaSR should been blocked to provide a more pathophysiological therapy. Calcilytic drugs are being studied to block the CaSR and thus provide a more pathophysiological approach in treating these patients. This could be a very promising novel therapeutic approach for ADH.[12]

In conclusion, we present a father and his son who were diagnosed with ADH. Their activating mutation in the *CaSR* gene leads to symptomatic hypocalcaemia. In both family members a new mutation in the CaSR was found. Possibly, a polymorphism of a gene related to alopecia is located near the *CaSR* gene and is also dominantly inherited.

## DISCLOSURES

The authors declare no conflicts of interest.

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