Novel Mutation in the CASR Gene (p.Leu123Ser) in a Case of Autosomal Dominant Hypocalcemia

Joana Regala1 Branca Cavaco2,3 Rita Domingues2,3 Catarina Limbert1 Lurdes Lopes1

1 Pediatric Endocrinology Unit, Dona Estefânia Pediatric Hospital, Hospital Centre of Central Lisbon, Lisbon, Portugal
2 Molecular Pathobiology Research Centre, Portuguese Institute of Oncology Francisco Gentil, Lisbon, Portugal
3 Chronic Diseases Research Center, NOVA Medical School, NOVA University of Lisbon, Lisbon, Portugal

Address for correspondence Catarina Limbert, MD, Unidade de Endocrinologia Pediátrica, Departamento de Pediatria, Hospital Dona Estefânia, Centro Hospitalar Lisboa Central (CHLC), Rua Jacinta Marto, 1169-045 Lisboa, Portugal (e-mail: climbert@gmail.com).

Abstract
Autosomal dominant hypocalcemia, caused by activating mutations of the calcium-sensing receptor (CASR) gene, is characterized by hypocalcemia with an inappropriately low concentration of parathyroid hormone (PTH). In this report, we describe the identification of a novel missense mutation in the CASR gene, in a boy with autosomal dominant hypocalcemia. Polymerase chain reaction (PCR)–single strand and DNA sequencing revealed a heterozygous mutation in CASR gene that causes a leucine substitution for serine at codon 123 (p.Leu123Ser). This mutation was absent in DNA from 50 control patients. In silico studies suggest that the identified variant was likely pathogenic. Sequencing analysis in the mother suggested mosaicism for the same variant, and she was clinically and biochemically unaffected. Clinical manifestations of the index case started with seizures at 14 months of age; cognitive impairment and several neuropsychological disabilities were noted during childhood. Extrapyramidal signs and basal ganglia calcification developed later, namely, hand tremor and rigidity at the age of 7 and 18 years, respectively. Laboratory analysis revealed hypocalcemia, hyperphosphatemia, and low-serum PTH with hypomagnesemia and mild hypercalciuria. After 2 years of treatment with calcium supplements and calcitriol, some brief periods of clinical improvement were reported; as well as an absence of nephrocalcinosis.

Keywords
► autosomal dominant hypocalcemia
► calcium-sensing receptor
► missense mutation
► mosaicism

Introduction
Autosomal dominant hypocalcemia (ADH; OMIM 601198) is a rare disease, caused by gain of function (activating) mutations in the calcium-sensing receptor (CASR) gene, located at chromosome 3q21.1.

CaSR is a 1078-amino acid cell membrane G protein–coupled receptor, mainly expressed in the parathyroid and in the cells lining the kidney tubule. Structurally, the CaSR comprises the following three principal structural domains: a 612 amino acid extracellular domain, which is critical for cotranslational processing, receptor dimerization, binding of ligands, and transmitting signals through the seven transmembrane domains, which comprises residues 613–862, and an intracellular domain (residues 863–1078).1 CaSR is able to sense small changes in circulating calcium concentration and to couple this information to intracellular signaling pathways, mediated by the Gq/11 protein–dependent stimulation of phospholipase C activity, that leads to accumulation of inositol 1,4,5-triphosphate, which in turn increases cytoplasmic calcium.1 This cascade results in a decrease in PTH secretion from the parathyroid cells, and at the renal level in an
inhibition of calcium reabsorption in the thick ascending limb and water reabsorption in the collecting duct. CaSR is also abundantly expressed in thyroidal calcitonin-secreting C cells and in neuronal and glial cells of several brain regions, such as, cerebral cortex, striatum, hypothalamus, hippocampus, and cerebellum. Activating CASR mutations result in increased sensitivity of parathyroid and renal cells to calcium concentrations, leading to hypocalcemia being perceived as normal.

ADH has a wide spectrum of clinical presentation, ranging from asymptomatic forms to severe neonatal seizures. Most patients with ADH are asymptomatic or mildly symptomatic and therefore are not diagnosed until adulthood when hypocalcemia is incidentally noted. Approximately 50% of patients have paresthesias, carpopedal spasm, and seizures; approximately 10% have hypercalciuria with nephrocalcinosis or nephrolithiasis, and more than 35% have ectopic and basal ganglia calcifications. Children, in particular, may become symptomatic with seizures and neuromuscular irritability during periods of stress, such as, a febrile illness, and may be mislabeled as having febrile seizures. Other possible associated problems may include intellectual disability, neuropsychiatric symptoms, basal ganglia calcification, nephrolithiasis, nephrocalcinosis, and reversible heart failure.

While the spectrum of ADH clinical features is broad, biochemical features are more uniform, including the following: hypocalcemia, usually in the range of 6 to 8 mg/dL (1.5–2.0 mmol/L), but as low as 5 mg/dL in some families; tendency toward hyperphosphatemia and hypomagnesemia; low or normal serum levels of PTH and normal or elevated levels of urinary calcium excretion, despite low serum calcium concentrations.

Case Report

The patient is the first son of a healthy, nonconsanguineous couple. Pregnancy and delivery were uneventful. A history of seizures between the ages of 14 months and 5 years was reported, which occurred mainly during infectious illnesses which was therefore interpreted as febrile seizures. At 5-year-old, in kindergarten, difficulties in social interaction with peers and in motor skills were noticed. Neuropsychological assessment by the Griffiths mental developmental scale highlighted language and gross motor skills impairments, a trembling and immature graphology and anxious behavior (general quotient 77.4). Serial neuropsychological assessments revealed learning disabilities, impaired verbal memory, visual working memory, visuospatial attention, and executive functions, as well as emotional hyper-reactivity, and low self-esteem. He started pedopsychiatric follow-up at the age of 6 years, and was later medicated with sertraline and quetiapine for anxiety symptoms and propranolol for hand tremor but with no improvement. Cognitive assessment using the Wechsler intelligence scale for children third edition (WISC-III) at 12-year-old yielded verbal, performance and full-scale intelligence quotient scores in the extremely low range (full-scale intelligence quotient score: 68). At the age of 15 years, a cranial computed tomography scan was performed, to exclude organic causes for the neuropsychiatric manifestations, displaying multiple calcifications in basal ganglia and cortico-subcortical frontal and temporal regions (►Fig. 1). Laboratory evaluation showed hypocalcemia (6.2 mg/dL), hyperphosphatemia (8.3 mg/dL), and low intact PTH levels (9.63 pg/mL; normal: 11.0–88 pg/mL), and he was referred to pediatric endocrinology consultation. The patient denied complains of paresthesia but previous blepharospasm was reported. No facial dysmorphisms, subcutaneous calcifications, or other muco-cutaneous changes were observed; Chvostek and Trousseau signs were absent. Neurologic examination revealed a slight hand resting tremor, more pronounced on the right side, particularly during fine movements. Lead-pipe rigidity was noted in both upper limbs, along with hypomimia and truncal ataxia. No other coordination disturbances were observed. Laboratory analysis was repeated confirming hypocalcemia, hyperphosphatemia, and low serum PTH with hypomagnesemia (1.30 mg/dL), slightly elevated 1,25-dihydroxyvitamin D (89.22 pg/mL; normal: 12–88 pg/mL) and mild hypercalciuria (urinary calcium/creatinine ratio 0.24). Adrenocorticotropic hormone and cortisol were normal. Antiparathyroid antibodies were

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**Fig. 1** Basal ganglia calcification. Computed tomography scan imaging showing multiple confluent calcifications in bilateral lenticular nuclei, extending to the right caudate nucleus and focal calcifications in cortico-subcortical transition, at bilateral frontal and left posterior temporal topography.
negative on several occasions. Fluorescence in situ hybridization for the chromosome band 22q11 deletion was negative. Renal ultrasound, osteodensitometry, and electroencephalogram were normal.

Treatment with calcium supplements and calcitriol was started, as well as magnesium supplements, which were used transiently to avoid worsening of hypocalemia. On the following clinical evaluation, hypocalemia persisted, as well as the clinical manifestations. Biperiden was also tried to treat rigidity with no significant improvement. After 2 years of treatment, some brief periods of clinical improvement were reported, coincident with the increase of serum calcium and no nephrocalcinosis was detected. None of the other family members reported symptoms associated with hypocalemia.

Written informed consent was obtained for blood collection and subsequent analyses of the CASR gene in the patient and his mother. In brief, the analysis of the CASR gene involved the genomic DNA extraction from the patient’s peripheral blood leukocytes, PCR amplification of exons 2–7, including the adjoining splice junctions (primer sequences are available on request), and direct sequencing of the PCR products.

A novel heterozygous point mutation, a T—C transition in the second base of codon 123, expected to result in the replacement of TTG (leucine) by TCG (serine) (c.368T>C; p.Leu123Ser), was identified in the patients with ADH (Fig. 2). This sequence abnormality was confirmed by repeat PCR and sequencing. DNA sequencing also disclosed the p.Leu123Ser heterozygous variant in the proband’s mother. However, sequencing analysis showed a lower proportion of the mutant allele (C) when compared with the wild-type allele (T), contrasting with the proband’s allele profile that showed a 1:1 wild-type/mutant allele proportion (Fig. 2). These results, although being merely qualitative, suggested that this could represent mosaicism in the mother. Restriction enzyme analysis with TaqI (T/CGA), confirmed the presence of the variant in the mother (data not shown). Therefore, we also investigated the presence of the variant in the mother’s oral mucosa epithelial cells, and an identical sequencing profile was obtained.

The patient’s mother was asymptomatic, normocalcemic, and had normal serum levels of phosphorus and PTH. The patient’s father did not give his consent for genetic study, but laboratory evaluation revealed normal serum levels of calcium, phosphorus, and PTH.

This genetic variant was not found in 50 normal controls of the Portuguese population, and was not present in the 1,000 genomes database, indicating that it is not a common polymorphism. In silico analysis with the software PolyPhen-2, using the HumDiv model, predicted that this mutation was probably damaging with a score of 0.990 (sensitivity 0.72 and specificity 0.97); in addition, using the HumVar model the mutation was predicted to be possibly damaging with a score of 0.903 (sensitivity 0.69 and specificity 0.90).

Discussion

Hypoparathyroidism is a diagnostic challenge. It may occur as a complex developmental disorder, such as DiGeorge syndrome (OMIM 188400) or hypoparathyroidism sensorineural deafness renal dysplasia syndrome (GATA3 gene; OMIM 146255), or as part of an autoimmune disorder, such as autoimmune polyendocrine syndrome type 1 (OMIM 240300). Our patient showed no dysmorphic features suggestive for DiGeorge syndrome and deletion of chromosome 22q11.2 was excluded by fluorescence in situ hybridization analysis. Neither deafness nor renal abnormalities were reported, making the diagnosis of hypoparathyroidism sensorineural deafness renal dysplasia syndrome unlikely. Autoimmune antibodies against the parathyroid gland were negative, serum cortisol levels were in the normal range, and there was no history of candidiasis, excluding a possible polyendocrine syndrome type 1.

When hypoparathyroidism occurs as a solitary endocrinopathy, it is called isolated hypoparathyroidism. Familial isolated hypoparathyroidism (OMIM: 146200) can be caused by activating mutations in the CASR gene, by homozygous inactivating or heterozygous dominant negative mutations in the PTH gene (OMIM 168450), or by homozygous or heterozygous mutations in the glial cells missing 2 gene (GCM2; OMIM 603716). The finding of low but detectable PTH

![Fig. 2 Detection of a novel heterozygous germline variant in the CASR gene in a case of autosomal dominant hypocalemia. The identified variant, a T>C transition in the second base of codon 123 (indicated by an arrow), expected to result in the replacement of TTG (leucine) by TCG (serine) (c.368T>C; p.Leu123Ser). Sequencing analysis in the mother of the index case (individual I.2) suggested mosaicism for the same variant. The father (individual I.1) was not assessed.]
levels in our patient suggested a possible diagnosis of ADH rather than a mutation of the PTH or GCM2 genes in which undetectable PTH levels are frequently observed.10 Moreover, CASR activating mutations causing ADH are much more common than other causes of familial isolated hypoparathyroidism.10 Activating antibodies anti-CaSR were also reported in patients with autoimmune hypoparathyroidism.12

ADH may be difficult to distinguish from isolated primary hypoparathyroidism on the basis of measurements of serum PTH and urinary calcium. Such patients are often labeled as having idiopathic hypoparathyroidism, unless mutational analysis of CASR gene is performed. Therefore, screening for mutations in the CASR gene is an important step in the work-up of this endocrine disorder, especially in hypocalcemic patients.12

At least 95 different activating mutations of CASR have been identified to date.14,15 Most reported mutations (>85%) occur in the extracellular domain, although some occur in the transmembrane domain, and the vast majority are missense substitutions.15 In addition to missense mutations, two deletions and one insertion mutation have also been reported.6,14

We have identified a novel heterozygous missense mutation, which consisted of a serine to leucine substitution at codon 123 (p.Leu123Ser). This mutation is located in the extracellular region of the CaSR, which has a bilobed venus flytrap domain, predicted to contain five calcium binding sites, and likely to have other roles, such as mediating receptor dimerization.15–17 Although the effect of p.Leu123Ser was not analyzed in vitro, in silico analysis suggested a damaging effect for this variant. In line with this hypothesis, several activating mutations located in the vicinity of amino acid 123, also affecting conserved Leucine residues (e.g., p.Leu125Pro and p.Leu125Phe), have been identified in cases with ADH.14,18–20 In vitro functional expression studies of CASR p.Leu125Pro, assessed by measuring CaSR-stimulated changes in intracellular calcium, revealed a reduction in the EC50 for extracellular calcium, compared with the wild-type gene, thus confirming that this was a gain-of-function mutant.18,19 Although a similar response to extracellular calcium could be expected for the mutation identified in our study, the effects on venus flytrap domain functions remain to be elucidated. Molecular analysis of CASR in the proband’s mother revealed that the same mutation of the proband was detected in subclones of her leukocytes and epithelial cells of oral mucosa, suggesting a possible somatic mosaicism. The fact that the proband’s mother was clinically and biochemically unaffected may be hypothetically explained by the absence of the mutation in one of the parathyroid and in the kidney tubule cells. Indeed, some cases of de novo mutations in ADH have also been identified,6 but to our knowledge, only one previous case of mosaicism in ADH was reported.21 The frequency of mosaic mutations may be underestimated because they often remain undetected during routine mutation analysis and the index cases are considered de novo cases. Therefore, a thorough genetic analysis of parents should be performed in apparently sporadic cases with ADH to uncover parental mosaicism, for an appropriate genetic counseling in subsequent offspring.

Clinical presentation of the index case included seizures during childhood, blepharospasm, intellectual disability, neuropsychological disturbances, truncal ataxia, extrapyramidal signs, and basal ganglia calcifications. Neuropsychological dysfunctions are present in up to one-third of patients with idiopathic hypoparathyroidism, with intellectual disability, psychomotor deficits, response inhibition, and impairment in visuo-spatial gestalt functioning, and visual perception constructive abilities.9 Neuropsychiatric abnormalities are present in about two-thirds of patients, namely, anxiety and depression.9 Approximately one-fifth of patients have the following motor disturbances: cerebellar dysfunction, extrapyramidal signs, or other movement disorders.9 Extrapyramidal symptoms, including an ataxic gait, have been reported in patients with ADH.22,23 Intracerebral calcification is recognized to be a feature of hypoparathyroidism, being most often described in the basal ganglia. While bilateral topography of cerebral lesions is often seen, asymmetry of neurologic signs has been frequently reported,23 as it was observed in our patient, with a hand resting tremor. A study of 25 patients with ADH did not report extrapyramidal symptoms, although calcification of the basal ganglia was observed in nine (36%) patients.23 Neurologic dysfunctions seem to correlate with duration of hypocalcemia symptoms, serum total calcium, and calcium-phosphorus maintained in follow-up, but not with the presence or extent of intracranial calcifications.9

Usually, extrapyramidal symptoms do not respond to classical drugs, but in some cases, there is an improvement after treatment with vitamin D and calcium,24 as it was observed in our patient. The goal of treatment in symptomatic patients is to maintain a serum calcium concentration enough to ameliorate the symptoms. This can be achieved by cautious calcium and calcitriol supplementation with careful monitoring of urinary calcium excretion to avoid nephrocalcinosis, nephrocalcinosis, or renal insufficiency.25 Thiazides diuretics can lower urinary calcium excretion in such patients.19 Although it is not currently approved, clinical trials have shown that recombinant PTH can increase serum calcium while maintaining a normal level of urinary calcium excretion, holding promise as a treatment for ADH and refractory hypercalcuria.26 Alternatively, calcilytics, compounds that act as negative allosteric modulators of CaSR, offer the possibility of a more physiologic correction of ADH through a decrease of sensitivity of the receptor to calcium, resulting in enhanced PTH secretion and renal calcium reabsorption.27,28 In our patients with ADH, treatment with calcium supplements and calcitriol has led to periods of clinical improvement, and after 2 years, he did not develop nephrocalcinosis.

In conclusion, this case of ADH presents a novel heterozygous missense mutation located in the extracellular domain of the CaSR (p.Leu123Ser), with neurologic presentation,
whose extrapyramidal signs did not respond to classical treatment. ADH represents an important differential diagnosis of basal ganglia calcification. When there is no conclusive family history, parental mosaicism should be considered before concluding a de novo mutation in index case.

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