Autosomal dominant hypocalcemia with a novel CASR mutation: a case study and literature review

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Abstract
Autosomal dominant hypocalcemia type 1 (ADH1) is a rare inherited disorder characterized by hypocalcemia with low parathyroid hormone (PTH) levels and high urinary calcium. Its clinical presentation varies from mild asymptomatic to severe hypocalcemia. It is caused by gain-of-function mutations in the calcium-sensing receptor gene (CASR) which affect PTH secretion from the parathyroid gland and calcium resorption in the kidney. Here, we describe a case who presented with symptoms of recurrent seizure caused by hypocalcemia with a novel CASR variant. We comprehensively analyzed the phenotypic features of this presentation and reviewed the current literature to better understand clinical manifestations and the genetic spectrum.

Keywords
Autosomal dominant hypocalcemia type 1, hypoparathyroidism, seizure, calcification, CASR, hyperphospheremia

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Introduction

Autosomal dominant hypocalcemia type 1 (ADH1) is a rare inherited disorder characterized by hypocalcemia with low parathyroid hormone (PTH) levels and high urinary calcium.\(^1\) It was first described in 1994, with patients mainly presenting with asymptomatic hypocalcemia.\(^2\) ADH1 is more common than ADH2, accounting for 70% of cases.\(^3,4\)

ADH1 is caused by mutations in the calcium-sensing receptor gene (\(CASR\)) which maps to chromosome 3q13.3-21 and is composed of six exons. CASR is a plasma membrane G protein-coupled receptor containing a large extracellular domain of 612 amino acids, seven transmembrane domains, and one intracellular domain.\(^5,6\) It plays an important role in maintaining systemic calcium homeostasis, and is mainly expressed in the parathyroid gland, kidney, bone and gut.\(^7\)

Herein, we describe a patient with a de novo \(CASR\) mutation and performed a detailed literature review of ADH1 to summarize clinical manifestations and biochemical characterizations, and to widen our knowledge of the genetic spectrum.

Case presentation

A 24-year-old man (Figure 1a) was admitted to our hospital with recurrent tonic-clonic seizures. He was born at term with a normal weight, length, and head circumference, following an uneventful pregnancy. He had his first tonic-clonic seizure with no obvious cause at the age of 9 months. At this time, he was admitted to hospital where biochemical analyses revealed low serum calcium and high serum phosphate levels. Blood glucose levels, and abdominal and parathyroid ultrasound were normal, so other causes of hypocalcemia were ruled out. Although he was prescribed phenobarbital, the epileptic seizures were not well controlled.

At the age of 1 year, he experienced recurrent seizures, which were more frequent when he had a fever. Computed tomography of the brain revealed multiple intracranial calcifications, and electroencephalography showed extensive epileptic discharges. Since then, he has been repeatedly hospitalized with recurrent seizures, and intracranial calcification was shown to be progressively aggravating. He was diagnosed with congenital cataracts at the age of 14 years.

Physical examination on admission at our hospital showed no obvious abnormalities. After written informed consent was obtained from the patient to participate in this study, clinical evaluation including laboratory testing and brain imaging were performed. Laboratory data on admission and 1 week after admission are shown in Table 1. Hypocalcemia, hyperphosphatemia, and hypoparathyroidism were clearly evident, and brain computed tomography showed multiple intracranial calcifications (Figure 1b). The patient had no further seizures during his hospitalization while being administered calcium and antiepileptic drugs.

Genomic DNA was extracted from the patient’s peripheral blood using the Gentra Puregene Blood kit (QIAGEN, Hilden, Germany). Whole-exome sequencing was performed using SureSelectXT reagents (Agilent, Santa Clara, CA, USA) for capturing exons and the Illumina platform for sequencing (Illumina, San Diego, CA, USA). This revealed a novel, heterozygous missense \(CASR\) variant, c.416 T>C (p.Ile139Thr), which was found to be absent from the following reference databases: 1000 Genomes Project, the dbSNP database, the Exome Aggregation Consortium, and the Human Gene Mutation Database. Variant pathogenicity was interpreted and classified following the...
American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines.\textsuperscript{8} MutationTaster and PolyPhen-2 algorithms predicted the variant to be pathogenic, and Sorting Intolerant From Tolerant predicted it to be a benign variant. Sanger sequencing and segregation analysis showed that the mutation was \textit{de novo} (Figure 1c). It was evaluated as ‘likely pathogenic (PS1 + PM1 + PP1)’ according to ACMG guidelines. The amino acid sequence at position 139 of the extracellular domain was shown to be highly conserved among different species (Figure 1d).

The reporting of this study conforms to CARE guidelines.\textsuperscript{9}

**Discussion**

Here, we report a novel heterozygous \textit{CASR} variant in a patient with severe hypocalcemia and hypoparathyroidism. ADH1 can manifest at any age, but most
commonly starts in childhood. Clinical manifestations vary from asymptomatic hypocalcemia to mild neuromuscular symptoms such as muscle cramps, tetany, cataracts, and baldness. Severe cases may present with epileptic seizures. Studies have found that approximately 50% of patients with ADH1 have symptomatic hypocalcemia and >30% have renal and/or intracerebral calcifications. Seizures are less common, and our review of the current literature found that 19.8% (50/253) of patients experienced them.

Our patient had both intracranial calcification and seizures, which is a relatively rare phenotype. Indeed, our literature review found that this was seen in only 8.7% (22/253) of patients (Table 2). The cause of epilepsy in patients with ADH1 is unclear. A previous study found that the severity of hypocalcemia was related to the severity of neurological symptoms, while other studies showed the occurrence of epilepsy to be independent of serum calcium levels. Of note, epileptic seizures appear to be a prominent feature of ADH1, suggesting that levels of electrolytes, especially serum calcium, should be measured to rule out ADH1 in patients with epilepsy.

CASR is a plasma membrane G protein-coupled receptor that is widely expressed in the peripheral tissue, including the parathyroid gland, pancreas, duodenum, kidney, bone, stomach and respiratory system. Its primary function is to maintain systemic calcium homeostasis. More than 400 CASR mutations have been reported to date according to the Human Gene Mutation Database. Loss- or gain-of-function CASR mutations lead to opposing clinical manifestations. For instance, heterozygous and homozygous loss-of-function mutations and inactivating variants cause familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism, respectively. Conversely, gain-of-function mutations and activating variants are a major cause of type-5 Bartter syndrome and ADH1. Type-5 Bartter syndrome and ADH1 have similar clinical features, although the former leads to electrolyte imbalances from hypokalemia. Their clinical manifestations differ from those of FHH.

Abnormal CASR function has previously been implicated in central nervous system disorders such as epilepsy where an undefined association between genotype and phenotype was observed. Clinical presentations also vary widely among members of the same family with identical CASR genotypes, indicating the possibility of interactions among genetic, epigenetic, and environmental factors. Studies revealed that approximately 95% of ADH1 cases are caused by missense substitutions, whereas 5% result from in-frame or frame-shift insertion/deletion mutations. Our literature review found that 7.5% (19/253) of ADH1 cases were caused by de novo CASR mutations. Missense CASR mutation hotspots cluster in exons 3, 4, and 7. The de novo heterozygous p.Ile139Thr variant identified in the present case is located in exon 3, within the extracellular domain. Amino acids 116 to 136 of CASR were shown to be ligand binding sites that are more sensitive to calcium levels than other amino acids. The variant identified in our patient is close to these ligand binding sites, suggesting it is likely to be more pathogenic than mutations in transmembrane and intracellular domains.

Once a diagnosis of ADH1 has been confirmed, the need for therapeutic correction of hypocalcemia is controversial. Recent studies reported that correction treatment should be avoided for asymptomatic patients. Indeed, it is thought necessary to begin treatment with the lowest amount of calcium and activated vitamin D when symptoms occur frequently because the therapeutic aim is to alleviate symptoms rather than restoring normal levels of
| Sex | Age of disease onset | Biomarker | Epilepsy seizure | Site of calcification | CASR variant | Exon | Het/Hom | Author |
|-----|---------------------|-----------|------------------|-----------------------|--------------|------|---------|--------|
| F   | 18y                 | Hypocalcemia | + Basal ganglia  | 1631G > A; p.Arg544Gln | 6 | Hom | Cavaco et al. (2018) |
| F   | 23y                 | Hypocalcemia | + Basal ganglia  | 2269G > A; p.Glu757Lys | 7 | Het | Kwan et al. (2018) |
| –   | 25y                 | Hyperphosphatemia | + Basal ganglia  | 354C > A; p.Asn118Lys | 3 | Het | Pearce et al. (1996) |
| F   | 44y                 | Hypocalcemia | + Basal ganglia  | 382T > C; p.Phe128Leu | 3 | Het | |
| M   | 8y                  | Normal calcium | + Frontal lobe  | p.Ala784Val | 7 | Het | Winer et al. (2018) |
| F   | 1y                  | Normal calcium | + Basal ganglia; Thalamus | p.Glu127Lys | 3 | Het | |
| M   | 2y                  | Normal calcium | + Basal ganglia | p.Phe128Cys | 3 | Het | |
| F   | 25y                 | Hypocalcemia | + Basal ganglia  | 2086C > G p.Leu696Val | 7 | Het | Gomes et al. (2020) |
| F   | 42y                 | Hypocalcemia | + Bilateral basal ganglia | 372C > A p.Asn124Lys | 2 | Het | Hu et al. (2002) |
| F   | 25y                 | Normal phosphorus | + Basal ganglia  | 372C > A p.Asn124Lys | 2 | Het | |
| F   | 25y                 | Normal phosphorus | + CNS calcification | 386G > A p.Cys129Tyr | 3 | Het | Burren et al. (2005) |
| M   | 4w                  | Hypocalcemia | + CNS calcification | 386G > A p.Cys129Tyr | 3 | Het | |
calcium,\textsuperscript{20} which could increase the risk of nephrocalcinosis and/or intracranial calcification. However, some reports suggest that twice- or thrice-daily subcutaneous PTH 1–34 injection is safe and effective.\textsuperscript{21} Human recombinant PTH 1-84 has also been used to treat patients with ADH1,\textsuperscript{22} and to avoid nephrocalcinosis and slow the progress of intracranial calcification.\textsuperscript{23} Although there is currently no standard therapeutic approach for ADH1, some reports suggest that slightly increasing

| Sex | Age of disease onset | Biomarker | Epilepsy seizure | Site of calcification | CASR variant | Exon | Het/Hom | Author |
|-----|---------------------|------------|------------------|-----------------------|--------------|------|---------|--------|
| F   | 3d                  | Hypocalcemia | Hyperphosphatemia Low PTH | Basal ganglia | 2530G > C p.Ala844Pro | 7 | Het | Nakajima et al. (2009)\textsuperscript{30} |
| M   | 54y                 | Hypocalcemia | Normal phosphorus Low PTH | Basal ganglia; Frontal lobe | 1666G > A p.Glu556Lys | 6 | Het | Livadariu et al. (2011)\textsuperscript{31} |
| F   | 5y                  | Hypocalcemia | Hyperphosphatemia Normal magnesium Low PTH Normal calcification | Basal ganglia | 734A > G p.Gln245Arg | 4 | Het | Raue et al. (2011)\textsuperscript{32} |
| M   | 8y                  | Hypocalcemia | Hyperphosphatemia Hypomagnesemia Normal calcification | Basal ganglia | 452C > G p.Thr151Arg | 3 | Het | |
| M   | 38y                 | –           | Basal ganglia | + | 662C > T p.Phe221Leu | 4 | Het | |
| F   | 7m                  | Hypocalcemia | Hyperphosphatemia Normal PTH Hypercalciuria | Basal ganglia | 354C > A p.Asn118Lys | 2 | Het | De Luca et al. (1997)\textsuperscript{33} |
| M   | 7d                  | Hypocalcemia | Normal PTH | Basal ganglia | 2363T > G p.Phe788Cys | 7 | Het | Watanabe et al. (1998)\textsuperscript{34} |
| F   | 6d                  | Hypocalcemia | Hyperphosphatemia | Basal ganglia | 2363T > G p.Phe788Cys | 7 | Het | |
| F   | 12d                 | Hypocalcemia | Hypomagnesemia Low PTH | Basal ganglia; Bilateral subfrontal cortex | 2486A > G p.Tyr829Cys | 7 | Het | Choi et al. (2015)\textsuperscript{5} |
| F   | 6y                  | Hypocalcemia | Hyperphosphatemia Hypomagnesemia Low PTH | Basal ganglia | 2204A > C p.Gln735Pro | 7 | Het | Wong et al. (2011)\textsuperscript{35} |

Het = heterozygous, Hom = Homozygous, F = female, M = male, y = years, m = months, w = weeks, d = days, PTH = parathyroid hormone, CNS = central nervous system.
serum calcium levels prevent hypercalciuria. Additionally, calcilytics are under development for the treatment of ADH1, while thiazide-like diuretics have been used to treat hypercalciuria. Our patient continued to receive calcium supplements and antiepileptic drugs to avoid the recurrence of epilepsy.

**Conclusion**

ADH1 is a rare genetic disease characterized by hypocalcemia. Genetic exploration of CASR should be considered during the initial work-up of hypocalcemia to aid earlier recognition, and the implementation of targeted preventive and therapeutic strategies. By reviewing ADH1 cases, we comprehensively analyzed the phenotypic and genetic features to better understand the genotype and phenotype spectra.

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**Declaration of conflicting interest**

The authors declare that there is no conflict of interest.

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**Ethical approval**

The study protocol was reviewed and approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People’s Hospital (approval no: 2021-219). Written informed consent was obtained from the patient for participation in the study and the publication of any potentially identifiable images or data included in this article.

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**References**

1. Kwan B, Champion B, Boyages S, et al. A novel CASR mutation (p.Glu757Lys) causing autosomal dominant hypocalcaemia type 1. *Endocrinol Diabetes Metab Case Rep* 2018; 2018: 18–0107.
2. Pollak MR, Brown EM, Estep HL, et al. Autosomal dominant hypocalcaemia caused by a Ca(2+)‑sensing receptor gene mutation. *Nat Genet* 1994; 8: 303–307.
3. Papadopoulou A, Gole E, Melachroinou K, et al. Clinical characterization of a novel calcium sensing receptor genetic alteration in a Greek patient with autosomal dominant hypocalcemia type 1. *Hormones (Athens, Greece)* 2017; 16: 200–204.
4. Bastepe M. Gain-of-function CASR mutation causing hypocalcaemia in a recessive manner. *J Clin Endocrinol Metab* 2018; 103: 3514–3515.
5. Choi KH, Shin CH, Yang SW, et al. Autosomal dominant hypocalcemia with Bartter syndrome due to a novel activating mutation of calcium sensing receptor, Y829C. *Korean J Pediatr* 2015; 58: 148–153.
6. Hendy GN, D’Souza-Li L, Yang B, et al. Mutations of the calcium-sensing receptor (CASR) in familial hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia. *Human mutation* 2000; 16: 281–296.
7. Rasmussen AQ, Jorgensen NR and Schwarz P. Identification and functional characterization of a novel mutation in the human calcium-sensing receptor that co-segregates with autosomal-dominant hypocalcemia. *Front Endocrinol (Lausanne)* 2018; 9: 200.
8. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; 17: 405–424.
9. Gagnier JJ, Kienle G, Altman DG, et al; CARE Group. The CARE guidelines: consensus-based clinical case reporting guideline development. *Headache* 2013; 53: 1541–1547.
10. Schouteten MK, Bravenboer B, Seneca S, et al. A new mutation in the calcium-sensing receptor gene causing hypocalcaemia: case report of a father and two sons. *Neth J Med* 2017; 75: 253–255.
11. Hannan FM, Kallay E, Chang W, et al. The calcium-sensing receptor in physiology and in calcitropic and noncalcitropic diseases. *Nat Rev Endocrinol* 2018; 15: 33–51.
12. Thim SB, Birkebaek NH, Nissen PH, et al. Activating calcium-sensing receptor gene variants in children: a case study of infant hypocalcaemia and literature review. *Acta Paediatr* 2014; 103: 1117–1125.
13. Rossi GC, Patterson AL, McGregor AL, et al. Intractable generalized epilepsy and autosomal dominant hypocalcemia: a case report. *Child Neurol Open* 2019; 6: 2329048x19876199.
14. Tan YM, Cardinal J, Franks AH, et al. Autosomal dominant hypocalcemia: a novel activating mutation (E604K) in the cysteine-rich domain of the calcium-sensing receptor. *J Clin Endocrinol Metab* 2003; 88: 605–610.
15. Cole DE, Yun FH, Wong BY, et al. Calcium-sensing receptor mutations and denaturing high performance liquid chromatography. *J Mol Endocrinol* 2009; 42: 331–339.
16. Hussain A, Atlani M, Goyal A, et al. Type-5 Bartter syndrome presenting with metabolic seizure in adulthood. *BMJ Case Rep* 2021; 14: e235349.
17. Díaz-Soto G, Rocher A, García-Rodríguez C, et al. The calcium-sensing receptor in health and disease. *Int Rev Cell Mol Biol* 2016; 327: 321–369.
18. García-Castaño A, Madariaga L, Pérez de Nancalares G, et al. Novel mutations associated with inherited human calcium-sensing receptor disorders: A clinical genetic study. *Eur J Endocrinol* 2019; 180: 59–70.
19. Lienhardt A, Bai M, Lagarde JP, et al. Activating mutations of the calcium-sensing receptor: management of hypocalcemia. *J Clin Endocrinol Metab* 2001; 86: 5313–5323.
20. Roszko KL, Bi RD and Mannstadt M. Autosomal dominant hypocalcemia (hypoparathyroidism) types 1 and 2. *Front Physiol* 2016; 7: 458.
21. Winer KK, Fulton KA, Albert PS, et al. Effects of pump versus twice-daily injection delivery of synthetic parathyroid hormone 1–34 in children with severe congenital hypoparathyroidism. *J Pediatr* 2014; 165: 556–563.
22. Hawkes CP, Shulman DI and Levine MA Recombinant human parathyroid hormone (1–84) is effective in CASR-associated hypoparathyroidism. *Eur J Endocrinol* 2020; 183: K13–k21.
23. Winer KK, Kelly A, Johns A, et al. Long-term parathyroid hormone 1-34 replacement therapy in children with hypoparathyroidism. *J Pediatr* 2018; 203: 391–399 e391.
24. Ji Y, Kang C, Chen J, et al. Identification of p.Arg205Cys in CASR in an autosomal dominant hypocalcaemia type 1 pedigree: A case report. *Medicine* 2021; 100: e26443.
25. Cavaco BM, Canaff L, Nolin-Lapalme A, et al. Homozygous calcium-sensing receptor polymorphism R544Q presents as hypocalcemic hypoparathyroidism. *J Clin Endocrinol Metab* 2018; 103: 2879–2888.
26. Pearce SH, Williamson C, Kifor O, et al. A familial syndrome of hypocalcemia with hypercalciuria due to mutations in the calcium-sensing receptor. *N Engl J Med* 1996; 335: 1115–1122.
27. Gomes V, Silvestre C, Ferreira F, et al. Autosomal dominant hypocalcaemia: identification of two novel variants of CASR gene. *BMJ Case Rep* 2020; 13: e234391.
28. Hu J, Mora S, Colussi G, et al. Autosomal dominant hypocalcemia caused by a novel mutation in the loop 2 region of the human calcium receptor extracellular domain. *J Bone Miner Res* 2002; 17: 1461–1469.
29. Burren CP, Curley A, Christie P, et al. A family with autosomal dominant hypocalcaemia with hypercalciuria (ADHH): mutational analysis, phenotypic variability and treatment challenges. *J Pediatr Endocrinol Metab* 2005; 18: 689–699.
30. Nakajima K, Yamazaki K, Kimura H, et al. Novel gain of function mutations of the calcium-sensing receptor in two patients with PTH-deficient hypocalcemia. *Intern Med* 2009; 48: 1951–1956.

31. Livadariu E, Auriemma RS, Rydlewski C, et al. Mutations of calcium-sensing receptor gene: two novel mutations and overview of impact on calcium homeostasis. *Eur J Endocrinol* 2011; 165: 353–358.

32. Raue F, Pichl J, Dörr H G, et al. Activating mutations in the calcium-sensing receptor: genetic and clinical spectrum in 25 patients with autosomal dominant hypocalcaemia – a German survey. *Clin Endocrinol* 2011; 75: 760–765.

33. De Luca F, Ray K, Mancilla EE, et al. Sporadic hypoparathyroidism caused by de Novo gain-of-function mutations of the Ca(2+)-sensing receptor. *J Clin Endocrinol Metab* 1997; 82: 2710–2715.

34. Watanabe T, Bai M, Lane C R, et al. Familial hypoparathyroidism: identification of a novel gain of function mutation in transmembrane domain 5 of the calcium-sensing receptor. *J Clin Endocrinol Metab* 1998; 83: 2497–2502.

35. Wong WC, Lam CW, Tong SF, et al. Persistent hypocalcaemia in a Chinese girl due to a novel de-novo activating mutation of the calcium-sensing receptor gene. *Hong Kong Med J* 2011; 17: 157–160.