JIG Journal of Interdisciplinary Genomics

https://doi.org/10.22742/JIG.2021.3.1.21
Journal of Interdisciplinary Genomics 2021;3(1):21-24
eISSN 2671-6771

Clinical and Molecular Features of Three Korean Cases of Activating Variants in the *CASR* Gene

Jung Kwan Eun[1], Mi Sun Lee[1,2], Ji Min Lee[1,2], Eun Joo Lee[1,3], Sook-Hyun Park[1,3], Cheol Woo Ko[1,2], Jung-Eun Moon[1,2]

[1]Department of Pediatrics, School of Medicine, Kyungpook National University, Kyungpook National University Hospital, Daegu, Korea
[2]Division of Pediatric Endocrinology, Kyungpook National University Children's Hospital, Daegu, Korea
[3]Division of Neonatology, Kyungpook National University Children's Hospital, Daegu, Korea

Purpose: Activating mutations of the calcium-sensing receptor (*CASR*) are a rare genetic disorder, and result in autosomal dominant hypocalcemia with hypercalciuria (ADHH). ADHH exhibited varying degrees of hypocalcemia. In this study, we report the clinical and molecular characteristics of activating variants in *CASR* patients diagnosed in Korea. **Methods:** This study included three patients with activating variants of *CASR* confirmed by biochemical and molecular analysis of *CASR*. Clinical and biochemical findings were reviewed chart retrospectively. Mutation analysis of *CASR* was performed by Sanger sequencing. **Results:** Subject 1 showed severe symptoms from the neonatal period and had difficulty in controlling the medications that were administered. Subject 2 was identified as having a novel variant of *CASR* with hypocalcemia and a low parathyroid hormone that were found in the neonatal period. During a course without medication, hypocalcemia occurred suddenly around 2 years of age. Subject 3 was diagnosed with hypoparathyroidism with hypocalcemic seizures starting from the neonatal period. About 4 years without taking medication with any symptom. However, at 10 years old revisited by repetitive hypocalcemic seizure events. Subject 1 and 3, were heterozygous for c.2474A>T (p.Y825F), c.2395G>A (p.E799K) located in the transmembrane domain (TMD) of *CASR*. Subject 2 was heterozygous for c.403A>C (S430L) located in the extracellular domain (ECD) of *CASR*. **Conclusion:** We reported 3 patients who have activating *CASR* variant with different onset and severity of symptoms. In the future, further study is needed to determine how the protein level according to the location of the mutation of *CASR* affects the degree of symptoms.

Key words: Calcium-sensing receptor, Activating mutations, Hypocalcemia, Hypercalciuria

ORIGINAL ARTICLE

Received: September 18, 2020
Revised: October 12, 2020
Accepted: October 14, 2020

Correspondence to: Jung-Eun Moon
Department of Pediatrics, School of Medicine, Kyungpook National University, Kyungpook National University Hospital, 807 Hoguk-ro, Buk-gu, Daegu 41404, Republic of Korea
Tel: +82-10-4782-8315
Fax: +82-53-425-6683
E-mail: subuya@daum.net

ORCID
Jung-Eun Moon: https://orcid.org/0000-0001-9786-7898
Eun Jung Kwan: https://orcid.org/0000-0002-2437-191X

INTRODUCTION

Calcium-sensing receptor (CASR), a cell membrane G protein-coupled receptor located in the parathyroid glands and renal tubules, detects changes in serum calcium levels and is responsible for the regulation of parathyroid secretions and positive ions in the kidneys [1,2]. CASR maintains calcium homeostasis by regulating extracellular calcium ion ($Ca2^{+}$) levels. Activating mutations of *CASR* are also called gain-of-function *CASR* mutations, and such mutations result in autosomal dominant hypocalcemia with hypercalciuria (ADHH) [3]. ADHH is a genetic disorder characterized by hypocalcemia and hypercalciuria with inappropriately low levels of parathyroid hormone (PTH) [4]. Patients with ADHH have varying degrees of hypocalcemia [5]. CASR is characterized by the presence of a large extracellular domain (ECD), transmembrane domain (TMD), and intracellular domain (ICD) [6]. Various *CASR* mutations have been reported. We encountered and reported three cases of activating variants of *CASR*, with major differences observed in the degree of symptoms according to the patients. In this study, we investigated the clinical and molecular characteristics of patients with activating variants in *CASR* in Korea.

METHODS

This study included 3 patients (1 male and 2 females) with an activating variants in *CASR*. Clinical features and biochemical profiles were collected by retrospective chart review. Each diagnosis of the *CASR* variants was confirmed by molecular analysis of the *CASR* gene. This study was approved by the Institutional Review Board (IRB) of Kyungpook National University Chilgok Hospital and was conducted according to the recommendations of the IRB. Blood samples for DNA analysis were collected after obtaining informed consent from the participants' parents.

RESULTS

Clinical and biochemical characteristics of patients with *CASR* activating variant (Tables 1, 2)

Subject 1, a 5-day-old female neonate, was referred for evaluation of neonatal seizures. She was born at 39 weeks of gestation with a birth weight of 3.2 kg. The serum calcium level was 4.7 mg/dL (9.0–10.6), ionized calcium level was 2.2 mg/dL (4.8–4.9), phosphate level was 13.2 mg/dL (4.8–8.2), magnesium level was 1.29 mEq/L (1.44–3.1), PTH level was 2.6 pg/mL (10–65), and vitamin D level was 13.95 ng/mL (8.0–51.9). The patient did not have any morphological abnormalities of the face, limbs, or fingers. When patients were given calcium carbonate, magnesium, calcitriol, and thiazide, the serum calcium level was maintained at 6–8 mg/dL (9.0–10.6), and the phosphate level was maintained at 8–10 mg/dL (4.8–8.2). When the dose for calcitriol was increased, serum calcium level was maintained low at 6–8 mg/dL (9.0–10.6), but the spot urine Calcium/Creatinine (Ca/Cr) increased at 2.0 and kidney stones were formed in the kidney USG. Therefore, calcitriol (0.5 mcg/day) treatment has been reduced and being maintained, and the levels are currently being monitored. Lithotripsy was performed in the urology department due to urine stones at eight months of age in the neonate.

Subject 2, a 2-day-old male neonate, was referred to neonatal intensive care unit due to tachypnea. He was born at 38 weeks of gestation and weighed 3.6 kg without any perinatal problems. He has been incidentally finding hypocalcemia in the neonatal intensive care unit. The serum calcium level was 6.8 mg/dL (9.0-10.6), ionized calcium level was 3.2 mg/dL (4.8–4.92), phosphate level was 6.3 mg/dL (4.8–8.2), magnesium level was 1.6 mEq/L (1.44–3.12), PTH level was 8.5 pg/mL (10–65), and vitamin D level was 9.98 ng/mL (8.0-51.9).

Table 1. Clinical and molecular characteristics of patients with *CASR* activating variant

Variable	Subject 1	Subject 2	Subject 3
Age at Diagnosis	1 month	1 month	14 yr
Current Age (yr)	3.5	3.3	17.1
Symptom start at	3 days after birth	3 yr old	3 days after birth and restart symptoms at 10 yr
Current medication	Calcium carbonate Magnesium Calcitriol Thiazide	Calcium carbonate Calcitriol	Calcium carbonate Calcitriol
Other finding	Kidney stone	None	Medullary nephrocalcinosis
CASR variant	c.2474A>T (p.Y825F)	c.403A>C (S430L)	c.2395G>A (p.E799K)
Domain in CASR	TMD	ECD	TMD

yr, year; CaSR, Calcium sensing receptor; TMD, Transmembrain domain; ECD, Extracellualr domain.

Table 2. Laboratory finding of 3 patients

Variable (reference ranges)	Subject 1		Subject 2		Subject 3	
	Initial	Post med	Initial	Post med	Initial	Post med
Calcium (mg/dL) (9.0-10.6)	4.7	6–8	6.8	7–8	4.6	7–8
Phosphate (mg/dL) (4.8-8.2)	13.2	8–10	6.3	6–7	9.8	6–7
Ionaized calcium (mg/dL) (4.8-4.9)	2.2	3.5–4	3.2	3.5–4.5	2.5	3.9–4.8
Intact PTH (pg/mL) (10-65)	2.6	1–5	8.5	3–9	8.5	1–3
Spot urine Ca/Cr (<0.2)	2.0	<0.2	0.2	<0.2	-	0.14

PTH, parathyroid hormone; Ca/Cr, Calcium Creatinine ratio.

This neonate had low intact PTH when being monitored without administration as spot urine Ca/Cr <0.2, but showed improvement with serum calcium level higher than 8 mg/dL and maintained normal ranges without medication. During following up, the serum calcium level decreased to 6.8 mg/dL (9.0–10.6) at the age of 2, and is currently being monitored with the administration of calcium carbonate and calcitriol. Currently, the patient is 3 years old, and maintaining low calcium levels at 7–8 mg/dL without any complication in the kidney USG.

Subject 3, a 4-day-old female neonate, was referred for evaluation of neonatal seizure. She was born at 38 weeks of gestation with a birth weight of 3.1 kg. The serum calcium level was 4.6 mg/dL (9.0–10.6), ionized calcium level was 2.5 mg/dL (4.8–4.92), phosphate level was 9.8 mg/dL (4.8–8.2), magnesium level was 1.5 mEq/L (1.44–3.12), and PTH level was 8.5 pg/mL (10–65). Administration of calcium gluconate (100 mg/kg/day) and low phosphate diet was initiated at the time of diagnosis of primary hypothyroidism. Serum calcium level was maintained at 7–8 mg/dL in the process, and there were no neurological symptoms. After 4 years without any symptoms and without medication by the patient's own. The patient has not visited the hospital for about 4 years, and there were no special symptoms in the meantime. The patient was readmitted to generalized tonic-clonic type seizure at the age of 10, and the laboratory findings at that time were as follows. Serum calcium 6.5 mg/dL, phosphate 7.4 mg/dL and intact PTH 2.5 pg/mL. The patient was receiving a calcitriol (1.5 mcg/day) and a calcium carbonate (3,000 mg/day), and medullary nephrocalcinosis diagnosed from the conducted kidney USG. The spot urine Ca/Cr of 0.14. The patient was being monitored. The serum calcium level was maintained at 7–8 mg/dL (9.0–10.6), and the phosphate level was maintained at 6–7 mg/dL (4.8–8.2).

Molecular analysis of the *CASR* gene (Table 1)

Targeted exome sequencing in the 3 patients was performed using the TruSight One Sequencing Panel, resulting in a heterozygous variant c.2474A>T (p.Y825F), c.403A>C (S430L), and c.2395G>A (p.E799K) in exon 7 of the *CASR* gene confirmed by Sanger sequencing. Subjects 1 and 3 were heterozygous for c.2474A>T (p.Y825F), and c.2395G>A (p.E799K) located in the TMD of *CASR*. Subject 2 was heterozygous for c.403A>C (S430L) located in the ECD of *CASR*.

DISCUSSION

This study presents the clinical, biochemical, and molecular analysis of patients with activating variants of *CASR*.

Most activating mutations of the *CASR* gene have been reported to be missense mutations in patients with ADHH [7]. Conditions of all patients in this study were due to missense mutation and we reported patient 1 as having a novel variant [8]. Cell function study was performed for the variant of patient 1, and it was confirmed that MAP kinase singnaling was increased. Patient 2 had a variant that has not been reported previously. The variant of patient 3 is in a pathogenic location that has been previously reported [6]. *CASR*, which is composed of 1,078 amino acids, consists of a large ECD comprising seven transmembrane-spanning domains and an intracellular tail, and is encoded by six exons of the *CASR* gene that are located on chromosome 3q13.3–21 [3].

Leach et al. [9] reported that many activating mutations of *CASR* coupled more strongly to $Ca2^{+}$. Research on the role of TMD in *CASR* is ongoing. There are studies that outline that *CASR* of the TMD has enhanced sensitivity depending on the calcium concentration, with research that also outlines that control is conducted by constitutive activation rather than enhanced $Ca2^{+}$ sensitivity [10,11]. In addition, reports outlining the characterization of a novel ADHH-causing mutation, Arg-680Gly, has led to the identification of a transmembrane salt bridge (Arg680–Glu767). Therefore, it was confirmed that the three-dimensional structure connected to the location of the TMD affects the function of *CASR* [12]. Moreover, Vezzoli et al. [13] reported that the Arg990Gly *CASR* single nucleotide polymorphism is associated with an increased risk of hypercalciuric nephrolithiasis; therefore, symptoms may vary depending on the location of the *CASR* mutation. In this study, subject 1 was heterozygous in the 6th loop, and the severe symptoms and excretion of calcium from the kidney were the most severe among the 3 patients from the neonatal period. In the case of subject 3, the TMD was located on the cyto-loop 3, which is between the 5th and 6th loop. Despite showing symptoms since birth, they were milder than those in subject 1. The location was at ECD for subject 2, and the symptoms were milder compared to the other two subjects. Hypocalcemia occurred at the age of 3, and the onset and severity of symptoms were observed to be different. Variants are located between loops 3 and 4 of the ECD, and there have been no reports of activating variants of *CASR* in this area. It is hypothesis that the number of cases confirmed through tests are few as

the symptoms were mild. However, we confirmed that the activating variant of this area eventually leads to hypocalcemia, and would like to emphasize that patients should regularly be monitored even if symptoms are mild at the time as the ECD is a variant during the neonatal period.

In conclusion, we report three patients with activating variants in *CASR*. The variants of each patient were as follows: Subjects 1 and 3 were heterozygous for c.2474A>T (p.Y825F), and c.2395G>A (p.E799K) located in the TMD of *CASR*. Subject 2 was heterozygous for c.403A>C (S430L) located in the ECD of *CASR*. Patients report different onset and severity of symptoms. Further studies are needed in the future to determine how protein levels affect the degree of symptom exertion, according to the location of the *CASR* mutation.

ACKNOWLEDGEMENT

Not Applicable.

DISCLOSURE

All authors have no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Moon JE, Ko CW. Data curation: Kwan EJ, Lee JM, Lee EJ, Park SH, and Moon JE. Formal analysis: Kwan EJ, Lee JM, Lee EJ, Park SH, and Moon JE. Methodology: Moon JE, Ko CW. Writing - original draft: Kwan EJ and Moon JE. Writing - review & editing: Kwan EJ and Moon JE.

REFERENCES

1. Janicic N, Soliman E, Pausova Z, Seldin M, Rivire M, Szpirer J, et al. Mapping of the calcium-sensing receptor gene (CASR) to human Chromosome 3q13.3-21 by fluorescence in situ hybridization, and localization to rat Chromosome 11 and mouse Chromosome 16. Mamm Genome 1995;6:798-801.
2. Hendy GN, D'Souza-Li L, Yang B, Canaff L, Cole DE. Mutations of the calcium-sensing receptor (CASR) in familial hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia. Hum Mutat 2000;16:281-96.
3. Janicic N, Soliman E, Pausova Z, Seldin MF, Riviere M, Szpirer J, et al. Mapping of the calcium-sensing receptor gene (CASR) to human chromosome 3q13. 3-21 by fluorescence in situ hybridization, and localization to rat chromosome 11 and mouse chromosome 16. Mamm Genome 1995;6:798-801.
4. Pollak MR, Brown EM, Estep HL, McLaine PN, Kifor O, Park J, et al. Autosomal dominant hypocalcaemia caused by a Ca2+-sensing receptor gene mutation. Nat Genet 1994;83:303.
5. D'Souza-Li L, Yang B, Canaff L, Bai M, Hanley DA, Bastepe M, et al. Identification and functional characterization of novel calcium-sensing receptor mutations in familial hypocalciuric hypercalcemia and autosomal dominant hypocalcemia. J Clin Endocrinol Metab 2002;87:1309-18.
6. Hu J, Spiegel AM. Structure and function of the human calcium-sensing receptor: insights from natural and engineered mutations and allosteric modulators. Journal of Cellular and Molecular Medicine 2007;11:908-22.
7. Brown EM. Clinical lessons from the calcium-sensing receptor. Nat Rev Endocrinol 2007;3:122.
8. Moon JE, Lee SJ, Park SH, Kim JS, Jin DK, Ko CW. De novo a novel variant of CASR gene in a neonate with congenital hypoparathyroidism. Ann Pediatr Endocrinol Metab 2018;23:107-11.
9. Leach K, Wen A, Davey AE, Sexton PM, Conigrave AD, Christopoulos A. Identification of molecular phenotypes and biased signaling induced by naturally occurring mutations of the human calcium-sensing receptor. Endocrinology 2012;159:4304-16.
10. Bai M, Quinn S, Trivedi S, Kifor O, Pearce SHS, Pollak MR, et al. Expression and characterization of inactivating and activating mutations in the human Ca2+(o)-sensing receptor. J Biol Chem 1996;271:19537-45.
11. Zhao XM, Hauache O, Goldsmith PK, Collins R, Spiegel AM. A missense mutation in the seventh transmembrane domain constitutively activates the human Ca2+ receptor. FEBS Lett 1999;448:180-4.
12. Hannan FM, Kallay E, Chang W, Brandi ML, Thakker RV. The calcium-sensing receptor in physiology and in calcitropic and noncalcitropic diseases. Nature Reviews Endocrinology 2019;15:33-51.
13. Vezzoli G, Tanini A, Ferrucci L, Soldati L, Bianchin C, Franceschelli F, et al. Influence of calcium-sensing receptor gene on urinary calcium excretion in stone-forming patients. J Am Soc Nephrol 2002;13:2517-23.