


CASE REPORT

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A case of familial hypocalciuric hypercalcemia type 1 due to CASR p.Pro55Leu mutation

Akira Sumida¹, Katsumi Iizuka^{1,2,3*} , Takehiro Kato¹, Yanyan Liu¹, Sodai Kubota^{1,4}, Saki Kubota-Okamoto^{1,4}, Teruaki Sakurai¹, Toshinori Imaizumi¹, Yoshihiro Takahashi¹, Masami Mizuno¹, Ken Takao¹, Takuo Hirota¹, Tetsuya Suwa¹, Yukio Horikawa¹, Mayumi Yamamoto⁵, Yusuke Seino⁶, Atsushi Suzuki⁶ and Daisuke Yabe^{1,3,7,8}

Abstract

Background: Familial hypocalciuric hypercalcemia (FHH) is a rare autosomal dominant disease, which requires differential diagnosis from relatively common primary hyperparathyroidism (PHPT) in order to avoid unnecessary surgery.

Case presentation: A 16-year-old female had been followed by the department of psychosomatic medicine at our institution. Throughout the follow-up period, her plasma calcium levels were high, plasma Pi levels were relatively low, and plasma intact PTH was relatively high. She was referred to our department to determine the cause of her hypercalcemia. Her 24 h urinary calcium excretion was as low as 100 mg/day, and calcium creatinine clearance ratio was below 0.01. Moreover, she had a family history of hypercalcemia (proband, her brother, and her father). The genetic testing for her family revealed that she, her brother, and her father were definitively diagnosed with FHH type 1 due to the heterozygous calcium-sensing receptor mutation (NM_003884:c.164C>T;p.Pro55Leu).

Conclusion: We experienced a 16-year-old female with FHH, in whom genetic testing identified the heterozygous calcium-sensing receptor mutation (NM_003884:c.164C>T;p.Pro55Leu) as pathogenic, permitting a definitive diagnosis of FHH type 1. The genetic testing for calcium sensing receptor is beneficial to distinguish asymptomatic primary hyperparathyroidism from FHH.

Keywords: Familial hypocalciuric hypercalcemia, FHH, Calcium creatinine clearance ratio, CCCR, Calcium-sensing receptor, CASR

Background

Hypercalcemia is a condition causing disorders in multiple organs, including bone, brain, and heart [1]. The causes of hypercalcemia include hyperparathyroidism, cancer, tuberculosis, immobility, and inappropriate vitamin D supplementation. Among these, primary hyperparathyroidism (PHPT) is a relatively

common disease (incidence rate; 1 in 1,000) and often requires parathyroid gland resection [2]. Familial hypocalciuric hypercalcemia (FHH) is a rare autosomal dominant disease (incidence rate; 1 in 78,000) that requires, in general, no treatment [2, 3]. Thus, differential diagnosis of FHH from PHPT is critical to avoid unnecessary surgery.

Urinary calcium excretion and calcium creatinine clearance ratio (CCCR) levels are widely used for differential diagnosis of FHH and PHPT; even so, it is sometimes difficult to distinguish the two diseases [2–6]. Approximately 80% of FHH patients

*Correspondence: katsumi.iizuka@fujita-hu.ac.jp

² Department of Clinical Nutrition, Fujita Health University, 1-98 Dengakugakubo, Kutsukake-Cho, Toyoake, Aichi 470-1192, Japan
Full list of author information is available at the end of the article



demonstrate urinary calcium excretion <100 mg/day and CCCR <0.01, while ~20% show CCCR 0.01–0.02 [6]. In contrast, PHPT patients usually have CCCR >0.02, while ~20%, who have concomitant vitamin D deficiency show CCCR <0.01 [6]. It is therefore difficult to distinguish FHH from PHPT based only on CCCR values. Thus, genetic testing becomes critical for the positive diagnosis of FHH.

To date, FHH-related mutations have been identified in genes related to the calcium sensing system that regulates calcium homeostasis [2]. The calcium-sensing receptor, CASR, is encoded by the *CASR* gene and belongs to a G protein-coupled receptor family that regulates parathyroid hormone (PTH) secretion, vitamin D synthesis, and calcium absorption and resorption [2, 5]. G-protein α_{11} (*GNA11*) is encoded by the *GNA11* gene and mediates CaSR signaling [2, 5]. Adaptor-related protein complex 2 (AP-2) is a membrane-associated heterotetramer complex and composed of two large chain, medium chain, and a small chain. The adaptor-related protein complex 2 sigma one subunit encoded by the *AP2S1* gene binds to CASR and facilitates CaSR internalization [2, 5]. Loss of function mutations in the *CASR*, *GNA11*, or *AP2S1* genes cause FHH type 1 (FHH1), FHH type 2 (FHH2), and FHH type 3 (FHH3), respectively [1, 4]. Approximately 60% of FHH patients have mutations in the *CASR* gene [2].

Here we report a case of FHH1 in which genetic testing identified the heterozygous *CASR* mutation p.Pro55Leu, which permitted the definitive diagnosis of

the disease. In this case with no symptom, family history and genetic test for CASR was beneficial for the diagnosis of FHH type1.

Case presentation

A 16-year-old female had been followed by the department of psychosomatic medicine at our institution for borderline personality disorder. Throughout the follow-up period, her plasma calcium levels were high, plasma Pi levels were relatively low, and plasma intact PTH (PTHi) was relatively high (Fig. 1).

Her height and body weight were 154 cm and 53.5 kg, respectively. Although she was asymptomatic, she was referred to our department to determine the cause of her hypercalcemia on June. Upon referral, her plasma calcium, inorganic phosphate (Pi) and PTHi were 11.5 (normal range; 8.8–10.1) mg/dL, 3.1 (normal range; 2.7–4.6) mg/dL, and 51 (normal range; 10–65) pg/mL, respectively (Table 1 and Fig. 1). Her 1,25-OH vitamin D (1,25-OHD) was elevated (59.7 [normal range; 20.0–60.0]) while 25-OH vitamin D (25-OHD) was low (9 [normal range; <25]) (Table 1). Thyroid echography and ^{99m}Tc-MIBI scintigraphy revealed no abnormalities in the parathyroid glands. Her 24 h urinary calcium excretion was as low as 100 (normal range; 100–300) mg/day, and CCCR was below 0.01 (1st measurement, 0.0053 and 2nd measurement, 0.0059) (normal range; >0.01) (Table 1). Her 24 h urinary Pi excretion was 0.58 (normal range; 0.5–1.0) g/day, and tubular reabsorption of phosphate was 89.9%

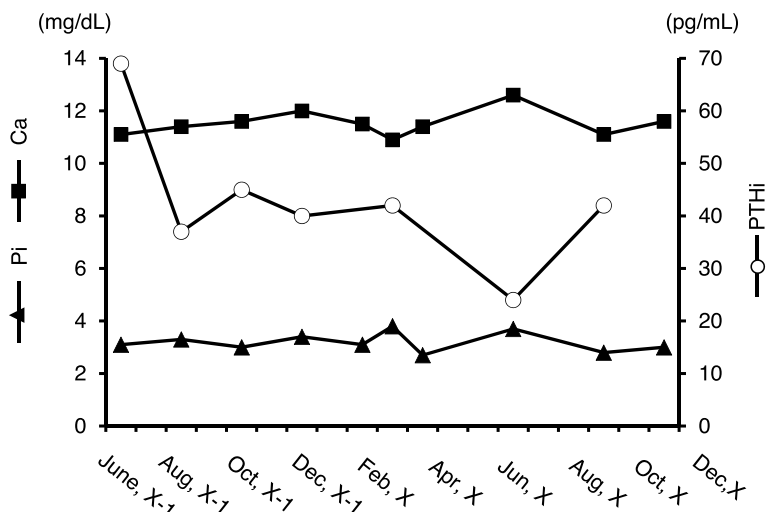


Fig. 1 Changes in plasma calcium, inorganic phosphate and intact parathyroid hormone in the current case. Square, plasma calcium; triangle, inorganic phosphate (Pi); circle, intact PTH (PTHi)

Table 1 Laboratory data of this patient

| | This patient | Normal ranges |
|---|---------------|---------------|
| Blood parameters | | |
| Albumin (g/dL) | 4.7 | 4.1–5.1 |
| Creatinine (mg/dL) | 0.54 | 0.46–0.79 |
| Na ⁺ (mEq/L) | 138 | 138–145 |
| K ⁺ (mEq/L) | 3.9 | 3.6–4.8 |
| Ca ²⁺ (mg/dL) | 11.5 | 8.8–10.1 |
| Pi ²⁻ (mg/dL) | 3.1 | 2.7–4.6 |
| Mg ²⁺ (mg/dL) | 2.2 | 1.8–2.4 |
| Alkaline phosphatase (U/L) | 236 | 106–322 |
| 1,25OHD (pg/mL) | 59.7 | 20.0–60.0 |
| 25OHD (pg/mL) | 9 | <25 |
| PTH intact (pg/mL) | 51 | 10–65 |
| PTH whole (pg/mL) | 44.8 | 8.3–38.7 |
| PTHrP (pmol/L) | < 1.0 | ≤1.1 |
| TSH (μIU/mL) | 0.49 | 0.54–4.26 |
| FT ₄ (ng/dL) | 0.93 | 0.76–1.65 |
| ACTH (pg/mL) | 32.1 | 7.2–63.3 |
| Cortisol (μg/dL) | 15.9 | 6.24–18.0 |
| Urine parameters | | |
| 24 h urinary Ca excretion (g) | 0.1 | 0.1–0.3 |
| 24 h urinary Pi excretion (g) | 0.58 | 0.5–1.0 |
| %TRP (%) | 89.9 | 80–94 |
| TmP/GFR (mg/dL) | 2.9 | 2.3–4.3 |
| Calcium creatinine clearance Ratio (CCCR) | 0.0059/0.0054 | > 0.01 |

TmP/GFR was estimated by nomogram for derivation of renal threshold phosphate concentration¹⁶. CCCR were calculated by plasma and 24-h urine samples for calcium and creatinine

Abbreviations: *Cre* Creatinine, *1,25OHD* 1,25-hydroxy-vitamin D, *25OHD* 25-hydroxy-vitamin D, *PTH* Parathyroid Hormone, *PTHrP* Parathyroid hormone related Peptide, *TRP* Tubular Reabsorption of Phosphate, *TmP/GFR* Tubular maximum Phosphate reabsorption per GFR, *CCCR* Calcium Creatinine Clearance Ratio.

(normal range; 80–94) (Table 1). Her TmP/GFR was almost same as plasma Pi levels, which were consistent with her normal renal function (Table 1).

Since her brother (II-1) and father (I-1) had hypercalcemia (Fig. 2A), we proposed that she receive a genetic test to diagnose with FHH and she and her family agreed with our proposal. Whole exon sequencing was performed by fragmentation of DNA, enrichment of exonic regions using capture-based hybridization, PCR amplification, and rapid sequencing using a next-generation sequencer in Kazusa DNA laboratory. Exon sequencing of hereditary hypercalcemia-related

genes (e.g., *MEN1*, *CDKN1B*, *RET*, *CASR*, *GNA11*, *AP2S1*, *CDC73*, *GCM2*) was performed and a pathogenic *CASR* mutation (*CASR*: NM_000388.4: exon2: c.164C>T: p. Pro55Leu) was noted in her father (I-1), brother (II-1), and the proband (II-3) (Fig. 2B), but no mutation in *GNA11* and *AP2S1* was identified. Based on the American College of Medical Genetics and Genomics Standards and Guidelines, the present variant was classified as pathogenic due to PS1 + PS3 + PM1 + PM2 [7]. She was then positively diagnosed as FHH1. As she was diagnosed with FHH, we followed up her with no medication. She and her family were relieved to hear that she needed no surgical treatment. Her plasma Ca²⁺ and Pi levels were almost unchanged for 18 months (Fig. 1).

Discussion and conclusions

We report here a case of hypercalcemia with family history and low CCCR with hypovitaminosis D, in which genetic testing permitted definitive diagnosis of FHH1.

FHH is a very rare benign inherited condition that typically does not require parathyroidectomy. Patients with FHH usually have no symptoms and are often diagnosed by chance during routine blood examination. Weakness, fatigue, issues with concentration, constipation, polyuria, headache, and polydipsia have been reported by some people with FHH. The calcimimetic drugs are sometimes effective to improve hypercalcemic symptoms such as muscle aches, anorexia, polydipsia and constipation [2]. Rarely, people with this disorder experience pancreatitis or a chondrocalcinosis [8]. Although she had no past-history of pancreatitis and bone fractures, we may follow up her risk of pancreatitis due to hypercalcemia through her life [8].

In the current study, a heterozygous *CASR* mutation p.Pro55Leu was found to be pathogenic in the patient's father (I-1), brother (II-1) and the proband (II-3). This mutation has previously been found in several individuals with FHH including one Japanese [2, 8–16]; it was classified as pathogenic in the ClinVar database (Variation ID: 279,731). The mutation is localized in the N-terminal extracellular domain (ECD) of CaSR, where ligand binding occurs; it causes a rightward shift of the PTH dose–response curve in response to extracellular calcium [11, 16]. Consistent with available information regarding the mutation, the patient showed relatively high PTHi despite the high plasma calcium level (Table 1 and Fig. 1).

In conclusion, we report a case of FHH1 due to *CASR* p.Pro55Leu mutation, in which only genetic testing

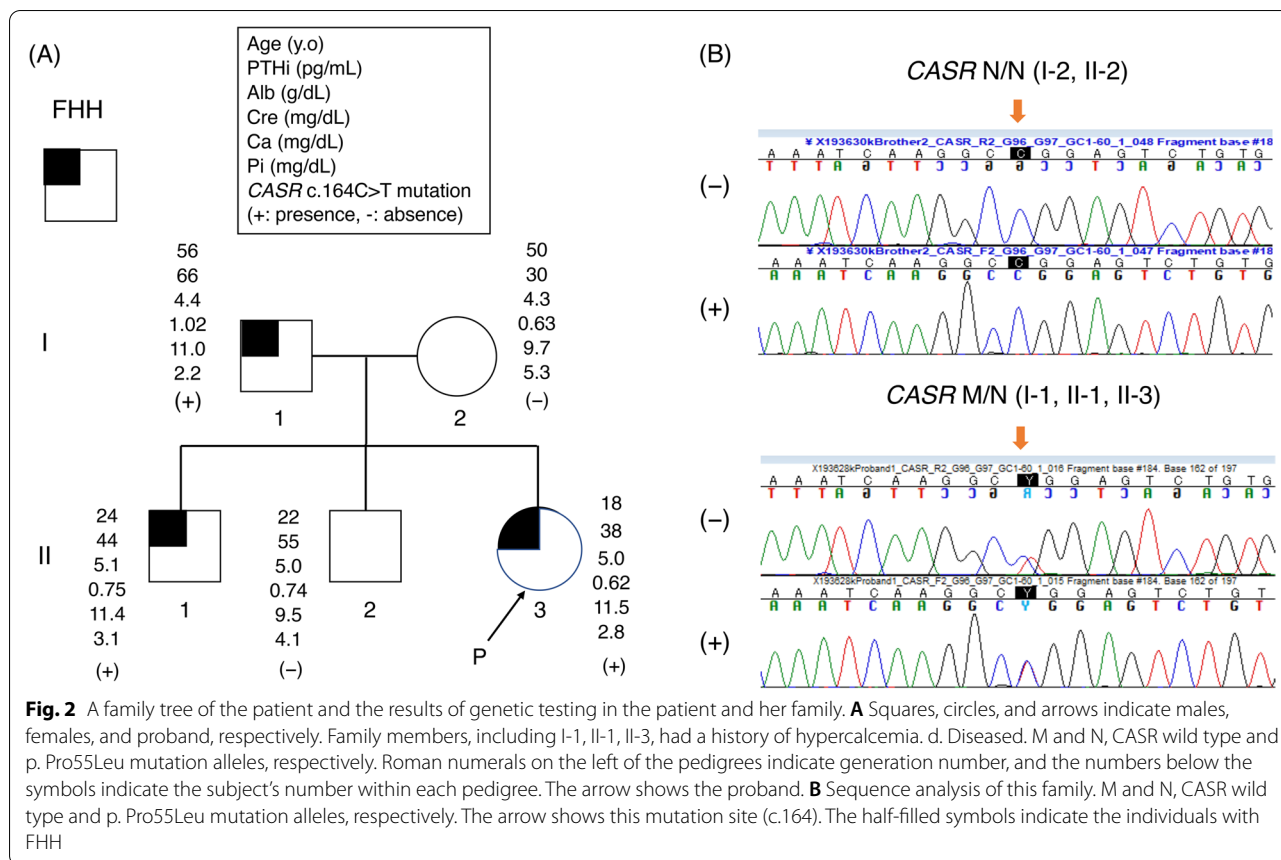


Fig. 2 A family tree of the patient and the results of genetic testing in the patient and her family. **A** Squares, circles, and arrows indicate males, females, and proband, respectively. Family members, including I-1, II-1, II-3, had a history of hypercalcemia. d. Diseased. M and N, CASR wild type and p. Pro55Leu mutation alleles, respectively. Roman numerals on the left of the pedigrees indicate generation number, and the numbers below the symbols indicate the subject's number within each pedigree. The arrow shows the proband. **B** Sequence analysis of this family. M and N, CASR wild type and p. Pro55Leu mutation alleles, respectively. The arrow shows this mutation site (c.164). The half-filled symbols indicate the individuals with FHH

allowed definitive diagnosis of FHH1 and thus avoided unnecessary surgery. The patient and family commented that they were very relieved to have found the cause of the disease. To diagnose with FHH by a genetic test is important to avoid unnecessary surgical treatment.

Availability of data and materials

Clinical data from the corresponding author will be available upon request.

Abbreviations

FHH: Familial hypocalciuric hypercalcemia; PHPT: Primary hyperparathyroidism; CCCR: Calcium creatinine clearance ratio; CASR: Calcium sensing receptor.

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Authors' contributions

AS, KI, and DY contributed to the analysis, collection, and interpretation of data and writing of the manuscript. KT, YL, SK, SK-O, TS, TI, YT, MM, KT, TH, TS, YH, MY, YS and AS contributed to the analysis, collection, and interpretation of data and critical revisions of the manuscript for important intellectual content.

All authors approved the version to be published. KI is a guarantor of this work. Corresponding authors: KI.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

We obtained approval from the ethics committee of Gifu University Graduate School of Medicine (Approval number 2020-201). The patient and her relatives provided written consent to participate in genetic screening for hereditary hypercalcemia.

Consent to publication

After she became at 18 years old, we obtained written informed consent of this case report from both herself and her parent. Genetic testing was carried out after counseling by a clinical genetic specialist. Her parents and her brothers were above 20 years old and we obtained written informed consent of this case report from themselves. We also obtained written informed consent for genetic test for her and her families. The patient and her families also gave written consent for their personal or clinical details along with any identifying images to be published in this study.

Competing interests

All authors have no conflict of interest.

Author details

¹Department of Diabetes, Endocrinology and Metabolism/Department of Rheumatology and Clinical Immunology, Gifu University Graduate School of Medicine, Gifu University Graduate School of Medicine, Gifu 501-1194, Japan. ²Department of Clinical Nutrition, Fujita Health University, 1-98 Dengakugakubo, Kutsukake-Cho, Toyoake, Aichi 470-1192, Japan. ³Yutaka Seino Distinguished Center for Diabetes Research, Kansai Electric Power Medical Research Institution, Kobe, Japan. ⁴Center for Diabetes, Metabolism, and Endocrinology, Kansai Electric Power Hospital, Osaka, Japan. ⁵Health Administration Center, Gifu University, Gifu, Japan. ⁶The Department of Endocrinology, Diabetes and Metabolism, Fujita Health University, Toyoake, Japan. ⁷Division of Molecular and Metabolic Medicine, Department of Physiology and Cell Biology, Kobe University Graduate School of Medicine, Kobe, Japan. ⁸Center for Healthcare Information Technology (C-HIT), Tokai National Higher Education and Research System, Nagoya, Japan.

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