

Case Report

A case of isolated hypocalciuric hypercalcemia and type 2 diabetes mellitus followed by Grave's disease

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A 73-year-old woman was found to have diabetes mellitus along with elevated levels of calcium and alkaline phosphatase. She had also sometimes exhibited slight elevation of parathyroid hormone (97 pg/ml) while receiving treatment for hypertension and heart failure with atrial fibrillation, which had been detected by the Department of Cardiology at 71 years of age. She was admitted with elevated blood glucose (422 mg/dl) and hemoglobin A_{1c} (12%) levels, as well as for further assessment of hyperparathyroidism. Her diabetes was treated with intensive insulin therapy (insulin lispro at 24 U per day). Assessment of hypercalcemia revealed that urinary calcium excretion was extremely low (0 mg/day) and the fractional excretion of Ca (FE_{Ca}) was 0%. Familial hypocalciuric hypercalcemia was suspected. No abnormalities of the parathyroid gland were found by ultrasonography and ^{99m}Tc+Tl scintigraphy. The patient and her family members did not have any mutations in the extracellular domain or transmembrane domain of the calcium-sensing receptor, which has been reported to play an important role in Ca binding and intracellular signaling. However, the patient did have a missense mutation of codon 990 (R990G) in the intracellular domain, which has been reported to be a site of polymorphism, as well as a mutation of intron 6 (16 base pairs downstream from the exon-intron boundary: IVS6+16). One year later, she developed Grave's disease that presented with fatigue and weight loss.

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Introduction

Familial hypocalciuric hypercalcemia (FHH) is a benign disorder that is characterized by asymptomatic hypercalcemia, slight elevation or an almost normal concentration of parathyroid hormone (PTH), and hypocalciuria. It is transmitted as an autosomal dominant trait with a high penetrance (1). The clinical importance of this disorder is that it requires differential diagnosis from primary hyperparathyroidism

since patients may otherwise undergo unnecessary treatment such as parathyroidectomy.

FHH has been reported to be associated with a heterozygous mutation of the calcium-sensing receptor (CaSR), which is predominantly expressed in the parathyroid glands and kidneys (2). However, CaSR mutations have only been reported in one-half to two-thirds of FHH patients (3), and have also been reported in sporadic cases without a family history (4). In addition, a similar clinical picture to FHH can

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be found when autoantibodies to the CaSR develop in patients with some autoimmune diseases (5).

This report presents an isolated case of hypocalciuric hypercalcemia without CaSR mutation, which was associated with type 2 diabetes mellitus and the subsequent onset of Grave's disease.

Case Report

The clinical course of the patient is outlined in Figure 1.

71-year-old woman was admitted to the Department of Cardiology of our hospital with cardiac failure due to atrial fibrillation and hypertension. After admission, she was found to have elevation of alkaline phosphatase (ALP) to 761 IU/l and diabetes mellitus (hemoglobin A_{1c} [HbA_{1c}]: 7.5%). Her serum calcium level was also slightly elevated to 10.8 mg/dl. She was placed on a diet for her diabetes. It was also noted that she occasionally showed slight elevation of intact-PTH (192 pg/ml). Seven months later, she was admitted to our center because of elevation of HbA_{1c} and for further examination of parathyroid function. On admission, the patient's was 145.2 cm tall and weighed 47.9 kg, with a body mass index of 22.72 kg/m². Her blood pressure was 127/61 mmHg. Physical examination was normal. Her blood glucose was

442 mg/dl and HbA_{1c} was 12.0%. To improve glycemic control, she was treated with intensive insulin therapy (insulin lispro at 24 U daily).

Her ALP level increased to 650 IU/l with elevation of ALP3 isoenzyme. In addition, serum calcium was elevated to 10.5 mg/dl and intact-PTH (97pg/ml) was at the upper limit of the normal range. Serum magnesium and 1,5(OH)₂ vitamin D3 were in the normal range at 1.7 mg/dl and 46.5 pg/ml, respectively. Urinary calcium excretion was extremely low (0 mg/day) and fractional excretion of Ca (FE_{Ca}) was 0%. No calcium excretion was detected (0 mg/day) when urine was collected three times. Because FHH was suspected, her family members were also examined.

With regard to possible primary hyperparathyroidism, no abnormalities of the parathyroid gland were found by parathyroid ultrasonography or by 99mTc+Tl parathyroid scintigraphy.

The pedigree of the patient's family is shown in Figure 2A. Her mother was admitted to another hospital for treatment of a fracture. The mother's FE_{Ca} was low, but within the normal range (0.02%), and secondary hyperparathyroidism (intact PTH: 164 pg/ml) was present due to chronic renal failure (serum creatinine: 2.7 mg/dl). Her sister (II-4) also had an FE_{Ca} in the low normal range (1.2%), while the other family members showed normal urinary calcium excretion.

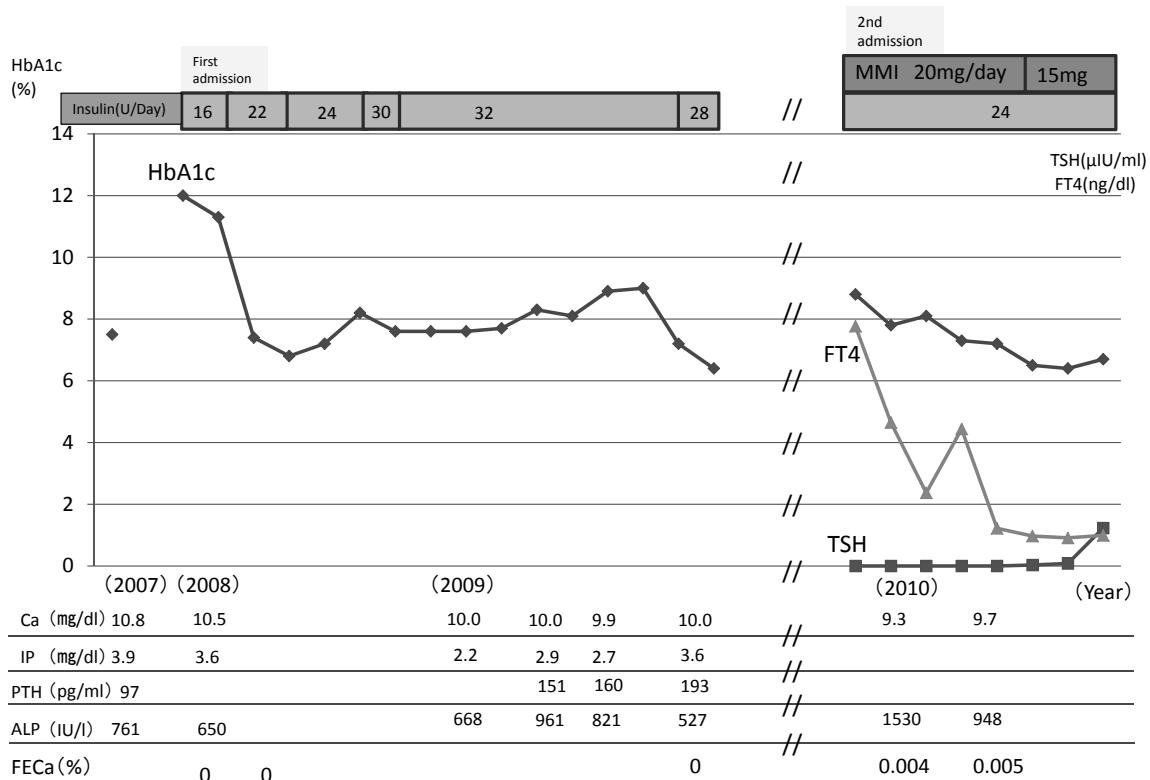


Figure 1.

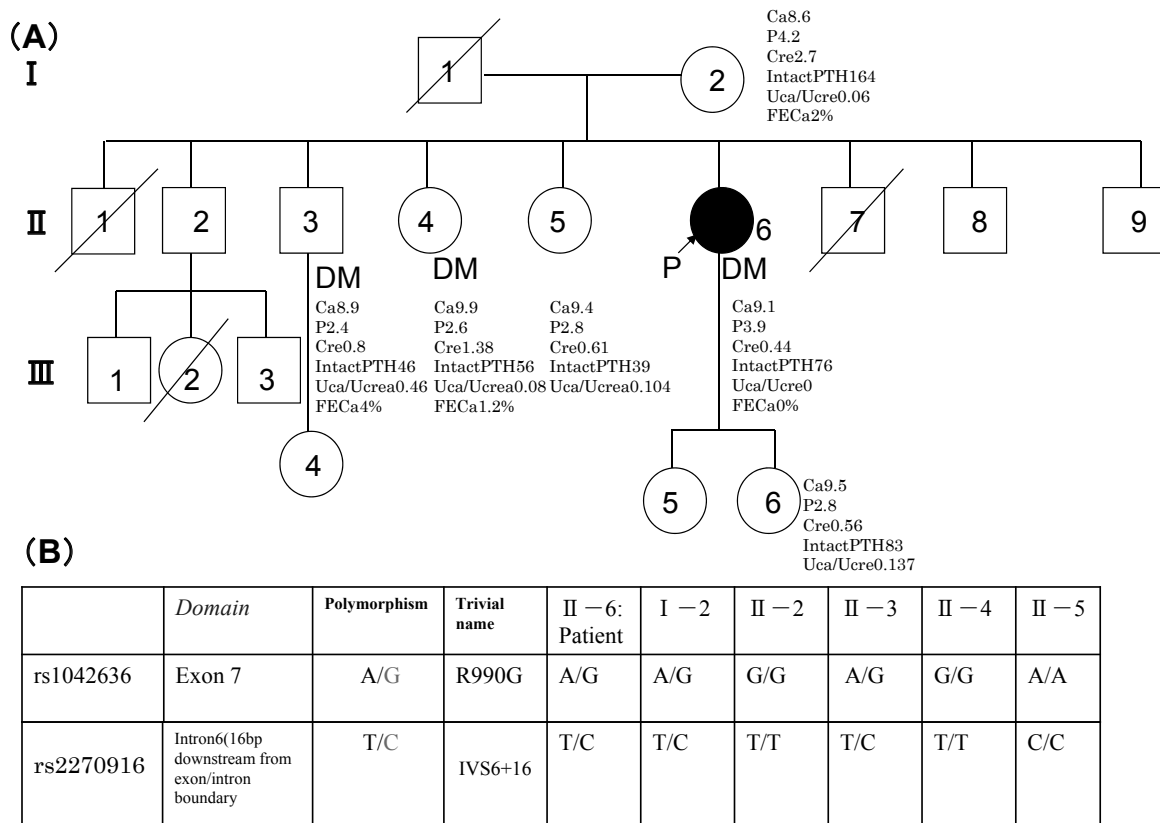


Figure 2.

Since FHH was not definitely ruled out in her mother and sister (II-4), genetic analysis of CaSR was performed in the patient and her relatives (I-2, II-2, II-3, II-4, II-5, and II-6) (Figure 2B).

In brief, genomic DNA was extracted from whole blood with a QIAmp DNA mini kit. The 11 primer sets used for PCR were designed to amplify exons 2-7 of the CaSR gene and about 100 bp of the flanking intronic regions as described previously (2). After PCR amplification, the products were subjected to electrophoresis on 1% agarose gels. Amplicons were sequenced using Big Dye terminator software version 1.3 (Figure 2B) as described previously (2).

Analysis of the CaSR gene revealed that the patient and her family members had no mutations in the extracellular domain or transmembrane domain of the calcium-sensing receptor (CaSR), which has been reported to have an important role in Ca binding or intracellular signaling. However, the patient had an A to G substitution at position two of codon 990, predicting a change from arginine to glycine (R990G) in the intracellular domain of CaSR (Figure 2B). Her mother (I-2) and her brother (II-3) were heterozygous (A/G) for this variation, while her sister (II-5) was homozygous (genotype A/A) (Figure 2B). The patient also had a T to C substitution at 16 bp downstream from the exon/intron

boundary of intron 6. The patient, her mother (I-2), and her brother (II-3) were all heterozygous (T/C) for this variation, while her sister (II-5) was homozygous (C/C).

After receiving intensive insulin therapy for 1.5 years, she stopped attending our hospital. She was re-admitted in December 2009 because of weight loss and fatigue with worsening glycemic control (HbA_{1c} of 9%). On examination, her thyroid gland was enlarged. Thyroid function tests revealed a hyperthyroid state, with the thyroxine (FT4) level being 4.22 ng/dl and the thyroid-stimulating hormone (TSH) level being less than 0.01 μ IU/ml. The TSH-receptor antibody titer was 77.2%. Ultrasonography showed an enlarged thyroid gland that was isoechogenic with multiple sites of orbicular calcification. Grave's disease was diagnosed. Her thyroid function was eventually normalized after treatment with thiamazole, but urinary calcium excretion remained low (FE_{Ca} 0.005%).

Discussion

The present patient initially showed occasional elevation of serum calcium and alkaline phosphatase, as well as slight elevation of PTH. Investigations revealed that urinary calci-

um excretion was undetectable despite repeated examination, hence FHH was suspected. The patient's mother and sister had urinary calcium excretion in the low normal range, and FHH could not be ruled out and genetic analysis of CaSR was therefore performed. Mutations of the extracellular and transmembrane domains of CaSR on chromosome 3q21 were not found. Two years after her first admission, this patient developed Grave's disease. The unique finding in this patient was extremely low urinary calcium excretion (0 mg/day on repeated examination) in spite of slight elevation of PTH, since urinary calcium excretion should be normal or slightly elevated in patients with hyperparathyroidism.

Clinically, FHH is characterized by inappropriately low calcium excretion in the presence of hypercalcemia along with elevation of PTH. The clinical importance of this disorder is that it requires differential diagnosis from primary hyperparathyroidism, and has been reported in patients who failed to respond to surgery such as parathyroidectomy. Unlike individuals with primary hyperparathyroidism and other hypercalcemic disorders, patient with FHH are generally asymptomatic and do not develop peptic ulcer, kidney stones, renal parenchymal damage, or skeletal complications (2).

The genetic abnormality causing FHH generally involves a loss-of-function mutation of CaSR on chromosome 3q21-q24, resulting in hypocalciuric hypercalcemia (6). In humans, CaSR is a 1078 amino acid cell-surface protein that is expressed in the parathyroid gland. It modulates renal tubular calcium reabsorption so that it is appropriate for the extracellular calcium concentration. Mutations involving the 3234-bp coding region of the CaSR gene have been found in only one-half to two-thirds of FHH patients (3).

Two other FHH loci that are not linked to chromosome 3q21 have been mapped to chromosome 19p13 (referred to as type 2 FHH) (7) and chromosome 19q13 (referred to as type 3 FHH) (8) by genetic linkage analysis, but the affected genes have not yet been identified. Thus, it appears likely that the present patient had another mutation involving the CaSR gene that has not yet been characterized (9).

Since urinary calcium excretion was at the low end of normal in the patient's mother and sister, FHH could not be ruled out, and analysis of CaSR was performed in both the

patient and her family members. However, none of them were found to have mutation of CaSR exons 2-7 encoding the extracellular and transmembrane domains, where mutations have previously been reported in FHH. Instead, the patient had a missense mutation in the intracellular domain (R990G) as well as a mutation of intron 6 (IVS6+16) (Figure 2B). In FHH, mutation at codon 990 has been reported to occur at a site of polymorphism that does not affect CaSR function (9,10). Such benign polymorphism of CaSR has also been reported at codons 536 and 926 by James et al. (10), as well as at codons 986 and 1101 by Hunter et al. (11). In addition, our patient had a T to C substitution at 16 bp downstream of the exon/intron boundary in intron 6, which has not been reported previously in FHH. It is unknown whether or not this mutation affected CaSR function, but the mutation of intron 6 was related to R990G polymorphism in our patient.

A syndrome of acquired hypocalciuric hypercalcemia, with features similar to those of FHH, has been reported in patients with autoimmune diseases such as Hashimoto's thyroiditis, celiac disease, and autoimmune hypophysitis (12). This syndrome is caused by antibodies directed against the extracellular domain of CaSR (5,12), and biochemical changes such as hypercalciuria and hypercalcemia are improved by glucocorticoid treatment (5). Our patient developed autoimmune manifestations at a later date when Grave's disease was diagnosed and was positive for antibodies to the TSH receptor. Glucocorticoid therapy was not performed and the CaSR antibody titer was not measured in our case, but the possibility of autoimmune hypocalciuric hypercalcemia cannot be ruled out.

A previous case of a sporadic hypocalciuric hypercalcemia has been reported (4), but that patient had no autoimmune manifestations.

In summary, we reported a sporadic case with the features of FHH, including hypocalciuric hypercalcemia, but without any mutation involving the extracellular or transmembrane domains of CaSR. In this patient, FHH manifestations were associated with type 2 diabetes and the subsequent onset of Grave's disease.

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