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Original Article

A Neonatal Case of Autosomal Dominant Hypoparathyroidism without Mutation of the *CASR* Gene

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Abstract. We experienced a case of familial hypoparathyroidism with an autosomal dominant pattern of transmission and performed molecular analysis of the calcium-sensing receptor (*CASR*) gene. The patient was a female neonate, born by cesarean section at term because of breech presentation. Her mother had been diagnosed with idiopathic hypoparathyroidism at the age of 9 yr and had been receiving vitamin D treatment since then. At birth, the patient's serum calcium concentration was 8.4 mg/dl, but it fell to 4.0 mg/dl on the fifth day after birth. Furthermore, her serum intact PTH level was inappropriately low, while hyperphosphatemia and hypomagnesemia were found. She was diagnosed with familial hypoparathyroidism, and was immediately started on oral administration of 1α(OH)D_3 (0.1 μg/kg/day) and continuous intravenous infusion of 8.5% calcium gluconate. Additionally, trichlormethiazide was administered because of elevated urinary calcium/creatinine (Ca/Cr) ratio. Her serum calcium concentration gradually improved thereafter. In this case, autosomal dominant hypocalcemia (ADH) due to abnormality in the *CASR* gene was clinically suspected, but DNA sequencing analysis revealed no mutation of the *CASR* gene in either the patient or her mother. This result suggests that the patient's hypoparathyroidism may have been caused by abnormality in a gene other than *CASR*.

Key words: hypocalcemia, autosomal dominant hypoparathyroidism, calcium-sensing receptor gene

Introduction

Idiopathic hypoparathyroidism is an unusual endocrine deficiency characterized by hypocalcemia with a decreased level of serum PTH. It consists of a heterogeneous group of sporadic and familial hypoparathyroidism. Familial hypoparathyroidism may occur as either isolated endocrinopathies with autosomal dominant, autosomal recessive, or X-linked recessive transmission, or as part of complex congenital anomalies such as the DiGeorge (MIM no.188400), Kenny-Caffey (MIM no.244460) and hypoparathyroidism-deafness-renal dysplasia (HDR) (MIM no.146255) syndromes (1–3). The mechanisms underlying at least some of the recessively inherited forms have been characterized as mutations in the *PTH* gene (4, 5). On the other hand, activating mutations of the calcium-sensing receptor (*CASR*) gene have

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been identified in some cases of autosomal dominant hypoparathyroidism or autosomal dominant hypocalcemia (ADH; MIM no.146200) (6–11). HDR syndrome, which is caused by mutations of the dual zinc finger transcription factor (*GATA3*) gene, is also an autosomal dominant disorder (3). Recently, we experienced a female neonatal case of familial hypoparathyroidism with an autosomal dominant pattern of transmission. Early diagnosis and treatment were required because the patient had severe hypocalcemia on the fifth day after birth. We report here the clinical course of our patient and describe the molecular analysis of the *CASR* gene in both the patient and her mother.

Case Report

The patient was a female neonate, delivered by cesarean section at 37 wk and 6 d of gestation because of breech presentation. Her birth weight was 3,006 g and her body length was 49.0 cm. Her Apgar scores were 8 at 1 min and 9 at 5 min. The patient's mother had been diagnosed with idiopathic hypoparathyroidism at the age of 9 yr, and had been receiving vitamin D treatment since then. Her serum calcium concentration was maintained between 7.2 and 8.5 mg/dl by oral administration of 6.0 μg/day of 1α(OH)D_3 and 4.0 mg/day of trichlormethiazide before and during pregnancy. Her serum phosphorus levels, serum magnesium levels, serum intact PTH levels, and urinary calcium/creatinine (Ca/Cr) ratio were 5.1–5.9 mg/dl, 1.6–1.8 mg/dl, 3–13 pg/ml and 0.104–0.305 mg/mg, respectively. She had no problems during pregnancy. Furthermore, there is no history of hearing impairment or renal disease on the mother's side. At birth, the patient's serum calcium concentration was 8.4 mg/dl, but it fell to 4.0 mg/dl on the fifth day after birth. She was then immediately transferred to our pediatric ward. She had no symptoms of hypocalcemia on admission. On physical examination, she had a body temperature of 37.1°C, a heart rate of 165 /min, and a respiratory rate of 50 /min. Small fistulas were found in front of both ears. There were no abnormal findings on the chest or abdomen.

Laboratory findings showed a low serum calcium level of 4.0 mg/dl (normal range: 8.6–10.2 mg/dl), an elevated serum phosphorus level of 9.6 mg/dl (normal range: 4.0–7.0 mg/dl), and a low serum magnesium level of 1.4 mg/dl (normal range: 1.8–2.6 mg/dl) (Table 1). Additionally, the patient's urinary Ca/Cr ratio was 0.3 mg/mg. On endocrinological investigation, serum concentrations of intact PTH and 1,25$(OH)_2$D were 21 pg/ml (normal range: 10–65 pg/ml) and 81.8 pg/ml (normal range: 20–70 pg/ml), respectively (Table 1). This intact PTH level was inappropriately low for hypocalcemia. The patient's thyroid function was normal. Brain computed tomography (CT) revealed no calcification. Based on these findings, the patient was diagnosed with familial hypoparathyroidism.

As shown in Fig. 1, oral administration of 1α(OH)D_3 (0.1 μg/kg/day) and continuous intravenous infusion of 8.5% calcium gluconate were immediately initiated after admission. Magnesium sulphate (0.5M, 0.5 ml/kg) was intravenously administered twice for hypomagnesemia. On day 9, 1α(OH)D_3 was increased to a dose of 0.2 μg/kg/day. Furthermore, trichlormethiazide (0.1 mg/kg/day) was orally administered because the patient's urinary Ca/Cr ratio was elevated to 0.7 mg/mg. The patient's serum calcium concentration improved thereafter and was normalized on day 25. Urinary Ca/Cr ratio also decreased.

The patient was discharged on day 31, and since then has been followed up at our outpatient clinic with oral medication of 1α(OH)D_3 (0.2 μg/kg/day) and trichlormethiazide (0.1 mg/kg/day). Abdominal CT and auditory brainstem responses (ABR) have revealed no abnormalities since discharge. Recent laboratory findings have shown a serum calcium level of 10.1 mg/dl, a serum phosphorus level of 5.9 mg/dl, a serum magnesium level of 1.8 mg/dl, a serum intact

Table 1 Laboratory findings on admission

(CBC)		(Biochemistry)		(Endocrinology)	
WBC	11200 /μl	GOT	35 IU/l	TSH	4.83 μIU/l
RBC	344×10^4 /μl	GPT	13 IU/l	FT_3	2.67 pg/ml
Hb	11.4 g/dl	LDH	605 IU/l	FT_4	1.40 ng/dl
Ht	33.4%	ALP	964 IU/l	intact PTH	21 pg/ml
PLT	43.6×10^4 /μl	T-Bil	10.3 mg/dl	$1,25(OH)_2D$	81.8 pg/ml
		CK	1625 IU/l	Osteocalcin	31 pg/ml
(BGA)	vein	TP	5.2 g/dl	Calcitonin	2.5 ng/ml
pH	7.358	Alb	3.6 g/dl		
pCO_2	47.0 mmHg	BUN	3.0 mg/dl	(Urinalysis)	
pO_2	33.3 mmHg	Cr	0.4 mg/dl	SG	1.028
HCO_3	25.8 mmol/l	Na	142 mmol/l	pH	5.5
BE	–0.1 mmol/l	K	4.7 mmol/l	Protein	(2+)
		Cl	103 mmol/l	Sugar	(–)
		Ca	4.0 mg/dl	Ketone	(–)
		IP	9.6 mg/dl	Occult	(±)
		Mg	1.4 mg/dl	RBC	1-9 /HPF
		CRP	< 0.04 mg/dl	WBC	5-9 /HPF
				Ca/Cr	0.3 mg/mg

PTH level of 5 pg/ml and a urinary Ca/Cr ratio of 0.19 mg/mg. At present (age of 5 yr), the patient's growth and psychomotor development are normal.

Genetic Analysis

In our patient, ADH due to an activating mutation in the *CASR* gene was suspected because of autosomal dominant hypoparathyroidism with hypomagnesemia and relative hypercalciuria. On the other hand, HDR syndrome is unlikely since the patient has neither hearing impairment nor kidney anomaly. We therefore examined the *CASR* gene of both the patient and her mother. This study was approved by the local ethics committee of Jikei University School of Medicine. First, genomic DNA was extracted from the patient's and her mother's white blood cells by standard procedures after informed consent was obtained from the patient's parents. Polymerase chain reaction (PCR) was then carried out using specific primers to amplify exons 2–7 of the *CASR* gene, encompassing the entire coding sequence (Table 2). Sequencing analysis was performed by the dideoxy method using a Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). In the present study, the sequences of all six exons of the *CASR* gene were investigated, but no mutation was found in either the patient or her mother.

Discussion

Familial dominant hypoparathyroidism is an uncommon condition that can come to clinical attention at any point in life. In 1994, Pollak *et al.* first reported that an activating mutation in the *CASR* gene can be a cause of ADH (6). The CASR is a G protein-coupled receptor that plays key roles in mineral ion, fluid and electrolyte homeostasis (12). The binding of calcium ion to CASR on parathyroid cells decreases PTH secretion; thus, CASR determines the set-point of PTH secretion by acting as a calcium sensor. Patients with activating mutations in the *CASR* gene exhibit hypocalcemia, hyperphosphatemia, hypomagnesemia, and abnormally low PTH

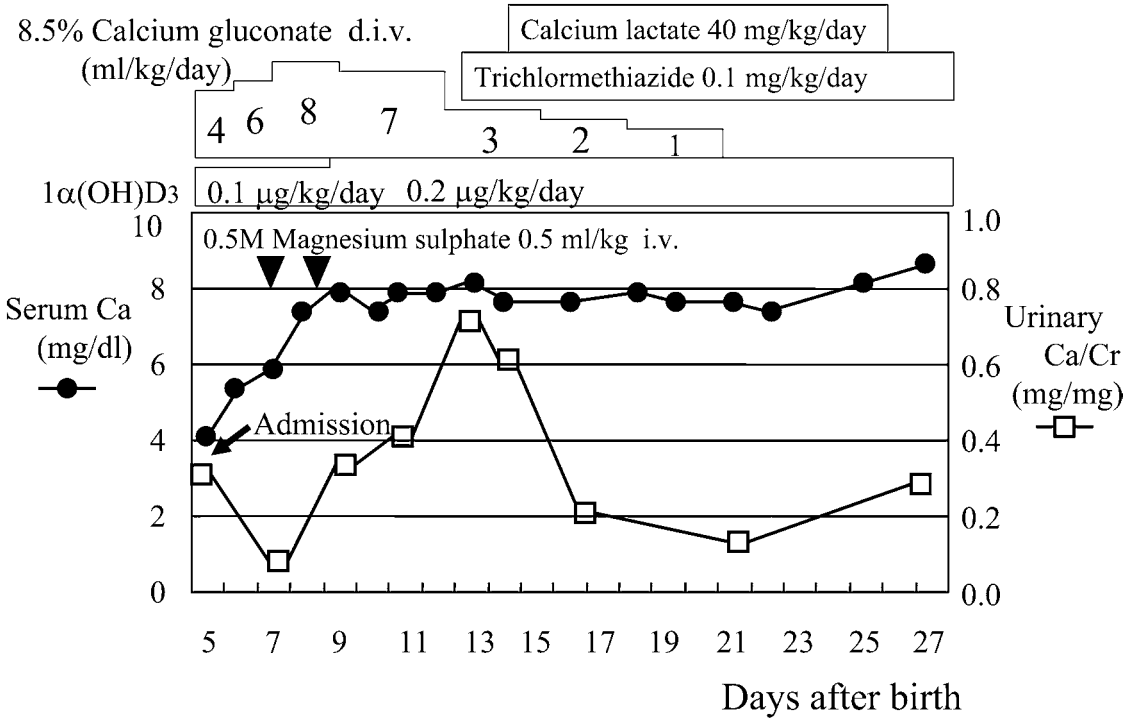

Fig. 1 Clinical course of the present case after admission to the pediatric ward.

Table 2 Primers designed for the amplification and sequencing of the DNA fragments of the *CASR* gene

Exons	Directions	Sequences (5' to 3')
2	Forward	TCCCTTGCCCTGGAGAGACGGCAGA
	Reverse	AGAGAAGAGATTGGCAGATTAGGCC
3	Forward	AGCTTCCCATTTTCTTCCACTTCTT
	Reverse	CCCGTCTGAGAAGGCTTGAGTACCT
4A	Forward	ACTCATTCACCATGTTCTTGGTTCT
	Reverse	GAATTCCCGGAAGCCTGGGATCTGC
4B	Forward	GCCATGCCTCAGTACTTCCACCTGG
	Reverse	CCCAACTCTGCTTTATTATACAGCA
5	Forward	GGCTTGTACTCATTCTTTGCTCCTC
	Reverse	GACATCTGGTTTTCTGATGGACAGC
6	Forward	CAAGGACCTCTGGACCTCCCTTTGC
	Reverse	GACCAAGCCCTGCACAGTGCCCAAG
7A	Forward	AAGTGCCCAGATGACTTCTGGTCCA
	Reverse	GGTAGGAGAGCTCTCGGTTGGTGGC
7B	Forward	TTCCGCAACACACCCATTGTCAAGG
	Reverse	GGATCCCGTGGAGCCTCCAAGGCTG
7C	Forward	GCAACGTCTCCGAAGCGGTCCAGCA
	Reverse	CCATGGCGTTCTTCTGAGGCTCATC
7D	Forward	CAGAAGGTCATCTTTGGCAGCGGCA
	Reverse	TCTTCCTCAGAGGAAAGGAGTCTGG

Internal primers in exons 4 and 7 were designed for sequencing.

levels (6, 8–10, 13–15). However, the clinical presentation is variable, ranging from asymptomatic forms to severe neonatal seizures (11).

Our patient presented severe hypocalcemia during the neonatal period. Hyperphosphatemia, hypomagnesemia, and a serum intact PTH level too low to maintain normocalcemia were also found. The urinary Ca/Cr ratio was gradually elevated by oral administration of $1\alpha(OH)D_3$. It is reported that vitamin D therapy in patients with activating *CASR* mutations often results in disproportionate hypercalciuria (7, 13, 16), and thiazide diuretics are therefore administered to reduce urinary Ca excretion at any level of serum Ca (17). Actually, in this case subsequent therapy with $1\alpha(OH)D_3$ and trichlormethiazide was effective for hypocalcemia and hypercalciuria. Moreover, a recent report suggested that screening of the *CASR* mutation should be considered in hypoparathyroidism patients with relative hypercalciuria or with a family history of hypocalcemia (18). In view of these findings, ADH due to the activating mutation in the *CASR* gene was clinically suspected for our patient and her mother, but molecular analysis found no mutation in either individual. This result leads us to speculate that the patient's hypoparathyroidism may have been caused by an abnormality in a gene other than *CASR*. However, the *GATA3* mutation causing HDR syndrome is unlikely in our case because of isolated hypoparathyroidism without hearing impairment or kidney anomaly. To clarify the molecular basis of familial hypoparathyroidism such as we report here, further investigation is required.

In summary, we have described a neonatal case of autosomal dominant hypoparathyroidism without mutation of the *CASR* gene. Regular measurement of serum calcium, phosphorus, PTH concentrations and urinary Ca/Cr ratio after birth is necessary for neonatal patients who have a parent with idiopathic hypoparathyroidism.

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