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Genetic and Clinical Characteristics of Korean Patients with Isolated Hypoparathyroidism: From the Korean Hypopara Registry Study

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Isolated hypoparathyroidism (IH) shows heterogeneous phenotypes and can be caused by defects in a variety of genes. The goal of our study was to determine the clinical features and to analyze gene mutations in a large cohort of Korean patients with sporadic or familial IH. We recruited 23 patients. They showed a broad range of onset age and various values of biochemical data. Whole exome sequencing was performed on two affected cases and one unaffected individual in a family. All coding exons and exon-intron borders of *GCMB*, *CASR*, and *prepro-PTH* were sequenced using PCR-amplified DNA. In one family who underwent the whole exome sequencing analysis, approximately 300 single nucleotide changes emerged as candidates for genetic alteration. Among them, we identified a functional mutation in exon 2 of *GCMB* (C106R) in two affected cases. Besides, heterozygous gain-of-function mutations in the *CASR* gene were found in other subjects; D410E and P221L. We also found one single nucleotide polymorphism (SNP) in the *prepro-PTH* gene, five SNPs in the *CASR* gene, and four SNPs in the *GCMB* gene. The current study represents a variety of biochemical phenotypes in IH patients with the molecular genetic diagnosis of IH.

Key Words: CASR; GCMB; Hypocalcemia; Hypoparathyroidism; Prepro-PTH

INTRODUCTION

Hypoparathyroidism, a disorder of parathyroid hormone (PTH) absence or deficiency, causes hypocalcemia and hyperphosphatemia (1, 2). Postoperative hypoparathyroidism is the most common etiology of hypoparathyroidism (3). Other causes are autoimmune destruction of the parathyroid glands and congenital disorder, such as DiGeorge syndrome (4, 5).

Isolated hypoparathyroidism (IH) is defined as hypoparathyroidism related to an isolated endocrinopathy. IH is a rare disease and its molecular and clinical properties are known to be heterogeneous. In order to overcome the rarity and complexity of IH, several studies have been conducted as a multi-center survey (6, 7). Clinically, patients with IH have shown a broad spectrum of features, from severe symptomatic hypocalcemia in early infancy to asymptomatic, incidental finding in adulthood. Biochemically, IH presents with a wide range of serum calcium and PTH levels. Molecular genetic studies have reported on identification of several genetic mutations, including the *prepro-PTH*, calcium-sensing receptor (*CASR*), and glial cell missing B (*GCMB*) in patients with hypoparathyroidism, however, in most idiopathic cases of hypoparathyroidism, the genetic defect remains unknown (8-11).

Exome sequencing is useful for analysis of selective coding mutations of the human genome for discovery of novel genes for either rare or common disorders. Recently, use of exome sequencing has led to successful discovery of multiple causative gene mutations of genetic diseases (12).

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In this study, we recruited 23 patients with hypocalcemia and low serum levels of PTH from eight adult endocrine units in Korea (the Korean Hypopara Registry Study). We present mutational analysis, which can be attributed to the genetic basis of hypoparathyroidism by adopting a whole exome sequencing approach in Korean patients with IH and demonstrate the phenotypic features.

MATERIALS AND METHODS

Subjects

Since 2010, 23 subjects have been included in the Korean Hypopara Registry Study. The inclusion criteria were the presence of hypocalcemia, an inappropriately normal or low level of serum intact PTH, and normal renal function. Patients with postsurgical hypoparathyroidism or other endocrine diseases causing PTH defects were excluded. Blood samples were collected from subjects.

Serum levels of calcium, phosphate, creatinine, and intact PTH were evaluated in all subjects. Ionized calcium, albumin, magnesium, vitamin D metabolites (25-hydroxyvitamin D [25(OH)D], or 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]) and renal calcium excretion per day were determined in selected subjects.

Whole exome sequencing

Whole exome sequencing was performed using genomic DNA of the affected cases (patients No. 1 and No. 2 in Table 1) and the younger brother of patient No.1 as a negative control at the Theragen BiO Institute (Suwon, Korea). The qualified genomic DNA sample was randomly fragmented using Covaris and the size of the library fragments was mainly distributed between 150 to 200 bp. Adapters were then ligated to both ends of the resulting fragments. The adapter-ligated templates were purified using Agencourt AMPure SPRI beads and fragments with an insert size of approximately 250 bp were excised. Extracted DNA was amplified using ligation-mediated PCR (LM-PCR), purified, and hybridized to the SureSelect Biotinylated RNA Library (BAITS) for enrichment. Hybridized fragments were bound to strepavidin beads, whereas non-hybridized fragments were washed out after 24 hr. Captured LM-PCR products were subjected to an Agilent 2100 Bioanalyzer for estimation of the magnitude of enrichment. Each captured library was then loaded onto a Hiseq2000 platform, and high-throughput sequencing was performed for each captured library in order to ensure that each sample met the desired average sequencing depth. Raw image files were processed by HCS1.4.8 for base-calling with default parameters and the sequences of each individual were generated as 90 bp pair-end reads.

Table 1. Clinical and laboratory features of 23 patients with isolated hypoparathyroidism (IH) in the Korean Hypopara Registry Study

Ref. range Pt. No.	Sex	Age (yr)	Onset age (yr)	Alb 3.3-5.3 (g/dL)	Ca 8.2-10.8 (mg/dL)	iCa 1.12-1.3 (mM/L)	P 2.5-4.5 (mg/dL)	Mg 1.76-2.8 (mg/dL)	Cr 0.5-1.4 (mg/dL)*	iPTH 15-65 (pg/mL)	1,25$(OH)_2$D 25-66 (pg/mL)	25(OH)D 10-70 (ng/mL)	24 hr U Ca 100-250 (mg/d)
1	F	44	0	4.4	7.6	1.8	3.4	1.8	0.9	< 1.0	55.9	31.1	-
2	M	17	0	4.1	5.1	1.4	7.3	2.0	0.7	< 1.0	88.8	-	-
3	M	38	35	4.1	5.9	1.2	6.3	1.9	0.8	0.84	-	-	-
4	M	55	53	4.1	4.9	1.2	6.3	1.6	1.1	1.00	14.2	18.1	12.7
5	F	68	52	4.1	5.1	2.3	5.4	1.5	0.9	< 7.8	-	25.9	-
6	F	24	19	4.7	7.4	3.9	6.5	1.6	0.8	< 8.0	16.7	14.1	-
7	M	35	0	4.3	4.4	2.0	4.8	2.0	1.1	-	-	33.6	200.6
8	F	57	51	4.3	7.7	0.9	5.7	-	0.8	9.1	-	42.1	441
9	F	33	28	4.3	5.5	2.8	6.1	1.7	0.7	9.36	25.6	12.4	15
10	F	41	23	4.1	6.6	-	4.9	-	0.5	1.08	-	-	-
11	F	34	32	4.4	5.7	0.8	8.1	1.7	0.7	11.1	40.9	10.5	168
12	F	42	25	4.5	5.1	-	5.8	1.4	0.7	< 7.8	-	47.2	-
13	M	33	18	4.7	5.3	2.4	5.5	1.3	0.9	< 7.8	-	15	-
14	F	68	66	4.5	7.8	3.8	3.2	1.2	0.9	24.0	46.0	4	232.8
15	F	28	14	-	7.3	0.9	5.7	2.0	-	4.9	13.2	20.2	126
16	M	32	20	-	7.5	1.0	6.1	1.9	-	6.2	-	-	204
17	M	58	54	-	6.4	0.8	4.6	2.1	-	13.5	-	-	-
18	M	54	36	4.2	5.2	2.2	6.4	1.3	1.0	< 7.5	-	22.1	-
19	M	68	64	4.3	5.2	0.6	3.9	-	0.9	3.7	65.5	14.3	25.2
20	F	69	67	4.1	6.6	1.5	4.0	1.5	0.9	7.4	18.0	-	142
21	M	42	37	5.0	4.8	1.1	8.5	1.9	1.2	1.4	49.4	17.8	-
22	M	61	60	4.7	6.4	0.8	7.3	1.4	0.8	3.5	-	-	-
23	M	50	41	4.3	5.8	0.6	4.9	1.6	0.9	< 2.5	27.99	17.10	21.6

Familial type 1, patient No.1 and patient No.2; Familial type 2, patient No.8; Familial type 3, patient No.15, patient No.16, and patient No.17. *Serum creatinine reference range is given for adults. Alb, albumin; Ca, calcium; iCa, ionized calcium; P, phosphate; Mg, magnesium; Cr, creatinine; iPTH, intact parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D.

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Raw data filtering

Prior to performance of data analysis, removal of dirty raw reads, which only harbor the sequence of the adapter, is essential. The raw data filtering steps were; 1) removal of reads with the adapter, 2) removal of low quality reads (the percentage of low quality bases of quality value ≤ 5 is more than the read). We confirmed a dirty reads rate of 6.6% and a clean reads rate of 93.4%. The mean read depth for the exome sequence was 49x, with 98% of the exome covering clean reads.

Bioinformatics analysis

To improve raw alignment BAMs for single nucleotide variation (SNV) and InDel calling, we performed depth 5-200 × and HARD_TO_VALIDATE: MQ0 ≥ 4 & (MQ0/[1.0*DP]) > 0.1) with StandBiasFilter: SB ≥ -1.0 of condition using GATK. In the next step, patient sample variants were filtered against variants of the control sample and dbSNP data with variants for the InHouse database.

Genetic analysis

Genomic DNA was extracted from peripheral blood from all patients using a standard method. All coding exons and intron-exon junctions for the *prepro-PTH* (NM_000315.2, NP_000306.1), *CASR* (NM_000388.3, NP_000379.2), and *GCMB* (NM_004752.3, NP_004743.1) genes were amplified and sequenced. PCR was performed using 5 mM $MgCl_2$, 200 μM deoxyribonucleotides, 0.5 μM of each primer, 1 unit of *Taq* polymerase, and 100 ng of genomic DNA as a template. The sequences of primers are available upon request. The PCR products were electrophoresed through polyacrylamide gels and read in both directions using an ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Ethics statement

The study was approved by the institutional review board (IRB) of Gachon University Gil Medical Center, Korea (IRB No. GIRBA2151). Informed consent was obtained from all subjects before participation.

RESULTS

Clinical and biochemical features

We describe baseline clinical and biochemical characteristics of 23 patients (12 males and 11 females) with IH; six subjects of familial type from three families and 17 sporadic cases (Table 1). Three families with IH showed an association with the *GCMB* mutation (C106R) and *CASR* mutation (D410E and P221L), respectively. The mean age at onset was 34.6 ± 21.2 yr (range of onset age, 0-67 yr), and three patients had hypocalcemia in early infancy. All patients had a mean calcium level of 6.1 ± 1.1 mg/dL (range 4.4-7.8 mg/dL; Table 1), with a mean phosphate concentration of 5.7 ± 1.4 mg/dL (range 3.2-8.5 mg/dL). For the 22 patients with available data, serum levels of intact PTH were below 8.0 pg/mL in 17 cases or inappropriately normal despite the presence of hypocalcemia (n = 5). Vitamin D metabolites were available for 18 patients; mean $1,25(OH)_2D$ level, 38.5 ± 23.6 pg/mL (n = 12) and mean 25(OH)D level, 21.6 ± 11.7 ng/mL (n = 16), respectively.

Whole exome sequencing

We focused on a family in which a proband and her affected offspring were diagnosed with autosomal dominant hypoparathyroidism (patients No. 1 and No. 2 in Table 1), and performed whole exome sequencing on these two individuals and the proband's sibling, an unaffected individual. A summary of the filtration strategy and numbers of resulting variants is shown in Fig. 1. We obtained 216,260 variants from patient No. 1 and 409,709 variants from patient No. 2, respectively (Fig. 1). We selected the intersection of two sets (patient No. 1 and patient No. 2) and obtained 33,883 variants. Then, we filtered out 27,811 overlapping variants identified from the unaffected individual. The remaining 6,072 variants were then filtered against dbSNP data and the InHouse database, removing all previously reported variants. After filtering, a total of 294 variants (181 single nucleotide variations and 73 insertions with 40 deletions) were identified as being shared by the two affected subjects. All genetic data on the variants are available upon request. Of the 294 variants, we selected 22 candidate genes that reside in coding regions and have lower BLOSUM62 (BLOcks of Amino Acid SUbstitution Matrix) score (http://icb.med.cornell.edu/education/courses/introtobio/BLOSUM62), higher PolyPhen2 score,

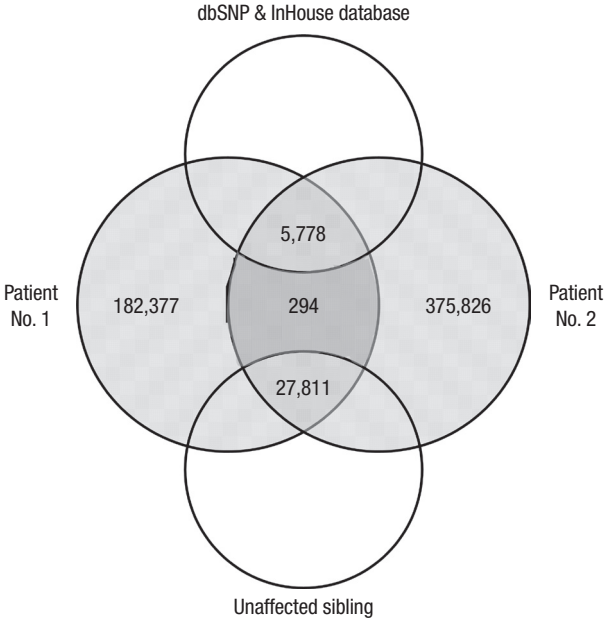

Fig. 1. Filters used in analysis of the exome data and numbers of candidate variants.

Table 2. Summary of single nucleotide variation (SNV) found from exome sequencing in patient No. 1 and No. 2

Ch'me	Genes	SNV		hom/het	B	*P*	S	Primates	Placental	Vertebrate
		Nucleotide	Amino acid							
chr1	*DISC1*	c.2228A > G	K743R	hetero	2		-		-	-
chr1	*PTCH2*	c.3088G > A	V1030I	hetero	3	0.999	0.02	0.538	0.659	1
chr4	*CCDC96*	c.1580C > T	S527F	hetero	-2	0.865	0.01	0.834	0.335	0.165
chr5	*NHP2*	c.153C > G	I51M	hetero	1	0.87	0	0.994	1	1
chr5	*PDZD2*	c.2146C > T	R716W	hetero	-3	0.996	0	0.025	0.967	0.952
chr6	*GCM2*	c.316T > C	C106R	hetero	-3	0.999	0	0.865	1	1
chr6	*CD83*	c.397C > T	R133C	hetero	-3	0.44	0.04	0.003	0	0.001
chr6	*HLA-DRB1*	c.71C > A	S24Y	hetero	-2	0.117	0	0.032	0.085	0.003
chr7	*FLNC*	c.2839G > C	G947R	hetero	-2	1	0	0.365	0.997	1
chr8	*ST3GAL1*	c.650T > G	V217G	hetero	-3	0.309	0	0.996	1	1
chr9	*COL27A1*	c.5237G > A	R1746Q	hetero	1	0	0.03	0.999	1	1
chr11	*FBXO3*	c.926G > T	R309L	hetero	-2	0.982	0.02	0.925	1	1
chr11	*OR9G1*	c.522T > G	D174E	hetero	2	0.808	0.05	0.403	0.991	0.914
chr11	*OR6Q1*	c.713G > A	R238H	hetero	0	0.998	0	0.951	0.777	0.438
chr11	*MTL5*	c.1138A > G	N380D	hetero	1	0.922	0	0.202	0.878	1
chr12	*OR10A7*	c.512C > A	P171Q	hetero	-1	0.059	0.01	0.546	0.051	0
chr14	*DHRS4L1*	c.591G > T	K197N	hetero	0	-	-	0.074	1	1
chr16	*BFAR*	c.508G > A	E170K	hetero	1	0.11	0	0.92	0.842	0.996
chr20	*CDH26*	c.191G > T	R64I	hetero	-3	0.558	0	0.009	0.096	0.128
chr20	*LAMA5*	c.5386C > T	R1796C	hetero	-3	0.998	0	0.878	1	1
chr20	*SRMS*	c.508C > T	R170W	hetero	-3	0.998	0	0.251	0.588	0.929
chrX	*BCORL1*	c.1735C > T	H579Y	hetero	2	0.337	0.02	0.13	0.488	0.989

The PolyPhen2 score ranges from 0 to 1. A high score indicates a variant that is more likely to be damaging (benign, possibly damaging, or probably damaging). SIFT score ranges from 0 to 1. The amino acid substitution is predicted to be damaging if is the score is ≤ 0.05, and tolerated if the score is > 0.05. Primates, Placental, Vertebrate, conservative ratio among primates, placental mammals, and vertebrate species, respectively. Ch'me, chromosome; SNV, single nucleotide variation; hom/het, homozygous/heterozygous; B, Blosum62; P, PolyPhen2 score; S, SIFT score.

Table 3. Summary of indels found from exome sequencing in patient No. 1 and No. 2

Ch'me	Type	Start	End	Sequences	Genes	Hom/Het	Frameshift	Primates	Placental	Vertebrate
chr3	INS	73111482	73111482	A	*EBLN2*	homo	frameshift	0.005	0.005	0.006
chr9	DEL	35813448	35813451	TCT	*HINT2*	hetero	inframe	0.99	1	0.993
chr19	INS	9236699	9236699	ATGGT	*OR7G3*	hetero	frameshift	0.004	0.001	0
chr21	INS	40883672	40883672	AGA	*SH3BGR*	homo	inframe	0.015	0.002	0.002

Primates, Placental, Vertebrate, conservative ratio among primates, placental mammals, and vertebrate species, respectively. Ch'me, chromosome; INS, insertion; DEL, deletion; hom/het, homozygous/heterozygous.

and lower SIFT score, which might be related to significant amino acid changes and therefore may disease-causing (Table 2). Tables 2 and 3 show final candidate variants related to hypoparathyroidism in patients with familial IH. Among the 22 variants, we noted the C106R mutation in the *GCMB* gene and confirmed this mutation by direct target gene-sequencing. Functional studies confirmed that *GCMB* C106R mutant as a loss-of-function mutation could explain hypoparathyroidism in the previous study (11).

Mutational analysis

Direct sequencing analysis detected a total of 12 different genetic defects. They are five variants in the *GCMB* gene, including one functional mutation, six variants in the *CASR* gene, including two activating mutations and one variant in the *prepro-PTH* gene. In addition to the C106R mutation of the *GCMB* gene, four single nucleotide polymorphisms (SNPs) were found in the *GCMB* gene (c.-44T > C [rs16870746], c.91-242A > G [rs9379881], c.343+163G > A [rs9393726], and c.583-72A > T [rs2076257]) in our cohort (Table 4).

We also found an activating mutation of the *CASR* gene (c.1230T > A [D410E]) (patient No. 8 in Table 4), which we reported as a novel mutation in the previous study (13). Another mutation of the *CASR* gene (c.662C > T [P221L]) was detected in one family (patient No. 15, patient No. 16, and patient No. 17 in Table 4); this mutation was previously known as a functional mutation (14). In addition, five SNPs in the *CASR* gene (c.2205G > A, c.2824G > A [rs76327999], c.2956G > T [rs1801725], c.2968A > G [rs1042636], and c.3031G > C [rs1801726]) were identified from six subjects. Finally, one SNP in the genes encoding *prepro-PTH* (c.247C > A [rs6256]) was identified in two patients (Table 4).

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Table 4. Results of genetic analysis of 23 patients with IH

Patient No.	*Prepro-PTH*	*CASR*	*GCMB*
1	(-)	(-)	**c.316T > C → C106R**
2	(-)	(-)	**c.316T > C → C106R**
3	(-)	(-)	c.583-72A > T
4	c.247C > A → R83R	(-)	c.343+163G > A c.91-242A > G c.583-72A > T
5	(-)	(-)	c.343+163G > A
6	(-)	(-)	(-)
7	(-)	(-)	(-)
8	(-)	**c.1230T > A → D410E** c.2968A > G → R990G	(-)
9	(-)	c.2968A > G → R990G	c.91-242A > G c.583-72A > T
10	(-)	c.2968A > G → R990G	c.91-242A > G c.583-72A > T
11	(-)	(-)	c.91-242A > G c.583-72A > T
12	(-)	(-)	c.91-242A > G c.343+163G > A
13	c.247C > A → R83R	c.2956G > T → A986S	c.91-242A > G c.583-72A > T c.-44T > C
14	(-)	(-)	c.91-242A > G c.583-72A > T
15	(-)	**c.662C > T → P221L**	(-)
16	(-)	**c.662C > T → P221L**	(-)
17	(-)	**c.662C > T → P221L**	(-)
18	(-)	(-)	(-)
19	(-)	c.2968A > G → R990G c.2824G > A → E942K	(-)
20	(-)	c.2205G > A → Q735Q	(-)
21	(-)	(-)	(-)
22	(-)	(-)	(-)
23	(-)	c.3031G > C → Q1011E	(-)

Bold, mutations; roman, SNP.

DISCUSSION

Here we report on a nation-wide study representing genetic and clinical assessment in 23 patients with IH. IH is a heterogeneous group of disorders with various genetic mutations; mainly *PTH* and *CASR* genes affecting PTH secretion, and *GCMB* genes related to parathyroid embryogenesis (15). In our series, IH results from three different mutations which have been described in the literature (P221L in the *CASR* gene) (14), including two by our group (C106R in the *GCMB* gene and D410E in the *CASR* gene) (11, 13). We showed that IH patients possessed several SNPs in the *PTH, CASR*, or *GCMB* genes regardless of accompanying functional mutations.

In our search for candidate genes in patients with IH, we made combined use of genomic tools, such as exome sequencing and direct target gene-sequencing. Whole exome sequencing is a new and powerful tool for elucidation of the genetic basis of unsolved Mendelian diseases and could be considered for use as a diagnostic method in congenital diseases (12). However, identification of disease-related variants among a large background of non-pathogenic polymorphism is always challenging (16). In this study, whole exome sequencing revealed that heterozygous c.316T > C (C106R) in the *GCMB* gene was one of the causal variants responsible for IH. However, other candidate genes identified by whole exome sequencing suggested that each gene might provide insights regarding contributions to hypoparathyroidism. For example, the frequency of HLA-DRB1*01 and DRB1*09 alleles was reported to be higher among patients with idiopathic hypoparathyroidism and the *HLA-DRB1* gene showed an association with CASR autoantibody positivity (17). Therefore, testing for *HLA-DRB1* mutation might be helpful in the effort to obtain additional information on an autoimmune component of disease among patients with IH in our cohort.

Using a direct sequencing approach, we found four SNPs in the *GCMB* gene from nine cases. Although mutations in *GCMB* genes are known to have a role in pathogenesis of hypoparathyroidism, considerable allelic variability for the *GCMB* gene has been detected in healthy individuals (15); and noncritical single-nucleotide change or non-functional heterozygous mutation in the *GCMB* gene is easily identified in IH patients (15, 18).

Direct sequencing analysis of the *CASR* gene in our IH cohort revealed two activating mutations, the D410E mutation and the P221L mutation. In addition, we found four SNPs in the *CASR* gene. Among these *CASR* variants, two SNPs (R990G and A986S) were reported as relatively common exonic variants (19). In our study, the R990G allele was detected in four patients (17.4%) and A986S was detected in only one patient. Some studies have reported an association of the A986S polymorphism with serum calcium levels in healthy adults, however, other studies did not report this association (20-23). Genome-wide association scans in European and Indian Asian cohorts indicated an association of A986S with higher serum calcium levels (24). One hypocalcemic patient in our cohort carried A986S as a non-significant polymorphism with allelic alterations in the *GCMB* and *prepro-PTH* genes.

We gathered clinical data including biochemical values and levels of vitamin D metabolites. The hypoparathyroidism patients in this study showed similar sex ratio and a broad spectrum of onset age. Most patients in this study showed relatively normal or decreased urine calcium levels. Urinary excretion of calcium is decreased in idiopathic hypoparathyroidism and elevated in patients with gain-of-function mutation of *CASR* (25). One patient with *CASR* D410E mutation was hypercalciuric with urinary calcium over 300 mg and other two patients carrying *CASR* P221L mutation showed normal urine calcium levels. Serum calcium level or vitamin D status was not associated with urinary calcium excretion.

Measurement of 25(OH)D levels is essential to rule out vitamin D deficiency as a cause of hypocalcemia. In classic vitamin

D deficiency, PTH hypersecretion compensates for the tendency for the blood calcium to fall (1). Vitamin D insufficiency, serum 25(OH)D level < 20 ng/mL, is reported to be relatively common in Korea (26). Vitamin D insufficiency was found in nine patients (56% of measured) in our subjects with low PTH levels. $1,25(OH)_2D$ is regulated by several factors including PTH, calcium and 25(OH)D levels. Serum $1,25(OH)_2D$ is usually reduced in hypoparathyroidism because PTH stimulates the renal 1α-hydroxylase (27). However, synthesis of $1,25(OH)_2D$ can be determined by 25(OH)D concentration in the absence of PTH (27). The level of $1,25(OH)_2D$ in our study varied, probably depending on PTH levels or substrate concentration.

Until now, several studies have attempted to identify the genetic background, prevalence, clinical characteristics and the disease course of IH by conducting a multi-center survey or via a nation-wide registry database (6, 7, 16, 18, 28-30). A German survey recruiting autosomal dominant hypocalcemia patients focused on identification of the *CASR* gene mutations and clinical phenotypes (7). The Italian Register of Primary Hypoparathyroidism analyzed biochemical data in relation to the Gsα encoding gene, *GNAS1* polymorphism (6). The epidemiologic study reported the prevalence of IH was 7.2 per million population in Japan (29). Our study showed the variation of phenotypic expression of IH, which was similar to previous studies. However, we could not identify the prevalence of IH or Korean-specific genetic mutations due to relatively small numbers of cases.

Our study has several limitations. In general, sampling strategies for exome sequencing involve a family-based approach which means sampling from multiple, affected individuals within a pedigree or parent-child trios and an extreme phenotypic approach which means that individuals at both ends of a phenotype distribution are selected for sequencing (16). We performed exome sequencing with relatively small sample numbers. Therefore, further validation of SNP detected from our study should be performed in 23 cases with sufficient additional controls. We only collected baseline clinical data of cohort subjects. Follow-up consequences of IH and details for treatment are needed in order to provide information on the long-term course of disease. Although our study did not report a new cause for PTH-deficient hypoparathyroidsm, genetic assessment showed that patients with IH carried many nonfunctional variants in the *GCMB*, *CASR* or *PTH* genes.

The presence of CASR autoantibodies or anti-parathyroid gland antibodies is known to be involved in the pathogenesis of IH (10, 31). However, the causal unique markers of autoimmune hypoparathyroidism are not yet established; some anti-parathyroid antibodies have the low specificity for parathyroid tissue or anti-CASR antibody positivity varied because of differences in technique (10). Therefore, we focused on the validation of genetic basis in this IH cohort. Further study to investigate the presence of autoantibodies related to hypoparathyroidism might be helpful to identify causes of IH in which the genetic defect remains unrevealed.

The current study, a multi-center collaborative survey conducted for the first time in Korea, demonstrates a variety of phenotypes in patients and detects the genetic mutations responsible. We show that some patients with IH carry significant nucleotide changes in the *GCMB, CASR*, or *prepro-PTH* genes. However, in an appreciable number of cases of IH, an advanced diagnostic approach such as whole exome sequencing for identification of unsolved genetic mutations may be needed.

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DISCLOSURE

The authors have no conflicts of interest to disclose.

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