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(Epi)genotype-Phenotype Analysis in 69 Japanese Patients With Pseudohypoparathyroidism Type I

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Context: Pseudohypoparathyroidism type I (PHP-I) is divided into PHP-Ia with Albright hereditary osteodystrophy and PHP-Ib, which usually shows no Albright hereditary osteodystrophy features. Although PHP-Ia and PHP-Ib are typically caused by genetic defects involving α subunit of the stimulatory G protein (Gsα)–coding *GNAS* exons and methylation defects of the *GNAS* differentially methylated regions (DMRs) on the maternal allele, respectively, detailed phenotypic characteristics still remains to be examined.

Objective: To clarify phenotypic characteristics according to underlying (epi)genetic causes.

Patients and Methods: We performed (epi)genotype-phenotype analysis in 69 Japanese patients with PHP-I; that is, 28 patients with genetic defects involving Gsα-coding *GNAS* exons (group 1) consisting of 12 patients with missense variants (subgroup A) and 16 patients with null variants (subgroup B), as well as 41 patients with methylation defects (group 2) consisting of 21 patients with broad methylation defects of the *GNAS*-DMRs (subgroup C) and 20 patients with an isolated *A/B*-DMR methylation defect accompanied by the common *STX16* microdeletion (subgroup D).

Results: Although (epi)genotype-phenotype findings were grossly similar to those reported previously, several important findings were identified, including younger age at hypocalcemic symptoms and higher frequencies of hyperphosphatemia in subgroup C than in subgroup D, development of brachydactyly in four patients of subgroup C, predominant manifestation of subcutaneous ossification in subgroup B, higher frequency of thyrotropin resistance in group 1 than in group 2, and relatively low thyrotropin values in four patients with low T4 values and relatively low luteinizing hormone/follicle-stimulating hormone values in five adult females with ovarian dysfunction.

Conclusion: The results imply the presence of clinical findings characteristic of each underlying cause and provide useful information on the imprinting status of Gsα.

Freeform/Key Words: congenital hypothyroidism, (epi)genotype-phenotype analysis, molecular classification, pseudohypoparathyroidism

Abbreviations: AHO, Albright hereditary osteodystrophy; Br, brachydactyly; DMR, differentially methylated region; GH, growth hormone; GHRH, GH-releasing hormone; Gsα, α subunit of the stimulatory G protein; ID, intellectual disability; mRNA, messenger RNA; Ob, obesity; PHP-I, pseudohypoparathyroidism type I; PPHP, pseudopseudohypoparathyroidism; PTH, parathyroid hormone; RF, round face; SO, subcutaneous ossification; SS, short stature; TSH, thyrotropin; UPD(20)pat, paternal uniparental disomy 20.

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Pseudohypoparathyroidism type I (PHP-I) is a rare endocrine disorder caused by parathyroid hormone (PTH) resistance due to compromised expression of the α subunit of the stimulatory G protein (Gsα) encoded by exons 1 to 13 of *GNAS* [1]. Gsα is predominantly expressed from the maternal allele and is barely expressed from the paternal allele in several tissues such as the proximal renal tubules, thyroid, gonads, and pituitary, whereas it is biallelically expressed in most tissues [2]. Thus, PHP-I develops when the maternal Gsα expression is compromised in the proximal renal tubules. Furthermore, because Gsα plays a pivotal role in signal transduction of multiple G protein–coupled receptors, including PTH receptor 1, thyrotropin (TSH) receptor, luteinizing hormone/choriogonadotropin receptor, follicle-stimulating hormone receptor, and growth hormone (GH)–releasing hormone (GHRH) receptor [1], PHP-I can be associated not only with PTH resistance but also with variable degrees of resistance to the corresponding ligand hormones. In particular, TSH resistance is relatively frequent and is often recognized as the first endocrine abnormality of PHP-I [3, 4]. Indeed, high TSH values are often identified in patients with PHP-I by the neonatal mass screening [5–7].

PHP-I is clinically divided into two types: PHP-Ia, which is accompanied by Albright hereditary osteodystrophy (AHO) characterized by short stature (SS), round face (RF), brachydactyly (Br), obesity (Ob), subcutaneous ossification (SO), and intellectual disability (ID); and PHP-Ib, which usually shows no AHO features [8]. Because most AHO features are rather nonspecific, the diagnosis of PHP-Ia is usually made when two or more of the six AHO features are identified in patients with PHP-I [3]. PHP-Ia is typically caused by abnormalities involving the maternally derived Gsα-coding *GNAS* exons (loss-of-function mutations or deletions), whereas PHP-Ib almost invariably results from methylation defects of the maternally inherited *GNAS* differentially methylated regions (DMRs) [1, 8]. Furthermore, PHP-Ib is divided into two forms: a sporadic form with broad methylation defects of the *GNAS*-DMRs, that is, loss-of-methylation type epimutations at the *A/B*-DMR, *XLas*-DMR, and *AS*-DMR, and gain-of-methylation type epimutation at the *NESP55*-DMR; and an autosomal-dominant form with an isolated loss-of-methylation type epimutation at the *A/B*-DMR, which is almost invariably accompanied by a common microdeletion involving *STX16* (exons 4 to 6) at the position ~220 kb upstream of *GNAS* [1, 8]. The broad methylation defects are also occasionally caused by paternal uniparental disomy 20 [UPD(20)pat] [9–11], as well as by rare types of microdeletions involving maternally derived *AS* (exons 3 to 4) with or without accompanying loss of *NESP55* [12, 13]. Such a difference in the underlying factors between PHP-Ia and PHP-Ib is primarily consistent with the notion that PHP-I develops as an imprinting disorder, whereas AHO is usually caused by reduced Gsα expression in tissues where Gsα is biparentally expressed [14]. Indeed, reduced paternal Gsα expression leads to pseudopseudohypoparathyroidism (PPHP), which is associated with AHO features and lacks hormonal resistance [14].

However, recent (epi)genotype-phenotype analyses have revealed a phenotypic overlap between patients with abnormalities involving Gsα-coding *GNAS* exons and those with methylation defects [3, 15, 16]. Indeed, a subset of patients with methylation defects exhibits some AHO features [3, 15]. Nevertheless, detailed phenotypic characteristics according to underlying (epi) genetic causes still remain to be clarified. Thus, we classified 69 patients with PHP-I according to the underlying (epi)genetic causes and performed (epi)genotype-phenotype analyses.

1. Patients and Methods

A. Patients

This study consisted of 69 Japanese patients from 52 families (29 males and 40 females; 60 hitherto unreported patients and 9 previously reported patients [17–21]) with clinically diagnosed PHP-I (Tables 1 and 2). Of the nine previously described patients, patient no. 40 exhibited Beckwith–Wiedemann syndrome and PHP-Ib phenotypes in the presence of severe loss-of-methylation type epimutations at the *Kv*-DMR and *GNAS*-DMRs [18]. The previously reported patient with PHP-Ib caused by a maternally inherited complex genomic rearrangement within the *NESP55* to *GNAS* region was excluded from this study because of the unique methylation defects [24].

Table 1. Molecular Findings in 69 Japanese Patients With PHP-I

Pt.	Family	Subject	Sex	Genetic Diagnosis[a]	Inheritance	Ref.
Group 1: Genetic defects involving Gsα-coding *GNAS* exons						
Subgroup A: Missense variants						
1	Family 1	Proband	F	Exon 1, p.(M1I)[b]	NE	
2	Family 2	Proband	F	Exon 1, p.(L30P)	*De novo*	
3	Family 3	Proband	F	Exon 1, p.(R42C)	Maternal	
4	Family 3	Sibling	F	Exon 1, p.(R42C)	Maternal	
5	Family 3	Sibling	F	Exon 1, p.(R42C)	Maternal	
6	Family 3	Sibling	F	Exon 1, p.(R42C)	Maternal	
7	Family 4	Proband	F	Exon 5, p.(M110T) (novel)	Maternal	
8	Family 5	Proband	M	Exon 7, p.(P192L)	NE	
9	Family 6	Proband	M	Exon 10, p.(E259K) (novel)	*De novo*	
10	Family 7	Proband	F	Exon 12, p.(I341T) (novel)	Maternal	
11	Family 8	Proband	F	Exon 13, p.(H362Y) (novel)	NE	
12	Family 9	Proband	F	Exon 13, p.(A366T)	*De novo*	
Subgroup B: Null variants						
13	Family 10	Proband	F	c.312+5G>A[c]	Maternal	
14	Family 11	Proband	F	Exon 1, p.(Q12X)[d]	Maternal	
15	Family 12	Proband	M	Exon 1, p.(Q29X)[d]	Maternal	
16	Family 13	Proband	M	Exon1, p.(Q35X)[d]	NE	
17	Family 14	Proband	F	Exon 5, p.(V117RfsX23)[d]	*De novo*	
18	Family 15	Proband	M	Exon 6, p.(A152GfsX6)[d] (novel)	Maternal	
19	Family 15	Sibling	F	Exon 6, p.(A152GfsX6)[d] (novel)	Maternal	
20	Family 16	Proband	M	Exon 7, p.(V191AfsX5)[d]	NE	
21	Family 17	Proband	M	Exon 11, p.(V287QfsX7)[d] (novel)	Maternal	
22	Family 18	Proband	F	Exon 12, p.(A337PfsX10)[e] (novel)	Maternal	
23	Family 18	Sibling	M	Exon 12, p.(A337PfsX10)[e] (novel)	Maternal	
24	Family 19	Proband	F	Microdeletion (*BMP7–CDH26*)[f]	*De novo*	
25	Family 20	Proband	F	Microdeletion (*VAPB–C20orf197*)[f]	Maternal	
26	Family 21	Proband	F	Microdeletion (*NPEPL1–EDN3*)[f]	Maternal	[17]
27	Family 21	Sibling	M	Microdeletion (*NPEPL1–EDN3*)[f]	Maternal	[17]
28	Family 22	Proband	F	Microdeletion (*GNAS* exons 1–13 only)[f]	Maternal	
Group 2: Methylation defects						
Subgroup C: Broad methylation defects at the *GNAS*-DMRs						
29	Family 23	Proband	F	Epimutations	Sporadic	
30	Family 24	Proband	M	Epimutations	Sporadic	
31	Family 25	Proband	M	Epimutations	Sporadic	
32	Family 26	Proband	M	Epimutations	Sporadic	
33	Family 27	Proband	M	Epimutations	Sporadic	
34	Family 28	Proband	F	Epimutations	Sporadic	
35	Family 29	Proband	M	Epimutations	Sporadic	
36	Family 30	Proband	F	Epimutations	Sporadic	
37	Family 31	Proband	M	Epimutations	Sporadic	
38	Family 32	Proband	M	Epimutations	Sporadic	
39	Family 33	Proband	F	Epimutations	Sporadic	
40	Family 34	Proband	F	Epimutations	Sporadic	[18]
41	Family 35	Proband	M	Epimutations	Sporadic	[19]
42	Family 36	Proband	F	Epimutations [UPD(20)pat not excluded]	NE	
43	Family 37	Proband	F	Epimutations [UPD(20)pat not excluded]	NE	
44	Family 38	Proband	F	Epimutations [UPD(20)pat not excluded]	NE	
45	Family 39	Proband	F	Epimutations [UPD(20)pat not excluded]	NE	
46	Family 40	Proband	M	Epimutations [UPD(20)pat not excluded]	NE	
47	Family 41	Proband	F	Epimutations (UPD(20)pat not excluded)	NE	
48	Family 42	Proband	F	Epimutations (UPD(20)pat not excluded)	NE	
49	Family 43	Proband	M	Epimutations (UPD(20)pat not excluded)	NE	
Subgroup D: Isolated *A/B*-DMR methylation defects						
50	Family 44	Proband	M	Epimutation with *STX16* microdeletion[g]	Maternal	
51	Family 44	Sibling	F	Epimutation with *STX16* microdeletion[g]	Maternal	
52	Family 44	Mother	F	Epimutation with *STX16* microdeletion[g]	NE	
53	Family 45	Proband	F	Epimutation with *STX16* microdeletion[g]	NE	

Table 1. Continued

Pt.	Family	Subject	Sex	Genetic Diagnosis[a]	Inheritance	Ref.
54	Family 45	Child	F	Epimutation with *STX16* microdeletion[g]	Maternal	
55	Family 46	Proband	M	Epimutation with *STX16* microdeletion[g]	NE	
56	Family 47	Proband	M	Epimutation with *STX16* microdeletion[g]	Maternal	
57	Family 47	Sibling	F	Epimutation with *STX16* microdeletion[g]	Maternal	
58	Family 47	Sibling	M	Epimutation with *STX16* microdeletion[g]	Maternal	
59	Family 47	Mother	F	Epimutation with *STX16* microdeletion[g]	NE	
60	Family 48	Proband	F	Epimutation with *STX16* microdeletion[g]	NE	
61	Family 48	Child	M	Epimutation with *STX16* microdeletion[g]	Maternal	
62	Family 49	Proband	M	Epimutation with *STX16* microdeletion[g]	NE	
63	Family 50	Proband	F	Epimutation with *STX16* microdeletion[g]	Maternal	
64	Family 50	Mother	F	Epimutation with *STX16* microdeletion[g]	NE	
65	Family 51	Proband	M	Epimutation with *STX16* microdeletion[g]	NE	[20]
66	Family 51	Sibling	M	Epimutation with *STX16* microdeletion[g]	NE	[20]
67	Family 52	Proband	F	Epimutation with *STX16* microdeletion[g]	NE	[21]
68	Family 52	Child	M	Epimutation with *STX16* microdeletion[g]	Maternal	[21]
69	Family 52	Child	M	Epimutation with *STX16* microdeletion[g]	Maternal	[21]

Abbreviations: F, female; M, male; NE, not examined; Pt., patient.
[a]Based on NM_000516 for GNAS (exons, 13; cDNA, 1972 bp; amino acids, 394).
[b]This variant affecting the translation start codon has been reported as a hypomorphic mutation with some residual activity [22].
[c]Immunoblot analysis has shown an ~50% reduction in the level of erythrocyte Gsα protein, indicating that the splice donor site mutation does not produce a normal Gsα protein [23].
[d]Predicted to undergo nonsense-mediated mRNA decay.
[e]Predicted to escape nonsense-mediated mRNA decay, and to produce a truncated protein missing a part of the ras-like domain that interacts with G protein–coupled receptors.
[f]The locus order is: centromere–BMP7–VAPB–STX16–NPEPL1–GNAS–NELFCD–EDN3–CDH26–C20orf197–telomere.
[g]The common ~3-kb microdeletion involving *STX16* exons 4–6.

B. Ethical Approval

This study was approved by the Institutional Review Board Committee at the National Center for Child Health and Development and was performed after obtaining written informed consent.

C. Molecular and In Silico *Analyses*

Molecular studies were performed for hitherto unreported 60 patients, using leukocyte genomic DNA samples. The molecular diagnostic flowchart is shown in Fig. 1, and the primers used are summarized in Supplemental Table 1.

We first performed direct sequencing for the Gsα-coding *GNAS* exons 1 to 13 and their splice sites in all 60 patients. For a reference, we used the *GNAS* transcript variant 1 (GenBank; NM_000516.5) (https://www.ncbi.nlm.nih.gov/genbank/) consisting of 13 exons and encoding 394 amino acids. Identified intragenic sequence variants were examined for the frequency in the public databases, and missense variants were studied for the pathogenicity by *in silico* analyses (Supplemental Table 2).

We also performed methylation-specific multiple ligation-dependent probe amplification (SALSA kit ME031A, MRC-Holland) to examine the copy number variants involving the *STX16* to *GNAS* region and the methylation patterns of the *GNAS*-DMRs in all 60 patients. Furthermore, we carried out array-based comparative genomic hybridization using a catalog human array (1 × 1M format, catalog no. G4447A; Agilent Technologies) in patients with copy number variants, pyrosequencing analysis for the *GNAS*-DMRs in patients with methylation defects, and microsatellite analysis to differentiate between epimutations and maternal uniparental disomy 20 in patients with broad methylation defects.

Table 2. Clinical Findings in 69 Japanese Patients with PHP-I

Pt.	Reason for Exam.	Clinical Dx.[a]	AHO Features	PTH Resistance	Intact PTH (pmol/L)	Ca (mmol/L)	IP (mmol/L)	Age at Exam. (y)	TSH Resistance	TSH (mU/L)	FT4 (pmol/L)	Age at Exam. (y)	Ref.
Group 1: Genetic defects involving Gsα-coding *GNAS* exons													
Subgroup A: Missense variants													
1	AHO (SS)	PHP-Ia	SS, Br	Yes	**8.2**	2.35	1.63	4.1	Yes	**5.6**	15.4	4.1	
2	Incidental finding[b]	PHP-Ia	SS, RF, Br	Yes	**60.5**	***2.05***	**1.98**	19.0	No	2.8	14.2	19.0	
3	Elevated TSH	PHP-Ia	SS, RF, Br, Ob, ID	Yes	**37.9**	2.23	1.18	13.2	Yes	**7.7**	16.8	1.0	
4	Family history	PHP-Ia	SS, RF, Br, SO, ID	Yes	**21.2**	2.28	1.22	25.5	Yes	**8.3**	10.3	25.5	
5	Family history	PHP-Ia	SS, RF, Br, Ob, ID	Yes	**29.5**	***2.13***	1.63	21.7	Yes	**12.0**	***7.7***	21.7	
6	Family history	PHP-Ia	SS, RF, Br, Ob, ID	Yes	**53.7**	***2.08***	1.15	17.4	Yes	**10.1**	11.6	17.4	
7	AHO (SS)	PHP-Ia	RF, Br, Ob, ID	Yes	**22.2**	2.38	1.86	6.6	Yes	**15.5**	***7.5***	6.6	
8	Seizure	PHP-Ia	RF, SS, Br, ID	Yes	**13.1**	***2.03***	**2.05**	11.0	No	3.5	11.6	11.0	
9	Elevated TSH	PHP-Ia	SS, RF, Ob, ID	Yes	**31.0**	***2.19***	**2.19**	2.8	Yes	**22.6**	***2.8***	Neonatal[d]	
10	AHO (SS)	PHP-Ia	SS, RF, Br, Ob, ID	Yes	**7.7**	2.50	1.47	8.3	No	2.6	***9.1***	8.3	
11	AHO (Ob)	PHP-Ib	Ob	Yes	**14.0**	2.60	1.70	1.0	Yes	**4.7**	11.1	1.0	
12	AHO (SS, Br)	PHP-Ia	SS, RF, Br, Ob, ID	Yes	**7.6**	2.40	1.34	16.0	No	3.5	18.0	16.0	
Subgroup B: Null variants													
13	AHO (Br)	PHP-Ia	SS, RF, Br	Yes	**44.4**	***1.95***	**1.95**	15.0	Yes	**57.1**	11.6	Neonatal[d]	
14	AHO (SO)	PHP-Ia	SS, RF, Br, SO, ID	Yes	**7.8**	2.58	1.73	0.6	Yes	**10.4**	***10.0***	0.2	
15	AHO (SO)	PHP-Ia	SS, RF, Br, SO, ID	Yes	**9.9**	2.60	**2.24**	0.1	Yes	**27.9**	***9.0***	Neonatal[d]	
16	AHO (SO)	PHP-Ia	SS, RF, Br, SO, ID	Yes	**31.8**	***1.90***	**2.56**	10.0	Yes	**16.5**	10.3	2.0	
17	AHO (SO)	PHP-Ia	RF, Ob, SO	Yes	**23.4**	2.43	1.73	3.0	Yes	**5.7**	14.8	3.0	
18	Incidental finding[b]	PHP-Ia	RF, Br, SO, ID	Yes	**34.3**	***1.83***	**2.72**	3.8	Yes	**10.0**	12.9	Neonatal[d]	
19	Family history	PHP-Ia	RF, SO	Yes	**48.4**	2.18	1.86	6.6	Yes	**11.6**	12.9	6.6	
20	Seizure	PHP-Ia	SS, RF, Br, ID	Yes	**74.7**	***2.15***	**2.05**	12.0	Yes	**8.1**	***9.4***	12.0	
21	Syncope	PHP-Ia	SS, RF, Br, SO	Yes	**10.8**	***1.40***	NE	11.2	No	3.9	23.7	11.2	
22	Seizure	PHP-Ia	SS, RF, Br, Ob, SO, ID	Yes	**50.6**	***1.68***	**2.50**	7.3	Yes	**4.4**	11.6	7.3	
23	Family history	PHP-Ia	RF, Br, Ob, SO, ID	Yes	**77.9**	2.48	**2.24**	0.2	Yes	**19.6**	18.0	Neonatal[d]	
24	Elevated TSH	PHP-Ia	RF, Br, Ob, SO, ID	Yes	**162.0**	***1.98***	**2.14**	6.5	Yes	**21.7**	16.7	Neonatal[d]	
25	Tetany on fever	PHP-Ia	RF, Br, Ob,	Yes	**140.0**	***1.40***	NE	6.5	Yes	**11.8**	***9.0***	6.5	
26	AHO (SO)	PHP-Ia	SS, RF, Br, Ob, SO	Yes	**16.0**	2.23	1.60	10.0	Yes	**9.1**	25.2	10.0	[17]
27	Family history	PHP-Ia	SS, RF, Ob, SO	Yes	**39.7**	***1.75***	**2.43**	10.0	Yes	**6.9**	10.3	10.0	[17]
28	AHO (SO)	PHP-Ia	RF, Br, Ob, SO,	Yes	**8.5**	2.40	1.57	1.4	Yes	**8.4**	12.9	1.4	
Group 2: Methylation defects													
Subgroup C: Broad methylation defects at the *GNAS*-DMRs													
29	Seizure	PHP-Ia	SS, RF, ID	Yes	**73.4**	***1.40***	**2.30**	11.1	No	1.7	17.6	11.1	
30	Seizure	PHP-Ia	RF, Br, ID	Yes	**60.9**	***1.78***	**2.75**	11.1	No	2.8	13.0	11.1	
31	Tetany	PHP-Ia	RF, Ob, Br	Yes	**56.7**	***1.70***	**2.78**	5.4	No	2.3	13.8	5.4	
32	Syncope	PHP-Ia	RF, ID	Yes	**77.6**	***1.40***	**2.94**	8.0	Yes	**6.5**	12.6	8.0	
33	Tetany	PHP-Ib	RF	Yes	**26.2**	***1.60***	**2.43**	9.2	No	3.1	18.7	9.2	
34	Tetany on fever	PHP-Ib	—	Yes	**62.5**	***1.63***	**3.04**	9.3	Yes	**5.2**	NE	9.3	
35	Tetany on fever	PHP-Ib	—	Yes	**47.2**	***1.50***	**2.02**	14.5	Yes	**5.1**	14.2	14.5	
36	DA	PHP-Ib	—	Yes	**100.9**	***1.53***	**2.62**	9.8	NE	NE	NE	9.8	
37	Incidental finding[b]	PHP-Ib	—	Yes	**28.3**	***1.65***	**2.24**	11.4	Yes	**6.6**	11.6	11.4	
38	Seizure	PHP-Ia	RF, ID	Yes	**21.1**	***2.08***	NE	1.0	No	0.9	11.3	1.0	
39	Incidental finding[b]	PHP-Ib	—	Yes	**80.1**	***1.90***	**2.10**	8.8	No	2.2	12.3	8.8	
40	BWS[c]	PHP-Ib	RF	Yes	**31.7**	***1.98***	**1.95**	13.0	Yes	**7.7**	***9.0***	13.0	[18]
41	Incidental finding[b]	PHP-Ib	—	Yes	**19.7**	2.40	1.98	6.0	No	2.1	16.2	6.0	[19]
42	Seizure	PHP-Ia	SS, RF, Br, ID	Yes	**37.2**	***1.58***	**3.26**	8.0	Yes	**12.9**	15.4	Neonatal[d]	
43	Seizure	PHP-Ia	RF, Br,	Yes	**24.1**	***1.53***	**2.11**	7.2	No	1.3	15.4	7.2	
44	AHO (Ob)	PHP-Ia	RF, Ob	Yes	**44.9**	2.30	1.66	3.3	Yes	**6.1**	13.4	3.3	
45	Incidental finding[b]	PHP-Ib	RF	Yes	**71.5**	***2.00***	1.89	5.6	No	1.9	***8.5***	5.6	
46	Seizure	PHP-Ib	RF	Yes	**31.5**	***1.68***	**2.40**	9.0	No	2.3	12.9	9.0	
47	Tetany on fever	PHP-Ib	—	Yes	**33.5**	***1.85***	1.15	35.0	No	2.9	10.4	35.0	
48	Tetany on fever	PHP-Ib	—	Yes	**78.9**	***2.08***	**2.34**	9.1	No	2.3	***9.0***	9.1	
49	Seizure	PHP-Ib	—	Yes	**25.3**	***1.90***	**3.60**	42.0	No	1.8	11.0	42.0	
Subgroup D: Isolated *A/B*-DMR methylation defects													
50	Tetany on fever	PHP-Ib	—	Yes	**34.4**	***1.23***	1.63	16.0	No	3.5	11.6	16.0	
51	Family history	PHP-Ib	—	Yes	**28.9**	***2.03***	**2.21**	13.0	No	1.6	10.3	13.0	
52	Family history	PHP-Ib	RF	Yes	**24.3**	***1.58***	1.57	46.0	Yes	**5.4**	***9.0***	46.0	
53	Tetany on fever	PHP-Ib	—	Yes	**41.2**	***1.60***	1.47	35.0	No	1.0	10.3	35.0	
54	Family history	PHP-Ib	—	Yes	**43.6**	***1.35***	**2.56**	5.2	No	0.5	11.7	5.2	
55	Tetany	PHP-Ib	—	Yes	**17.3**	***1.28***	**2.56**	13.9	Yes	**7.7**	14.0	13.9	
56	Syncope	PHP-Ib	—	Yes	**53.8**	***1.60***	1.70	13.7	No	4.0	13.1	13.7	
57	Family history	PHP-Ib	—	Yes	**38.0**	***1.60***	**3.04**	10.5	Yes	**7.9**	16.0	10.5	
58	Family history	PHP-Ib	—	Yes	**36.1**	***1.98***	**2.11**	8.9	Yes	**5.5**	13.1	8.9	

Table 2. Continued

Pt.	Reason for Exam.	Clinical Dx.[a]	AHO Features	PTH Resistance	Intact PTH (pmol/L)	Ca (mmol/L)	IP (mmol/L)	Age at Exam. (y)	TSH Resistance	TSH (mU/L)	FT4 (pmol/L)	Age at Exam. (y)	Ref.
59	Family history	PHP-Ib	—	Yes	**40.3**	***1.58***	1.25	39.0	No	3.8	13.3	39.0	
60	Tetany	PHP-Ib	—	Yes	**18.4**	***2.01***	1.41	51.0	Yes	**4.9**	11.6	51.0	
61	Family history	PHP-Ib	—	Yes	**42.4**	***1.55***	1.76	25.0	Yes	**4.7**	16.8	25.0	
62	Tetany	PHP-Ib	Ob	Yes	**31.2**	***1.73***	1.66	43.0	No	2.4	***8.9***	43.0	
63	Elevated TSH	PHP-Ia	RF, ID	Yes	**59.0**	***2.00***	**2.20**	12.0	Yes	**9.2**	14.2	3.0	
64	Family history	PHP-Ib	—	Yes	**43.1**	***1.70***	1.50	44.0	Yes	**5.1**	10.7	44.0	
65	Tetany on exercise	PHP-Ib	—	Yes	**26.7**	2.23	1.66	15.0	Yes	**5.6**	12.9	15.0	[20]
66	Family history	PHP-Ib	—	Yes	**45.3**	***1.60***	**2.91**	12.0	Yes	**4.1**	12.9	12.0	[20]
67	Tetany	PHP-Ib	—	Yes	**50.8**	***1.45***	1.76	25.0	Yes	**5.2**	13.0	25.0	[21]
68	Family history	PHP-Ib	SS	Yes	**31.0**	2.38	1.92	5.0	No	4.0	14.3	5.0	[21]
69	Family history	PHP-Ib	SS	Yes	**21.1**	2.38	1.92	5.0	No	2.1	16.6	5.0	[21]

Reference range: intact PTH, 1.0−6.8 pmol/L; calcium, 2.25−2.75 mmol/L (<1 years), 2.20−2.65 mmol/L (1−5 years), and 2.17−2.55 mmol/L (≥6 years); inorganic phosphate, 1.35−2.15 mmol/L (<1 year), 1.22−1.99 mmol/L (1−11 years), and 0.90−1.86 mmol/L (≥12 years); TSH 0.4−4.0 mU/L; and FT4, 10.3−29.6 pmol/L. The values above the age-matched reference ranges are boldfaced and underlined, and those below the age-matched reference ranges are boldfaced and italicized.

Abbreviations: BWS, Beckwith–Wiedemann syndrome; Dx., diagnosis; DA, dental abnormality; Exam., examination; IP, inorganic phosphate; Pt., patient.

[a]PHP-Ia is diagnosed when two or more AHO features are present.

[b]Identification of hypocalcemia by routine laboratory tests for PHP-unrelated features such as infections and operations.

[c]This patient exhibits Beckwith–Wiedemann syndrome and PHP-Ib phenotypes under the coexisting severe hypomethylations of the *Kv*-DMR and the *GNAS*-DMRs [18].

[d]Found to have increased blood TSH values at the neonatal mass screening (TSH cut-off values, 8−10 mU/L).

D. Clinical Studies

We collected detailed clinical findings in all 69 patients, including the previously reported 9 patients [17–21], from attending physicians using a comprehensive questionnaire. SS was defined as the length/height below –2 standard deviations of the age- and sex-matched Japanese standards [25], and Ob as the body mass index above +2 standard deviations of the age- and sex-matched Japanese standards [26]. ID was assessed as positive when the developmental quotient/intellectual quotient was <70. Br and SO were evaluated by physical and/or roentgenographic examinations. RF was evaluated subjectively. We also asked the physicians to report any clinical findings not covered by the questionnaire.

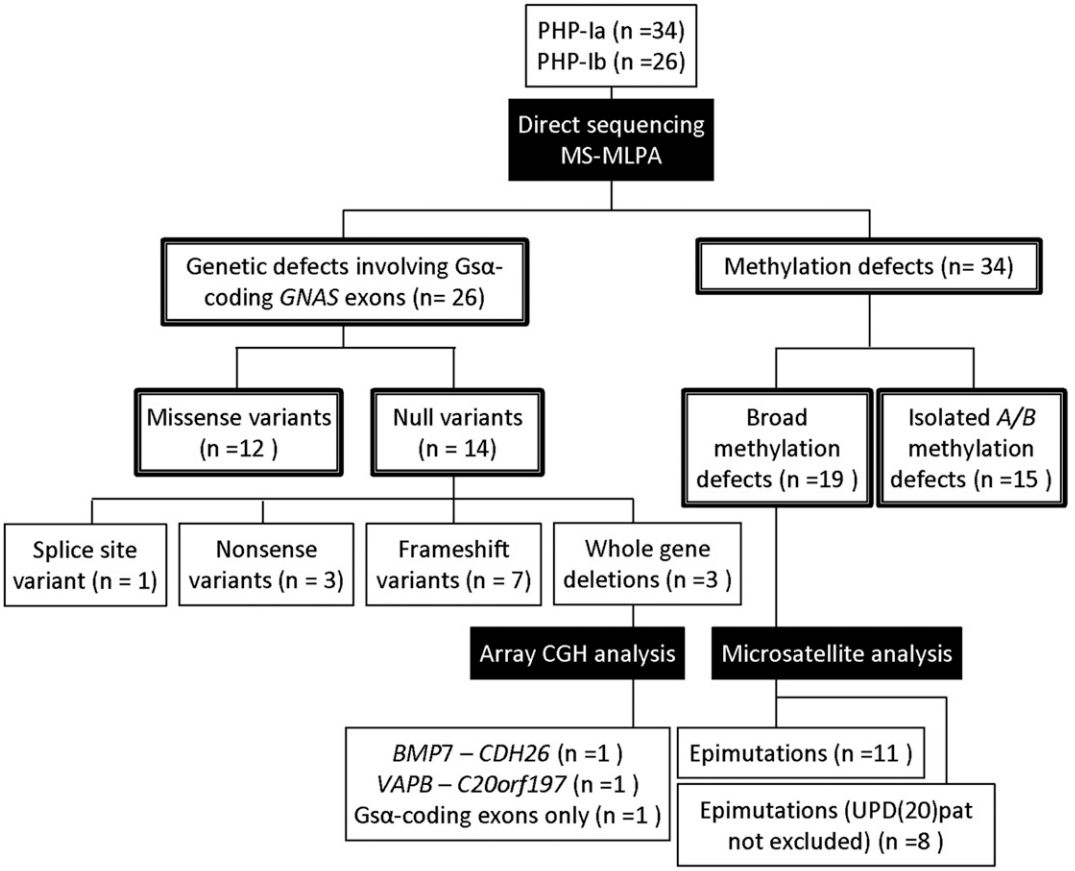
E. Statistical Analysis

Statistical significance of the median and the frequency between two groups or subgroups was examined by the Mann–Whitney *U* test and the Fisher's exact probability test, respectively, using the R environment (http://cran.r-project.org/bin/windows/base/old/2.15.1/). $P < 0.05$ was considered significant.

2. Results

A. Molecular and In Silico *Studies*

The results of the molecular studies for the 60 patients are shown in Table 1 and Fig. 1. Direct sequencing revealed a variant affecting the translation start codon in patient no. 1, eight types of missense variants in patient nos. 2 to 12, a variant affecting the splice donor site of intron 4



The number of patients in each group/subgroup	Total	This study	Previous studies
Group 1: Genetic defects involving Gsα-coding *GNAS* exons	28	26	2
Subgroup A: Missense variants	12	12	0
Subgroup B: Null variants	16	14	2
Group 2: Methylation defects	41	34	7
Subgroup C: Broad methylation defects	21	19	2
Subgroup D: Isolated *A/B* methylation defects	20	15	5

Figure 1. Molecular diagnostic flowchart and the number of patients in each group/subgroup.

in patient no. 13, three types of nonsense variants in patient nos. 14 to 16, and five types of frameshift variants in patient nos. 17 to 23. Of the total of 18 different types of variants, 7 were novel, whereas the remaining 11 have previously been reported, including those affecting the translation start codon and the splice donor site that were found to be a hypomorphic and an amorphic mutation, respectively [22, 23] (Supplemental Table 2). These intragenic variants were absent in the public databases, except for p.(Q12X) in patient no. 14, which has been registered as a clinically associated single nucleotide polymorphism in dbSNP 147, and the eight types of missense variants were assessed as pathogenic by *in silico* analyses (Supplemental Table 2). The nonsense and the frameshift variants in patient nos. 14 to 21 were predicted to cause nonsense-mediated messenger RNA (mRNA) decay, whereas the frameshift variant in sibling patient nos. 22 and 23 was predicted to produce a truncated protein missing a part of ras-like domain interacting with G protein–coupled receptor. Thus, all of the intragenic variants were assessed as pathogenic mutations.

Methylation-specific multiple ligation-dependent probe amplification analyses showed broad methylation defects in the presence of microdeletions encompassing the *GNAS* locus in patient nos. 24 and 25, normal methylation patterns in the presence of a microdeletion involving Gsα-coding *GNAS* exons 1 to 13 in patient no. 28, broad methylation defects in the absence of microdeletions involving *AS* and/or *NESP55* in patient nos. 29 to 39 and nos. 42 to 49, and isolated *A/B*-DMR methylation defects associated with the common ~3-kb microdeletions involving *STX16* exons 4 to 6 in patient nos. 50 to 64. Subsequently, array-based comparative genomic hybridization analysis revealed loss of a *BMP7* to *CDH26* region in patient no. 24, that of a *VAPB* to *C20orf197* region in patient no. 25, and that of Gsα-coding *GNAS* exons 1 to 13 in patient no. 28. Notably, the microdeletion of patient no. 28 did not involve the *GNAS*-DMRs including the *A/B*-DMR, with the centromeric and telomeric breakpoints being localized between chr20:57,465,955 bp and chr20:57,468,102 bp and between chr20:57,518,928 bp and chr20:57,522,236 bp, respectively (hg19; UCSC Genome Browser; http://genome.ucsc.edu/) (Supplemental Fig. 1). Pyrosequencing analysis demonstrated broad methylation defects in patient nos. 24 and 25, and normal methylation patterns of the *GNAS*-DMRs including the *A/B*-DMR in patient no. 28. Microsatellite analysis with parental DNA samples showed epimutations rather than UPD(20)pat in patient nos. 29 to 39, whereas parental DNA samples were not available in patient nos. 42 to 49.

B. Parent-of-Origin Analysis

Parent-of-origin analysis was performed in 40 of the 60 patients examined in this study, and the data were available in 6 of the 9 patients reported in the literature [17–21]. Collectively, it was found that: (1) pathogenic variants and deletions involving Gsα-coding *GNAS* exons were derived from the mother in 18 patients and occurred as *de novo* events in 5 patients; (2) broad methylation defects in the absence of deletions were caused by epimutations in all 13 patients in whom parental samples were available (it remained to be clarified whether the broad methylation defects were due to epimutations or maternal uniparental disomy in the remaining 8 patients); and (3) *STX16* microdeletions with the hypomethylated *A/B*-DMR were of maternal origin in all 10 patients in whom parental samples were available (Table 1).

C. Genetic Classification

On the basis of the molecular results of the 60 patients examined in this study and those of the previously reported nine patients, the 69 patients were classified as follows: (1) patient nos. 1 to 28 from 22 families (22 probands) with genetic defects involving Gsα-coding *GNAS* exons (group 1) consisting of patient nos. 1 to 12 from 9 families (9 probands) with missense variants (subgroup A) and patient nos. 13 to 28 from 13 families (13 probands) with null variants (splice site, nonsense, and frameshift variants, as well as deletions) (subgroup B); and (2) patient nos. 29 to 69 from 30 families (30 probands) with *GNAS* methylation defects (group 2) consisting of patient nos. 29 to 49 from 21 families (21 probands) with broad methylation defects of the *GNAS*-DMRs (subgroup C) and patient nos. 50 to 69 from 9 families (9 probands) with an isolated *A/B*-DMR methylation defect accompanied by the common *STX16* microdeletion (subgroup D) (Fig. 1; Table 1).

D. (Epi)genotype-Phenotype Analysis

Clinical findings of each patient are shown in Table 2, and those of each group and subgroup are summarized in Table 3. Phenotypic comparisons in the probands revealed that the age at examination for PHP was significantly younger in group 1 than in group 2, and that the reason of examination for PHP was mostly accounted for by AHO features in group 1 (mostly SS in subgroup A and invariably SO in subgroup B) and by hypocalcemic symptoms (tetany, seizures, and syncope) in group 2. Indeed, hypocalcemia was significantly more prevalent in group 2 than in group 1. Furthermore, the age at examination was significantly younger in subgroup C than in subgroup D as was the age at hypocalcemic symptoms, and

hyperphosphatemia was more frequent in subgroup C than in subgroup D, whereas there was no such significant difference between subgroup A and subgroup B.

Phenotypic comparisons in all patients showed that almost all patients of group 1 exhibited PHP-Ia phenotype, except for patient no. 11, who was just 1 year of age at the time of examination, whereas patients of group 2 primarily manifested PHP-Ib phenotype. Furthermore, patients of subgroup C showed both PHP-Ia and PHP-Ib phenotypes, whereas those of subgroup D almost invariably exhibited PHP-Ib phenotype, except for patient no. 63, who was assessed as having PHP-Ia because of the presence of RF and ID. For AHO features, all features including ID were more frequent in group 1 than in group 2. Of patients with PHP-Ia, Br or SO fairly characteristic of AHO was present not only in 10 of 12 patients of subgroup A and all patients of subgroup B, but also in 4 patients of subgroup C (patient nos. 30, 31, 42, and 43). Additionally, SO was more prevalent in subgroup B than in subgroup A, and positive AHO features per patient and RF were more frequent in subgroup C than in subgroup D. For PTH resistance, although all patients had increased serum PTH values, hypocalcemia and hypocalcemic symptoms were more frequent in group 2 than in group 1, and hyperphosphatemia was more prevalent in subgroup C than in subgroup D. Most patients, including all patients with hypocalcemia, were treated with active vitamin D analog (alfacalcidol) with or without calcium supplementation.

TSH resistance (increased serum TSH values) was more frequent in group 1 than in group 2, as was the detection rate of elevated TSH values at the newborn mass screening. However, four patients (patient nos. 10, 45, 48, and 62) had normal serum TSH values in the presence of low free T4 values. Sixteen patients received levothyroxine treatment. Notably, of seven patients (one in subgroup A, five in subgroup B, and one in subgroup C) who were found to have increased blood TSH values at the newborn mass screening, one patient (patient no. 15) was diagnosed as having PHP at that time, and the remaining six patients were found to have PHP at later ages (Table 4). Additionally, three of the seven patients exhibited hypothyroidism-compatible clinical features. Although six patients, including patient nos. 9 and 15 who had low free T4 values at the newborn screening, were treated with levothyroxine, three of them showed developmental delay and four of them showed Ob in infancy, and four of them showed ID and two of them showed Ob at the time of the latest examinations. Hypothyroidism-related autoantibodies were absent in all patients with TSH resistance.

Furthermore, other features were also identified in several patients. First, gonadotropin resistance was indicated in four adult females (patient nos. 3 to 5 and 12) of subgroup A and one adult female (patient no. 26) of subgroup B (Table 5), although autoimmune oophoritis and other underlying factors for ovarian dysfunction were not examined. The five female patients had primary or secondary amenorrhea or oligomenorrhea in the presence of moderate hypergonadotropism, and they exhibited breast development at Tanner stage 3 to 4 at the time of examinations. They were placed on hormone replacement therapy with estrogen and progesterone derivatives. Gonadotropin resistance was also suggested in one boy (patient no. 9) of subgroup A who had cryptorchidism in the presence of mild hypergonadotropinism. Second, GHRH resistance was suspected in patient no. 10 of subgroup A and sibling patient nos. 68 and 69 of subgroup D (Table 5). Indeed, apparent GH deficiency was indicated by GH provocation tests. Third, dental abnormality was described in patient nos. 24 and 26 of subgroup B and patient nos. 35 and 36 of subgroup C, and strabismus was reported in patient no. 2 of subgroup A, patient no. 24 of subgroup B, and one patient (no. 41) of subgroup C.

3. Discussion

We classified 69 Japanese patients with PHP-I on the basis of molecular findings and performed (epi)genotype-phenotype analysis. Although it remains to be clarified whether genetic and epigenetic defects resided on the maternally inherited allele in a substantial fraction of patients, it would be reasonable to assume compromised maternal Gsα expression in all 69 patients. Although the relative frequencies of each group and subgroup and (epi)genotype-phenotype findings are grossly similar to those reported in non-Japanese patients [3, 27], several findings are worth pointing out.

Table 3. Summary of Clinical Findings Based on Molecular Classifications

	Genetic Defects Involving Gsα-Coding *GNAS* Exons			Methylation Effects			*P* Value		
	Group 1	Subgroup A Missense Variants	Subgroup B Null Variants	Group 2	Subgroup C *GNAS*-DMRs[a]	Subgroup D *A/B*-DMR[b]	Group 1 *vs* Group 2	Subgroup A *vs* Subgroup B	Subgroup C *vs* Subgroup D
Probands									
Number	22	9	13	30	21	9			
Age at examination for PHP, y	6.9 (0.1–19.0)	8.3 (1.0–19.0)	6.5 (0.1–15.0)	11.1 (1.0–51.0)	9.1 (1.0–42.0)	16.0 (12.0–51.0)	<0.05	NS	<0.01
Reason for examination									
AHO features	12/22	5/9[d]	7/13[d]	1/30	1/21[d]	0/9	<0.01	NS	NS
Other features	0/22	0/9	0/13	2/30	2/21[e]	0/9	NS	NS	NS
Hypocalcemic symptoms[c]	5/22	1/9	4/13	22/30	14/21	8/9	<0.01	NS	NS
Age at hypocalcemic symptoms, y	11.0 (6.5–12.0)	11.0	9.2 (6.5–12.0)	12.4 (1.0–51.0)	9.1 (1.0–42.0)	20.5 (13.7–51.0)	NS	…	<0.01
High TSH at the newborn screening	5/22	1/9	4/13	1/21	1/16	0/5	NS	NS	NS
Incidental findings	2/22	1/9	1/13	4/30	4/21	0/9	NS	NS	NS
Hypocalcemia	11/22	3/9	8/13	27/30	19/21	8/9	<0.01	NS	NS
Hyperphosphatemia	10/20	3/9	7/11	18/29	16/20	2/9	NS	NS	<0.01
All patients									
Number	28	12	16	41	21	20			
PHP-Ia:PHP-Ib	27:1	11:1	16:0	9:32	8:13	1:19	<0.01	NS	<0.05
AHO features									
Positive AHO features per patient	4 (1–6)	4.5 (1–5)	4 (2–6)	0 (0–4)	1 (0–4)	0 (0–2)	<0.01	NS	<0.05
Short stature	19/28	10/12	9/16	4/41	2/21	2/20	<0.01	NS	NS
Round face	26/28	10/12	16/16	14/41	12/21	2/20	<0.01	NS	<0.01
Ob	16/28	8/12	8/16	3/41	2/21	1/20	<0.01	NS	NS
Brachydactyly	23/28	10/12	13/16	4/41	4/21	0/20	<0.01	NS	NS
Subcutaneous ossifications	14/28	1/12	13/16	0/41	0/21	0/20	<0.01	<0.01	NS
ID	17/28	9/12	8/16	6/41	5/21	1/20	<0.01	NS	NS
Endocrine-related features									
PTH resistance	28/28	12/12	16/16	41/41	21/21	20/20	—	—	—
Hypocalcemia	14/28	5/12	9/16	36/41	19/21	17/20	<0.01	NS	NS
Hypocalcemic symptoms[c]	5/28	1/12	4/16	22/41	14/21	8/20	<0.01	NS	NS
Hyperphosphatemia	12/26	3/12	9/14	23/40	16/20	7/20	NS	NS	<0.01
TSH resistance	23/28	8/12	15/16	18/40	7/20	11/20	<0.01	NS	NS
High TSH at the newborn screening	6/24	1/9	5/15	1/28	1/16	0/12	<0.05	NS	NS

The data are shown as the median (range) or the frequency. For the frequency, the denominators indicate the number of patients examined for the presence or absence of each feature, and the numerators represent the number of patient assessed to be positive for that feature; thus, differences between the denominators and numerators denote the number of patients evaluated to be negative for the feature.

Abbreviation: NS, not significant.

[a]Loss of methylation-type epimutations at the *AS*-DMR, the *XLas*-DMR, and the *A/B*-DMR, and gain of methylation-type epimutation at the *NESP55*-DMR.

[b]Isolated loss of methylation at the *A/B*-DMR accompanied by the *STX16* microdeletion.

[c]Hypocalcemic symptoms include tetany, seizures, and syncope.

[d]AHO features: subgroup A, SS (n = 3), SS plus Br (n = 1), and Ob (n = 1); subgroup B, Br (n = 1) and SOs (n = 6); and subgroup C, Ob (n = 1).

[e]Dental abnormality (n = 1) and Beckwith–Wiedemann syndrome (n = 1).

First, phenotypic comparisons in the probands showed that the age at examination for PHP was significantly younger in group 1 than in group 2. This would primarily be ascribed to the ascertainment bias that patients of group 1 were primarily examined because of AHO features that are discernible from infancy or early childhood [3, 4], whereas those of group 2 were primarily studied because of hypocalcemic symptoms that usually become recognizable from late childhood [3, 4]. Furthermore, the younger ages at examination for PHP and hypocalcemic symptoms and the higher frequency of hyperphosphatemia in subgroup C than in subgroup D would suggest that, in addition to the central role of the *A/B*-DMR, the *AS*-, *XLas*-, and *NESP55*-DMRs also play a certain role in the establishment of Gsα imprinting in renal tubules.

Table 4. Long-Term Clinical Findings in Seven Cases Found to Have Elevated TSH Values at the Neonatal Mass Screening

Patient			Neonatal Period				Infancy (1.0–2.0 y)		Latest examinations		
No.	Subgroup	Clinical Diagnosis	TSH (mU/L)	FT4 (pmol/L)	Hypothyroid-Like Clinical Findings	L-T4 Treatment	DD	BMI (SDS)[a]	Age (y)	ID	BMI (SDS)[a]
9[b]	A	PHP-Ia	**22.6**	***2.8***	Yes[c]	Yes	Yes	1 8.4 (–0.2)	7	Yes	31.3 (+3.1)
13	B	PHP-Ia	**57.1**	11.6	No	Yes	No	19.1 (+2.6)	16	No	23.1 (+0.8)
15	B	PHP-Ia	**27.9**	***9.0***	No	Yes	No	27.0 (+3.9)	2	No	20.5 (+3.3)
18	B	PHP-Ia	**10.0**	12.9	No	No	Unknown	Unknown	16	Yes	20.3 (+0.3)
23	B	PHP-Ia	**19.6**	18.0	Yes[d]	Yes	Yes	19.2 (+2.1)	14	Yes	18.9 (–1.3)
24	B	PHP-Ia	**21.7**	16.7	Yes[e]	Yes	Yes	25.2 (+5.6)	7	Yes	15.6 (+0.8)
42	C	PHP-Ia	**12.9**	15.4	No	Yes	No	18.3 (+1.6)	15	Yes	21.9 (+0.5)

The values above the reference ranges are boldfaced and underlined, and those below the reference ranges are boldfaced and italicized. Reference values: 1.7−9.1 mU/L for TSH and 11.6−33.5 pmol/L for FT4.
Abbreviations: BMI, body mass index; DD, developmental delay; L-T4, levothyroxine; SDS, standard deviation score.
[a]Assessed by the age- and sex-matched Japanese reference data (http://jspe.umin.jp/medical/taikaku.html).
[b]This patient had cryptorchidism, sleep apnea, and laryngotracheomalacia, which required tracheostomy, and died of cardiac arrest of unknown cause at 7 years of age.
[c]Macroglossia, hypothermia, and hypoactivity.
[d]Large posterior fontanelle.
[e]Jaundice.

Second, phenotypic comparisons in all patients revealed that patients of subgroup C exhibited both PHP-Ia and PHP-Ib phenotypes, whereas nearly all patients of group 1 showed PHP-Ia phenotype and nearly all patients of subgroup D manifested the PHP-Ib phenotype. This argues for the importance of molecular studies in PHP-I to reveal the precise underlying causes and to provide accurate genetic counseling, although the usefulness of molecular studies is rather limited in the prediction of phenotypic consequences. For AHO features, it is notable that Br was exhibited by four patients of subgroup C. This may suggest that Gsα is subject to a certain degree of imprinting in several, if not all, skeletal tissues. It is also notable that SO was predominantly manifested by patients of subgroup B. This finding is compatible with the previous report that SO is significantly higher in patients with truncating variants than in those with missense variants [28]. In this regard, SO constituted the major reason for the examination of PHP in the probands of subgroup B. Thus, together with the previous finding that SO is also predominantly identified from infancy in patients with PPHP and progressive osseous heteroplasia caused by truncating variants of paternal Gsα-coding *GNAS* exons [29], it appears that SO primarily develops from infancy to early childhood when Gsα expression is halved. In support of this, a heterozygous inactivation of *Gnas* in mice results in the development of SO probably by accelerating the osteoblast differentiation and promoting osteogenesis [30]. Furthermore, because positive AHO features per case and the frequencies of RF were significantly higher in subgroup C than in subgroup D, this would suggest the relevance of the methylation defects of the *GNAS*-DMRs other than the *A/B*-DMR to the development of the AHO features. Additionally, ID was occasionally observed in group 2 (primarily in subgroup C), although it was more prevalent in group 1 than in group 2. This finding, in conjunction with the previous report that ID is less prevalent in patients with PPHP than in those with PHP-I [31], may suggest that Gsα is subject to some degree of imprinting in the brain.

Third, TSH resistance and the detection of increased TSH values at the neonatal mass screening were more frequent in group 1 than in group 2. This would imply that Gsα is not completely imprinted in the thyroid [2]. Notably, most patients, who were found to have increased TSH values at the mass screening and were appropriately treated with levothyroxine, developed Ob and developmental delay in infancy and ID in later ages probably as

Table 5. Clinical and Endocrine Findings in Patients with Gonadotropin or GHRH Resistance

			Clinical Findings			Endocrine Findings						
								GH (μg/L)				
Patient No.	Sex	Subgroup	Salient Feature	Tanner Stage	Age at Exam. (y)	LH (IU/L) GnRH: Basal/Peak[a]	FSH (IU/L) GnRH: Basal/Peak[a]	Arginine Peak[b]	Clonidine Peak[c]	Glucagon Peak[d]	Age at Exam. (y)	Ref.
Gonadotropin resistance												
3	F	A	PA	B3	23	**15.3**/NE	**17.8**/NE	—	—	—	18	
4	F	A	SA[e]	B3−4	33	**10.2**/NE	**23.7**/NE	—	—	—	25	
5	F	A	PA	B3	32	**14.5**/NE	**22.4**/NE	—	—	—	21	
9	M	A	CYO	PH 1	4.8	**12.6**/NE	**10.2**/NE	—	—	—	4.8	
12	F	A	Oligo.	B3−4	26	**16.6**/**56.6**	**16.6**/**26.6**	—	—	—	16	
26	F	B	SA[e]	B3−4	24	0.6/**13.6**	**6.5**/**25.0**	—	—	—	10.0	[17]
GHRH resistance												
10	F	A	GHD	—		—	—	—	***5.2***	***3.5***	11.0	
68	M	D	GHD	—		—	—	***1.26***	***1.9***	—	5.0	[21]
69	M	D	GHD	—		—	—	***3.75***	***1.59***	—	5.0	[21]

Reference range: basal LH, 0.18−0.63 IU/L for prepubertal boys, 0.24−1.31 for prepubertal girls, and 2.0−9.0 for adult females (at the follicular phase); peak LH, 1.6−4.8 for prepubertal girls and 8.5−15.5 for adult females (at the follicular phase); basal follicle-stimulating hormone, 1.03−2.89 IU/L for prepubertal boys, 2.24−5.35 for prepubertal girls, and 1.8−11.2 for adult females (at the follicular phase); and peak follicle-stimulating hormone, 2.1−6.1 for prepubertal girls and 14.5−21.9 for adult females (at the follicular phase). The values above the age-matched reference ranges are boldfaced and underlined, and those below the age-matched reference ranges are boldfaced and italicized.

Abbreviations: B, breast; CYO, cryptorchidism; F, female; FSH, follicle-stimulating hormone; GHD, GH deficiency; LH, luteinizing hormone; M, male; NE, not examined; Oligo., oligomenorrhea; PA, primary amenorrhea; PH, pubic hair; SA, secondary amenorrhea.

[a]The peak value during GnRH stimulation tests: GnRH, 100 μg/m^2 (max. 100 μg) bolus i.v.; blood sampling at 0, 30, 60, 90, and 120 minutes.

[b–d]The peak values during GH provocation tests: arginine at 0.5 g/kg (max. 30 g) intravenously over 30 minutes or clonidine at 0.1–0.15 mg/m^2 (max. 0.15 mg) orally, blood sampling at 0, 30, 60, 90, and 120 minutes; glucagon at 0.03 mg/kg (max. 1 mg) subcutaneously, blood sampling at 0, 30, 60, 90, 120, 150, and 180 minutes. The diagnosis of GH deficiency is made when all the serum GH values in two or more provocation tests are <6 μg/L.

[e]Patient nos. 4 and 26 developed SA in their early 20s.

the AHO features. Thus, PHP-I should be considered in patients who are positive for neonatal mass screening for hypothyroidism and show Ob and/or developmental delay and ID in infancy to later ages. Additionally, it is also notable that serum TSH values remained normal in four patients with low serum-free T4 values. In this regard, previous studies have revealed blunted TSH response to exogenous TRH stimulation in patients with PHP-Ia [32], as well as the involvement of Gsα in the TRH signaling in the mouse [33]. Thus, although further human studies would be necessary, it might be possible that the impaired Gsα function leads to TRH as well as TSH resistance, resulting in the relatively low TSH secretion. For thyroid function in PHP-I, it is worth pointing out that patient no. 42 is the second case who was found to have elevated TSH at the time of neonatal mass screening, in the presence of broad methylation defects [7].

Fourth, other hormone resistances were also indicated in this study. Indeed, apparent gonadotropin resistance was identified in six patients of group 1 but not in group 2. In this context, gonadotropin resistance has also been identified in multiple patients with abnormalities involving Gsα-coding *GNAS* exons and in a few patients with methylation defects [3, 4, 15]. Thus, gonadal function should be examined in patients with PHP-I, especially in those with abnormalities involving Gsα-coding *GNAS* exons. Notably, gonadotropin values were not so increased even in patients with primary or secondary amenorrhea. This may be due to the involvement of Gsα in the GnRH signaling [34], because impaired Gsα function would cause GnRH resistance, resulting in more or less compromised gonadotropin secretion. Furthermore, the presence of apparent GH deficiency in patient no. 10 of subgroup A and in patient nos. 68 and 69 of subgroup D would argue for the imprinting of Gsα in pituitary somatotropes [35].

Lastly, dental abnormality and strabismus were identified in a few patients of different groups. These findings have also been reported previously [3, 19, 36–38]. Thus, dental abnormality, as well as strabismus, may represent possible AHO-related features.

In summary, we evaluated clinical findings in patients with PHP-I based on molecular classifications. In this regard, we note that (1) the patient number was small, especially after divided into four subgroups, so that this would have reduced the statistical power of the comparisons; (2) several missense variants can be severe as "null" variants, and several "frameshift" variants, especially the p.(A337PfsX10) that is predicted to escape nonsense-mediated mRNA decay, may have some functional activity; and (3) of the AHO features, RF and equivocal degree of Br were subjectively assessed by the attending physicians. Despite such caveats, this study implies the presence of clinical findings characteristic of each underlying cause, and it provides useful information on the imprinting status of Gsα in terms of clinical findings. Further studies will serve to define (epi)genotype-phenotype correlations.

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