Pseudohypoparathyroidism (PHP) is a group of disorders characterized by end-organ resistance to the parathyroid hormone (PTH). PHP type 1A includes multihormone resistance syndrome, Albright’s hereditary osteodystrophy, and obesity and is caused by mutations in \textit{GNAS} exon 1 through 13. PHP type 1B (PHP1B), caused by epigenetic changes in the \textit{GNAS} locus, was initially described as an isolated resistance to PTH. Epigenetic changes in \textit{GNAS} have also been reported in patients who display mild Albright’s hereditary osteodystrophy or mild thyroid-stimulating hormone (TSH) resistance without mutation of \textit{GNAS}. Here we report a case of PHP caused by epigenetic changes in \textit{GNAS} in a patient with congenital hypothyroidism. The patient was referred for a positive newborn screening for hypothyroidism (TSH 50 mIU/L). She exhibited severe clinical features of congenital hypothyroidism. The thyroid was in place, and etiologic explorations were negative. TSH was normalized under L-thyroxin, and the symptoms disappeared, except for a macroglossia. In childhood, PHP was suspected in addition to elevated PTH, obesity, brachydactyly, and a rounded face. Sequencing, methylation analysis, and large deletion research were performed in \textit{GNAS}. No genetic mutations were found. Methylation analysis revealed a broad epigenetic defect without deletion in \textit{GNAS} consistent with sporadic PHP1B. The multilocus methylation analysis was negative. This finding expands the known onsets of PHP1B and emphasizes the need for a new PHP classification system. This case report has important consequences for the etiologic diagnosis of congenital hypothyroidism because it adds a new cause of the disease.

abstract

Pseudohypoparathyroidism (PHP) is a group of rare genetic disorders that are characterized by end-organ resistance to parathyroid hormone (PTH). PHP type 1A (PHP1A) includes multihormone resistance syndrome, Albright’s hereditary osteodystrophy, and obesity and is caused by mutations in \textit{GNAS} exon 1 through 13. PHP type 1B (PHP1B), caused by epigenetic changes in the \textit{GNAS} locus, was initially described as an isolated resistance to PTH. Epigenetic changes in \textit{GNAS} have also been reported in patients who display mild Albright’s hereditary osteodystrophy or mild thyroid-stimulating hormone (TSH) resistance without mutation of \textit{GNAS}. Here we report a case of PHP caused by epigenetic changes in \textit{GNAS} in a patient with congenital hypothyroidism. The patient was referred for a positive newborn screening for hypothyroidism (TSH 50 mIU/L). She exhibited severe clinical features of congenital hypothyroidism. The thyroid was in place, and etiologic explorations were negative. TSH was normalized under L-thyroxin, and the symptoms disappeared, except for a macroglossia. In childhood, PHP was suspected in addition to elevated PTH, obesity, brachydactyly, and a rounded face. Sequencing, methylation analysis, and large deletion research were performed in \textit{GNAS}. No genetic mutations were found. Methylation analysis revealed a broad epigenetic defect without deletion in \textit{GNAS} consistent with sporadic PHP1B. The multilocus methylation analysis was negative. This finding expands the known onsets of PHP1B and emphasizes the need for a new PHP classification system. This case report has important consequences for the etiologic diagnosis of congenital hypothyroidism because it adds a new cause of the disease.
caused by a loss of methylation (LOM) of the A/B DMR. Some patients with PHP1B may manifest clinical features that overlap with PHP1A as brachydactyly or mild AHO. In sporadic PHP1B, epigenetic changes involve A/B DMR and ≥1 of the other DMRs in GNAS, whereas in autosomal dominant PHP1B, the loss of imprinting is restricted to the A/B DMR and associated in most cases with a recurrent 3-kb maternal deletion in the STX16 gene. The STX16 gene codes for syntaxin 16, is located in the cis region of A/B ∼220 kb upstream of GNAS, and harbors an imprinting control region (ICR) that is crucial for the establishment of the imprint at A/B.

Mild AHO and mild TSH resistance have been identified in patients with GNAS imprinting defects and PTH resistance in the absence of GNAS exon 1 through 13 mutations, indicating an overlap between the clinical features of PHP1A and PHP1B. Nevertheless, hypocalcemia and incidentally high PTH levels still typically reveal PHP1B; TSH resistance associated with GNAS epigenetic changes results in mildly elevated levels of TSH (<10 mIU/L) without symptoms of hypothyroidism. Here, we report the case of a child with congenital hypothyroidism and severe hypothyroidism symptoms who presented with PHP and broad epigenetic defects in GNAS during childhood.

**PRESENTATION**

The patient was born at 37 weeks’ gestational age from a nonconsanguineous union. She was small for her gestational age (2020 g [−1.9 SD], 46 cm [−0.7 SD]), attributed to preeclampsia. She was referred to our center because of an elevated TSH level on the newborn screening for congenital hypothyroidism (50 mIU/L, normal value [NV] <15 mIU/L). At day 7, the clinical features were consistent with hypothyroidism and included neonatal jaundice, lethargy, poor sucking, umbilical hernia, dry skin, macroglossia, and enlarged fontanels. Primary hypothyroidism was confirmed; the TSH levels were elevated (50 mIU/L, NV 2–10 mIU/L), with low levels of T4 (10.5 pmol/L, NV 7–20 pmol/L) and T3 (3.9 pmol/L, NV 3.8–6.8 pmol/L) in the absence of thyroid autoimmunity. Thyroid ultrasonography and iodine-123 scintigraphy with a perchlorate load revealed a eutopic thyroid without iodine organification defects.

The baby was started on L-thyroxine on her seventh day of life. While growing up, the young child suffered from childhood asthma, laryngomalacia, gastric reflux, and an atrial septal defect that was diagnosed at the age of 2 years. Although she had a euthyroid status, macroglossia persisted until the age of 2 years. She had a mild speech impediment without any hearing impairment that necessitated speech therapy. Her cognitive function was normal. She had never had seizures. She subsequently maintained statural growth in the higher portion of the normal range (Fig 2). Her parents were of normal height (mother: 1.60 m; father: 1.81 m). Her weight gain was excessive beginning at the age of 4 months. Dietary recommendations and physical activity improved her corpulence but failed to normalize her BMI.

At the age of 4 years, she exhibited signs consistent with AHO, including a rounded face and brachydactyly affecting the fourth and fifth rays. Biological exploration first emphasized the high PTH levels, normal calcium levels, and low vitamin D levels (Fig 2). Regular supplementation with vitamin D (1100 U/day) failed to decrease the PTH levels, which suggested resistance to PTH. Therefore, the diagnosis of PHP was suspected.

Because PHP1A is associated with multiple endocrine hormone resistance (eg, PTH, TSH, growth hormone, and gonadotropins), genetic testing of the genomic DNA from the patient and her mother was performed after the mother’s informed consent was obtained. No mutation was found in the coding sequence of the Gsα gene of the patient or her mother. According to the reported overlap between PHP1A and PHP1B phenotypes, we explored

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**FIGURE 1**

General organization of the GNAS locus. The single arrows indicate the transcribed allele and the direction of transcription. The dark gray and light gray boxes represent the coding and noncoding exons, respectively. The broken lines indicate the splicing patterns. The double arrows designate the regions affected by the maternal deletions identified in familial cases of PHP1B.19
the methylation patterns of the GNAS DMRs (Table 1). DNA methylation assays of the GNAS DMRs were assessed by pyrosequencing (Qiagen, Courtaboeuf, France) after bisulfite treatment (Epitect Bisulfite Kits, Qiagen).5,11 Confirmation of the methylation status of the GNAS DMRs and research on deletions in the GNAS and STX16 regions were performed via methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA; MRC-Holland, Amsterdam, Netherlands). We identified a LOM in the A/B DMR. The other DMRs exhibited a broad epigenetic defect with an LOM in the AS DMR, which was associated with a gain of methylation in the NESP DMR; the methylation pattern of the XL DMR was similar to that of controls. An MS-MLPA assay failed to detect deletions in the GNAS locus or the STX16 region.

Considering the unusual clinical features, we completed an analysis of the methylation patterns at ICR1 and ICR2 at 11p15, both loci involved in Beckwith–Wiedemann syndrome and Russell–Silver syndrome; the PEG1/MEST locus on chromosome 7 (involved in Russell–Silver syndrome); and DLK1/GTL2 intergenic germline-derived DMR on chromosome 14 (involved in Temple and Wang syndromes) with allele-specific methylated multiplex real-time quantitative polymerase chain reaction.12,13 The methylation patterns at the DLK1/GTL2, PEG1/MEST, 11p15 ICR2, and ICR1 loci were normal.

### TABLE 1 Results of the Methylation Assays of the DMRs at GNAS by Pyrosequencing and MS-MLPA

<table>
<thead>
<tr>
<th></th>
<th>NESP, %</th>
<th>AS, %</th>
<th>XL, %</th>
<th>A/B, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrosequencing assays</td>
<td>42–62</td>
<td>42–57</td>
<td>46–58</td>
<td>41–53</td>
</tr>
<tr>
<td>Patient</td>
<td>93</td>
<td>5</td>
<td>46</td>
<td>2</td>
</tr>
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<tr>
<td>Patient</td>
<td>95</td>
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<td>44</td>
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</table>

AS, antisense DMR; A/B, A/B DMR; NESP, NESP DMR; NV, normal values; XL, XL DMR.
The mother’s genomic DNA did not exhibit methylation abnormalities or deletions. Molecular analysis of the GNAS locus from the patient was consistent with the diagnosis of sporadic PHP1B with broad epigenetic defects in GNAS.

DISCUSSION
In contrast to PHP1A, PHP1B was initially thought to be an isolated form of PTH resistance. The first reported case of TSH resistance in patients with PHP1B occurred in a 5-year-old patient bearing a paternal isodisomy of chromosome 20q.14 Thereafter, Liu et al2 predicted the possibility of TSH resistance in patients with PHP1B, based on the monoallelic expression of Gsa in the thyroid. Later, imprinting defects in GNAS were revealed in several patients with mild TSH resistance, who had been misdiagnosed with PHP1A because of PTH resistance and the clinical features of AHO, despite the lack of GNAS exon 1 through 13 mutation.4,5 TSH resistance is a rare cause of congenital hypothyroidism. Inactivating mutations of the TSH receptor are a common cause of TSH resistance15 and were ruled out by sequencing for our patient. TSH resistance as diagnosed by the biochemical screening of newborns for congenital hypothyroidism has been reported in PHP1A; circulating TSH levels are moderately elevated (<60 mIU/L), and thyroid hormone levels are typically low or subnormal.16–18 Thus far, no epigenetic defect in GNAS has been reported with congenital hypothyroidism. Interestingly, our patient presented not only with an elevated TSH level at birth (50 mIU/L) but also with several clinical features of hypothyroidism.

Our patient exhibits an epigenetic defect in GNAS affecting all DMRs except for the XLoS DMR. This pattern has been previously described in 3 patients with sporadic PHP1B, all presenting with mild resistance to TSH.6,8 The XLoS protein has similarities to the Gsa protein, such as the ability to function as a distinct α-subunit of the G protein and to transduce signals via cAMP under certain conditions19; it has been suggested that this Gsa-like role should prevent TSH resistance in sporadic PHP1B with biallelic expressions of XL induced by LOMs in the XL DMR.8 Conversely, in our case the lack of any imprinting defect at XL might have been involved in the early TSH resistance because monoallelic XL expression cannot rescue the decrease in Gsa expression level.

Given the unusual clinical features of this patient, multilocus methylation assays were performed to rule out other imprinting disorders and multilocus imprinting defects.9,12,20,21 No additional epigenetic changes were observed, including those involved in Beckwith–Wiedemann syndrome, a pediatric overgrowth disorder that is a common cause of macroglossia.22 In contrast to patients with PHP1A, who are typically of short stature, patients with sporadic PHP1B are of normal height or taller. Patients with PHP1A or PHP1B can manifest obesity.7–9 Moreover, macroglossia has been reported in 3 cases of sporadic PHP1B.9 Perhaps this clinical feature is not as unusual in patients with sporadic PHP1B as is commonly assumed. However, the molecular mechanism of this feature remains unknown.

CONCLUSIONS
Recent studies have demonstrated an overlap between the clinical features of PHP1A and PHP1B. As shown in this report, the presence of TSH resistance or AHO is likely to mislead practitioners to the diagnosis of GNAS exon 1 through 13 mutation–negative PHP1A rather than PHP1B. This finding emphasizes the need for a new PHP classification system based on the molecular finding.23 Not only mild TSH resistance but also strong TSH resistance and congenital hypothyroidism can be present with GNAS imprinting defects. This finding has important consequences for the etiologic diagnosis of congenital hypothyroidism because it adds a new cause of the disease. In the absence of molecular diagnosis at birth, screening for PTH resistance must occur during childhood to establish the etiology of congenital hypothyroidisms, notably those associated with unusual clinical features such as persistent macroglossia.

ACKNOWLEDGMENTS
We thank Professor Richard Nicollas, pediatric ear, nose, and throat specialist; Professor Jean-Christophe Dubus, a pediatric pneumologist; and Professor Philippe Louis Devred, a pediatric radiologist, for their respective expertise in this case and their roles in the care process. We thank Nicole Peyrol of the Molecular Biology Laboratory for the GNAS genetic analysis.

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