PCR-Based Analysis of Differentially Methylated Regions of GNAS Enables Convenient Diagnostic Testing of Pseudohypoparathyroidism Type Ib

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BACKGROUND: Pseudohypoparathyroidism type Ib (PHP Ib) is characterized by parathyroid hormone (PTH) resistance, which can lead to hypocalcemia, hyperphosphatemia, and increased serum PTH. The disorder is caused by mutations in regulatory regions of the GNAS gene (GNAS complex locus) that lead to interferences in the methylation status of alternative GNAS promoters, such as exon A/B, NESP55, and XLα-s. PHP Ib comprises disorders that show distinctive changes in methylation status but share the same clinical phenotype: (a) loss of methylation only at exon A/B of the GNAS gene and involving no other obvious epigenetic abnormalities [e.g., those caused by heterozygous microdeletions in the STX16 (syntaxin 16) region and found in many patients with autosomal dominant (AD) PHP Ib]; (b) methylation abnormalities at several differentially methylated regions (DMRs), which are observed in most patients with sporadic PHP Ib and some families with AD PHP Ib.

METHODS: To permit early and reliable diagnosis of suspected PHP Ib, we designed methylation-sensitive restriction enzyme–based and bisulfite deamination–based PCR tests for exon A/B and NESP55 DMRs.

RESULTS: Both PCR strategies permit proper methylation testing of GNAS and NESP55 DMRs and elucidate different disease subtypes. We have identified a novel microsatellite repeat polymorphism within GNAS exon A/B, and pedigree analyses have shown its presence to be conclusive evidence for familial disease.

CONCLUSIONS: We provide a simple diagnostic test for PHP Ib, an imprinting disorder caused by different molecular changes within the GNAS complex locus. PHP Ib, a complex and diagnostically challenging clinical phenotype, can be treated successfully by taking steps before the manifestation of symptoms to avoid clinical complications in affected patients or asymptomatic members of affected families who show positive results in genetic tests.

Pseudohypoparathyroidism type I (PHP)3 is defined as parathyroid hormone (PTH) resistance that produces a compensatory increase in PTH concentration, hypocalcemia, hyperphosphatemia, and a decreased response of urinary cAMP to the administration of exogenous PTH.

The disease can be divided into different groups (1–4). In contrast to PHP type Ia (PHP Ia) and PHP Ic, patients with PHP Ib present with PTH resistance limited to the proximal renal tubules and lack the physical features of Albright hereditary osteodystrophy (AHO). This disorder is caused by mutations in regulatory regions that lead to a tissue-specific diminished transcription of the gene encoding the α subunit of the stimulatory G protein (Gsα) (1–5).

Alternative splice variants of GNAS (GNAS complex locus) seem to be involved in transcription regulation and are transcribed from different promoter regions and alternative first exons [e.g., NESP55, NESPas...
antisense transcript of NESP), XLα-s, and exon A/B (6–8)]. These variants also underlie a tissue-specific imprinting pattern, which is regulated by the methylation of differentially methylated regions (DMRs) and leads to allele-specific transcription. NESP55 and XLα-s exons are primarily expressed in neuroendocrine tissue and predominantly transcribed from the maternal allele and the paternal allele, respectively (Fig. 1). The ubiquitously transcribed but untranslated exon A/B (also known as exon 1A) is usually methylated at the maternal allele and thus predominantly transcribed from the paternal allele (6, 9). Gso is generally biallelically transcribed, but in some neuroendocrine tissues (such as the renal proximal tubules, the PTH responsive tissue) paternal transcription is silenced by a still unknown mechanism.

In PHPib, in addition to silencing of the paternal allele, the transcription of Gso from the maternal allele is also prevented (10–15). PHPib can be divided on the basis of the mode of inheritance into sporadic PHPib and autosomal dominant (AD) PHPib. Familial and sporadic forms of PHPib have distinct GNAS imprinting patterns that occur through different defects in the imprinting mechanism. Most known cases of AD PHPib are caused by heterozygous maternally inherited 3-kb or 4.4-kb deletions within the STX16 (syntaxin 16) region that lead to both a methylation defect limited to exon A/B and active exon A/B transcription from both paternal alleles (11). In 2 familial cases, a deletion in the NESP55 DMR that encompasses exon NESP55 and 2 of the antisense exons of NESPas leads to a loss of all maternal GNAS imprints and derepression of normally maternal silenced transcripts (16). Patients with sporadic PHPib appear to have mostly broader methylation defects, which can include all of the known DMRs of GNAS (13, 15, 17).
In many cases, diagnosis is made from clinically detected changes and changes in laboratory findings only; however, because many AD PHPIb cases remain asymptomatic for a long time and because disease manifestation with hypocalcemic seizures can be life threatening, screening is advised for all siblings of affected individuals [17]. Southern blotting is used for diagnostic testing in a few specialized laboratories, but such testing is time consuming and laborious. Because the complex molecular genetic background makes interpretation of results difficult, such testing has to be done by specialized laboratories with relevant experience. Although some published research has demonstrated bisulfite-based methylation testing via “bisulfite genomic sequencing” to be useful for determining aberrant methylation patterns, no convenient standard PCR diagnostic test has been published to the best of our knowledge. We describe 2 alternative PCR strategies for PHPIb-methylation testing that are both suitable for establishing routine testing and capable of distinguishing between 2 underlying genetic defects of clinically affected patients, as well as identifying unaffected female carriers.

### Materials and Methods

#### PATIENT SAMPLES AND CONTROLS

Wild-type control DNA derived from peripheral blood samples from healthy male and female individuals was extracted with the High Pure PCR Template Preparation Kit (Roche Applied Science). DNA samples obtained for PHPIb pedigree analysis and to be used in diagnostic testing were isolated from peripheral leukocytes with the QIAquick DNA kit (Qiagen).

#### DNA-METHYLATION TESTING

Unmethylated and methylated fractions of DNA from healthy control individuals and patient samples were digested in a total volume of 15 μL. For methylated DNA fractions in which unmethylated DNA was digested, 500 ng DNA was digested with 5 U each of AcI (New England Biolabs), Hin6I (MBI Fermentas), and HpaII in buffer Y (MBI Fermentas) supplied with the HpaII restriction enzyme. For unmethylated fractions, methylated DNA was digested with 7 U of McrBC (New England Biolabs) in NEBuffer 2 to which GTP and BSA were added, as recommended by the supplier. In addition, 0.3 μL of 10× T4 DNA Ligation Buffer (MBI Fermentas) were added to this restriction enzyme digestion to improve McrBC fidelity (see Supplemental Data S1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol54/issue9). Both unmethylated

### Table 1. Phenotypic and biochemical changes observed in index patients and family members.

<table>
<thead>
<tr>
<th>Patient ID (sex, PCR result)</th>
<th>Age at diagnosis, years</th>
<th>Serum Ca, mmol/L</th>
<th>Serum Ph, mmol/L</th>
<th>PTH, pg/mL</th>
<th>PTH test</th>
<th>TSH, U/L</th>
<th>FT3, pmol/L</th>
<th>FT4, pmol/L</th>
<th>Clinical signs</th>
<th>Family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (F, a)</td>
<td>12.5</td>
<td>1.4</td>
<td>9</td>
<td>912</td>
<td>Pos</td>
<td>5.6</td>
<td>5.4</td>
<td>11.5</td>
<td>ncc, shX, ncEEG, cECG</td>
<td>Mother and brother with PHPIb; no other family members</td>
</tr>
<tr>
<td>B (F, a)</td>
<td>6 (confirmed at 25)</td>
<td>NK</td>
<td>NK</td>
<td>NK</td>
<td>Pos</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>NK</td>
<td>Mother of patient A</td>
</tr>
<tr>
<td>C (F, b)</td>
<td>8.5</td>
<td>1.42</td>
<td>2.95</td>
<td>809</td>
<td>Pos</td>
<td>1.43</td>
<td>6.52</td>
<td>1.43</td>
<td>cc, shX, ncEEG, cECG</td>
<td>No PHPIb in parents, both brothers, aunt and uncle, and their children</td>
</tr>
<tr>
<td>I (M, b)</td>
<td>9.3</td>
<td>1.6</td>
<td>3.0</td>
<td>265</td>
<td>ND</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>cc, shX, ncEEG, ncECG</td>
<td>No PHPIb in parents and siblings</td>
</tr>
</tbody>
</table>

* PCR testing revealed PHPIb in patients A and B ([a, loss of methylation only at exon A/B but no other epigenetic abnormalities within these patients]. Patients C and I showed pattern b (methylation abnormalities at all GNAS DMRs). A 3-kb STX16 deletion found in patients A and B was previously described by Bastepe et al. ([13]; kindred E within this reference]). All concerned patients received substitution treatment with calcitriol, some received calcium therapy, and the hypothyroidism patient underwent levothyroxine treatment.

*Ph*, thyroid-stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine; Pos, positive; N, nonpathologic; NK, not known; ND, not determined; ncc, no signs of cerebral calcification; shX, signs of hyperparathyroidism in radiograph; ncEEG, no signs of changes in electroencephalogram; cECG, changes in electrocardiogram; cc, cerebral calcification; cEEG, changes in EEG; ncECG, no signs of changes in ECG.
and methylated restriction digests were incubated for 16 h at 37 °C.

We carried out bisulfite DNA deamination on 500 ng of DNA for 4 h at 55 °C with an oil overlay, as has previously been described (18, 19). After deamination, we purified the bisulfite-treated DNA on QIAquick Spin Columns (Qiagen) and used it for PCRs.

PCR analyses were performed either on native DNA after methylation-sensitive restriction enzyme digestion or on bisulfite-deaminated DNA. Details on the PCR protocols are provided in the online Data Supplement.

We identified a GGCGC pentanucleotide repeat within GNAS exon A/B via direct sequencing of the specific amplification products, which was carried out by a commercial sequencing service (IBL).

Results

Design of Control and Diagnostic PCRs

As we have outlined in the Introduction, PHP1b cases consist of either (a) loss of methylation at exon A/B of the GNAS gene alone or (b) methylation abnormalities at all GNAS DMRs, including the DMR at exon A/B, which is caused by a deletion that removes the NESP55 DMR (see table within Fig. 1). Thus, at minimum a test should identify both parental alleles from these 2 DMRs. As given in the scheme, complete restriction enzyme–based methylation analyses require distinct amplifications of the unmethylated and methylated DNA fractions (Fig. 1). This requirement is met by using McrBC to digest selectively methylated 5′CpG sites and a combination of AciI, Hin6I, and HpaII (to retain methylated 5′CpG sites), respectively. In line with these assumptions, we selected sequences from that gene region and defined PCR targets in these CpG islands with frequent recognition sites for sites of methylation-sensitive restriction enzymes AciI, Hin6I, and HpaII. By the same design strategy, we have adopted a multiplex control PCR that covers CpG islands or DMRs of genes of known methylation status as a control to confirm the completion of restriction enzyme digests.

We have used a series of DNA samples from healthy male and female individuals and from a blinded series of confirmed PHP1b patients and family members in control PCRs to test samples for complete methylation-sensitive digestion.

IGF2 [insulin-like growth factor 2 (somatomedin A)], H19 [H19, imprinted maternally expressed transcript (non-protein coding)], and XIST [X (inactive)-specific transcript (non-protein coding)] gene products were amplified when the methylated DNA fraction was used as template, and the absence of gene products for ABL1 (c-abl oncogene 1, receptor tyrosine kinase) and PITX2 (paired-like homeodomain 2) confirmed the complete digestion of unmethylated DNA. The FMR1 (fragile X mental retardation 1) gene product is derived from the inactive X chromosome and is present in female samples only. Amplification of the unmethylated DNA fraction after McrBC digestion confirms the completeness of digestion when XIST products are not amplified from male samples but are present only in female samples. Such products are derived from the unmethylated XIST gene located on the inactive X chromosome. On the active X chromosome, XIST is methylated and thus absent from male unmethylated amplicons.

In theory, the H19 amplicon should be present in all unmethylated samples, but it was absent from each sample analyzed. When we tested 30 different control DNAs from healthy individuals, we obtained the same pattern of a lack of H19 amplification in unmethylated samples (see Supplemental Data S1 in the online Data Supplement). Thus, the absence of H19 amplicons confirms the completion of McrBC digestion.

Methylation Testing of Samples

We were able to use the multiplex control PCR to confirm proper restriction enzyme digestion and to identify the sex of the individuals for the PHP1b samples, which we analyzed blinded (Fig. 2).

For methylation analyses of GNAS DMRs covering exon A/B and NESP55, we optimized reaction conditions to amplify both products within a single PCR reaction. Duplex PCRs with both unmethylated and methylated DNA fractions from controls showed the proper detection of both products, confirming the typical methylation patterns of the analyzed regions. Methylated amplicons derived from patient samples A, B, C, and I, however, lacked the 302-bp GNAS exon A/B amplification product, but the amplification of the NESP55 product excluded the possibility of PCR failure. This band pattern suggests the absence of the unmethylated GNAS exon A/B allele. Amplicon patterns derived from the unmethylated fraction of the control DNAs show the presence of both products. Both amplicons are also present in unmethylated PCRs of samples A and B, thereby confirming PHP1b according to the constellation of characteristics [A in Fig. 1], which has been found to be due to a maternal STX16 gene deletion. Despite the analysis of other samples, samples from patients C and I lack the NESP55 amplicon. This result resembles configuration B (methylation abnormalities at all GNAS DMRs due to a deletion that removes a NESP55 DMR) in the scheme (Fig. 1). When blinded methylation tests were finished, patient data were made available for comparison. The unblinded results were in line with those of Southern blot analyses and confirmed the clinical findings (Table 1). Thus, both daughter (A) and mother (B) from family 1 are
affected and have hereditary PHPIb, whereas patient C from family 2 (samples C–G) and patient I from family 3 (samples H–K), have sporadic PHPIb. These data are in line with results from diagnostic testing performed in external laboratories. The data for family 1 have been published previously, and the diagnosis of PHPIb has been confirmed at the molecular level for both patient A and patient B (11, 13, 20). All concerned patients received substitution treatment with calcitriol, some received calcium therapy, and the hypothyroidism patient underwent levothyroxine treatment.

IDENTIFICATION OF A NOVEL MICROsatellite POLYMORPHISM

Although the unmethylated amplicon of sample C lacks the 362-bp NESP55 product, we obtained an additional PCR product with a band intermediate in size between the expected GNAS exon A/B (302 bp) and NESP55 (362 bp) products. The same additional band was also present in sample A (Fig. 3A). These additional bands are not present in the lanes for the methylated product. As expected, these products were derived from the unmethylated GNAS exon A/B allele when these products were produced by a single PCR (i.e., NESP55 primers omitted). Amplicons derived from undigested DNA samples (A, C, F, I) showed the same product patterns as those obtained from unmethylated DNA. This finding excludes the possibility of any artificial generation of the additional GNAS exon A/B product band caused by the restriction enzyme treatment. The band was approximately 330 bp in size on an agarose gel, but sequencing of these products yielded a size of 307 bp. The larger size was due to a 5-bp insertion of a GGGCGC repeat unit. Samples A and C were heterozygous for 2 alleles with 2 and 3 GGGCGC repeat units. This polymorphism, which is located upstream of base position 56 897 684 on chromosome 20 (+ strand; according to the UCSC Genome Browser, Mar. 2006 Assembly; HG18), has not been annotated in databases and is different from an annotated 36-bp insertion about 34 kb downstream from this polymorphic site (see Fig. 1 in the online Data Supplement).

We used this polymorphic site as a marker for pedigree analysis of a family consisting of individuals C–G within our patient series. In addition to the methylation tests, this marker unequivocally identified the sporadic nature of PHPIb in patient C. Several years before we performed this methylation test on samples from the patient and his relatives, we studied the family extensively with microsatellite analysis to exclude hereditary disease (data not shown). Both the affected index male and his healthy brother carry the same allele, with the GGGCGC repeat insertion derived from their mother, who was a suspected PHPIb carrier. The typical methylation pattern obtained for the healthy brother G and his mother E excludes a PHPIb clinical diagnosis as well as hereditary disease. The polymorphic insertion is also present in patient A but is not seen in the mother (sample B), who is also affected clinically. Our test confirms hereditary disease in both mother and daughter, but the insertion allele is not present in

**Fig. 2. Control multiplex PCR.**

Gel images of the control PCR to test completion of digestion with the methylation-sensitive restriction enzymes AciI, Hin6I, and HpaII [methylated DNA (ME) is not digested] and with McrBC [unmethylated DNA (UM) is not digested]. Presented are DNA data for male and female control individuals (1–11) and patients (A–K). Indicated are amplified PCR products, amplicon sizes, and the methylation status in typical DNA (X and X denote PCR products derived from the inactive and active X chromosomes, respectively). Amplified products are in boldface; FMR1 products identify the sex of the patient. The H19 (gray) UM amplicon is missing after McrBC digestion (see Supplemental Data S1 in the online Data Supplement). ABL1 and PITX2 products for H samples (UM) are shifted to lower sizes on the gel because of overrepresentation of smaller amplicons in the multiplex amplification. m, male; f, female; S, 100-bp size marker; —, PCR negative control.
the mother and is apparently derived from the father (not available for testing).

To exclude any major disease-causing effect, we analyzed additional control DNA samples from healthy individuals via the PCR and agarose gel electrophoresis and found 18 heterozygotes among 54 samples (11 of 25 female samples and 7 of 29 male samples).

**Bisulfite-based Methylation Test**

To set up a convenient methylation-specific PCR (MS-PCR) test, we designed primers specific for deaminated DNA by targeting the same sequences used for the restriction enzyme–based test. We initially optimized the PCR reaction conditions with deaminated DNA from healthy individuals and DNA methylated in vitro to obtain the desired band patterns for amplifying both unmethylated and methylated GNAS exons A/B and NESP55 in 2 distinct duplex MS-PCR reactions. We then subjected deaminated PHPIb DNA samples to these MS-PCRs. The methylation pattern indicated after agarose gel electrophoresis unequivocally corroborated the results of the restriction enzyme–based testing. In addition, the GNAS exon A/B MS-PCR test revealed the identified polymorphic insertion. Both patients A and C presented a relatively faint additional GNAS unmethylated band of increased size on agarose gels, which was derived from a second unmethylated allele (Fig. 3B). This result is in line with the restriction enzyme–based test results for amplicons produced by amplification of the unmethylated (i.e., triply digested with AcI, HpaII, and Hin6I) DNA fraction (Fig. 3A).

In the NESP55 MS-PCR, affected patients C and I presented only the NESP55 methylated band on agarose gels (Fig. 3B; see Supplemental Data S2 in the online Data Supplement). Furthermore, we unequivocally established a PHPIb diagnosis for 12 clinically confirmed cases with the GNAS exon A/B MS-PCR. When the NESP55 MS-PCR was used to identify the molecular genetic defect, 8 of 11 patients presented only the NESP55 methylated-product pattern produced by an underlying 4.7-kb NESP deletion. No information regarding the familial background of disease was avail-

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**Fig. 3. Methylation testing of samples and controls.**

(A), Restriction enzyme–based PCR test. Both methylated (ME) and unmethylated (UM) DNA fractions are amplified in 2 distinct reactions. (B), MS-PCR. Deaminated DNA is amplified within the GNAS exon A/B and NESP55 MS-PCRs; products derived from UM and ME alleles are indicated. Notable in (B) was our analysis of DNA from a bone biopsy from an AHO patient (L), which revealed the typical methylation pattern. Amplification controls with native DNA and with no DNA served as negative controls and showed no bands on agarose gels (data not shown). PCR reactions were all duplexed, and the respective products are indicated along with amplicon sizes. The absence of methylated GNAS exon A/B products easily identifies the PHPIb patients; the diagnosis can be further refined by examining NESP55 product patterns (type A, patients A and B; type B, patients C and I; see text). Lettering is as in Fig. 2 legend.
able for these 8 cases. The other 4 PHPIb cases presented both unmethylated and methylated NESP55 products. These findings are consistent with disease caused by an STX16 deletion. Two of these patients were from the same family; no additional family members of the 2 remaining PHPIb patients were available for testing.

Discussion

DNA-methylation testing via bisulfite deamination not only has become a standard application for detecting aberrant de novo methylation of cancer genes in somatic tumor tissues but also has been useful for uncovering hereditary genetic traits, such as Prader–Willi syndrome (MIM 176270), Angelman syndrome (MIM 105830) (21), Beckwith–Wiedeman syndrome (MIM 130650), and Silver–Russel syndrome (MIM 180860) (22, 23).

Because the melting temperatures of C-rich (and CG-rich) DNA regions are tremendously reduced after the conversion of unmethylated cytosines to uracils with bisulfite deamination, this technique has been successfully applied in tests of expansions of repeats with high GC contents for both methylated and unmethylated repeats (18, 19, 24, 25). Thus, this technique also permits testing for repeat-expansion traits such as fragile X syndrome (MIM 309550) (19), myoclonus epilepsy, type Unverricht–Lundborg repeat expansion in CTSB [cystatin B (stefin B)] (MIM 254800) (18), and testing for congenital central hyperventilation syndrome [PHOX2B (paired-like homeobox 2b) polyalanine expansion; MIM 209880] (25). In addition, the microsatellite of the human androgen receptor gene located on the X chromosome (MIM 313700) has frequently been used for clonality studies and would also be suitable for testing of Kennedy spinal and bulbar muscular atrophy (MIM 313200) caused by a repeat expansion in AR [androgen receptor (dihydrotestosterone receptor; testicular feminization; spinal and bulbar muscular atrophy; Kennedy disease)] (19, 24, 26). These applications exemplify the practical relevance of methylation testing in research and molecular genetic diagnostics.

Because PCR tests are easily set up with the primers described in publications of relevant methods, such tests are the methods of choice for relatively rare hereditary syndromes and diseases. Such may be the case for molecular genetic identification of PHPIb, a relatively rare disease produced by the distortion of the methylation patterns within the imprinted GNAS gene complex.

PHPIb diagnosis based only on clinical and laboratory results is difficult, because the expression of disease features can be very subtle. Hypocalcemia is often not present at birth or during early childhood but may manifest during development, often in dramatic, occasionally life-threatening ways, such as hypocalcemic seizures. Moreover, because PHPIc and type II PHP patients also exhibit typical Gsα protein activity and because AHO features may not be well expressed in these patients, the differential diagnosis of these PHP subtypes can be difficult. Thus, early and reliable diagnosis is required in cases of suspected PHPIb. Moreover, molecular genetic diagnosis is required to determine the basis for genetic counseling of the affected patients and their families. Because a lack of GNAS exon A/B methylation on the maternal allele has been found in all investigated PHPIb patients and because methylation changes involve several transcripts in some AD PHPIb patients and in most sporadic PHPIb patients, methylation testing is a powerful and reliable tool for safe and early PHPIb diagnosis (5, 16, 17, 27–29).

In cases of suspected PHPIb, molecular analysis is warranted for diagnostic purposes because the clinical and laboratory changes are sometimes variable and often develop during childhood. To elucidate aberrant methylation patterns derived from these conditions and to allow certain and early diagnosis in family members of a PHPIb patient, we designed both methylation-sensitive restriction enzyme–based and bisulfite deamination–based PCR tests of GNAS and NESP55 DMRs. Both strategies permit reliable PHPIb diagnosis by elucidating abnormal methylation patterns within the amplified imprinted regions. Our analysis of patient samples with these PCR tests have confirmed the clinical PHPIb diagnoses and the molecular genetic findings produced in Southern blotting tests (11, 13, 20). Thus, testing native DNA by PCR analysis after restriction enzyme digestion and MS-PCR testing of bisulfite-deaminated DNA enable reliable diagnostic analyses for both disease-causing conditions, thereby obviating cumbersome Southern blotting tests. Either of the 2 PCR tests can be used, depending on the preferences of the interested institutions, but the restriction enzyme–based PCR test can serve as a control, especially for initial evaluations of the MS-PCR tests. This approach might be of particular importance for a rare disease such as PHPIb, for which the availability of positive controls is limited.

Both PCR assays for the analysis of GNAS exon A/B cover a polymorphic 5-bp GGGCG insertion, which heretofore has not been annotated. After extensive review of the relevant literature, we found a reference to this site in a publication by Demura et al. (30). Our PHPIb pedigree analysis and estimates of the heterozygote frequency in healthy controls allow us to exclude any (major) disease-causing effect of the polymorphic site. This site may be helpful in research and
The elucidation of methylation patterns of the GNAS gene complex of patients and control individuals with bisulfite-deaminated DNA has been used in several related research projects (13, 30, 31). To the best of our knowledge, however, a combined test that reveals both GNAS exon A/B and NESP55 methylation patterns and that has been designed for practical PHPIb MS-PCR testing has not been published. This test can be performed very easily in standard laboratories with expertise in MS-PCR testing. DNA deamination may be the preferred test compared with the restriction enzyme–based approach, especially when DNA deamination is done routinely on series of DNA samples analyzed in parallel. The restriction enzyme–based approach may be an option for laboratories that have not established DNA-deamination protocols. Combining both tests would allow each test to serve as a control for the other, which would be helpful during test setup and initial evaluation. Both strategies might also be of interest to those who specialize in molecular genetic and clinical research. Like other PCR techniques, both the restriction enzyme and bisulfite-deamination PCR tests we have described are sensitive and work with minimal amounts of DNA, and our experience suggests that the MS-PCR approach also works with limited quantities of DNA. Thus, this test is well suited for routine diagnostic testing and for analyses of archived material, such as paraffin blocks or cells from cytogenetic analyses that have been fixed with methanol and acetic acid. Because of the minimal DNA quantities required, both tests are suited for analyses of GNAS-methylation patterns in DNA from limited sources; such analyses may be helpful for defining tissue-specific methylation patterns.

PHPIb diagnosis is based on expert clinical examination and a combination of specialized electrophysiological and biochemical tests. Because of the highly variable PHPIb phenotype, establishing a PHPIb diagnosis and especially distinguishing it from PHPIc or PHP type II may be difficult. A reliable PHPIb diagnosis is very important for guiding hormone treatments to prevent intracranial calcification, the cause of most of the complications of manifested disease. PHPIb is thus an example of a hereditary trait in which the genetic diagnosis enables efficient treatment. We have described PCR-based DNA-methylation tests suitable for revealing known molecular genetic abnormalities that underlie a PHPIb clinical diagnosis. These technically simple tests permit reliable diagnosis of an otherwise complex and diagnostically challenging clinical phenotype, which, when diagnosed early, can be successfully treated to avoid clinical complications in affected patients or in asymptomatic members of affected family members who have tested positive in genetic tests.

Grant/Funding Support: This study was funded in part by the project on AHO (BMBF no. GM1G0315) granted to O. Hiort.

Financial Disclosures: None declared.

Acknowledgments: We thank Harald Jueppner and Murat Bastep for providing molecular genetic data in their laboratory for PHPIb DNA samples.

References


