Progressive osseous heteroplasia: a model for the imprinting effects of GNAS inactivating mutations in humans.

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Progressive Osseous Heteroplasia: A Model for the Imprinting Effects of GNAS Inactivating Mutations in Humans


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Context: Heterozygous GNAS inactivating mutations are known to induce pseudohypoparathyroidism type 1a when maternally inherited and pseudopseudohypoparathyroidism when paternally inherited. Progressive osseous heteroplasia (POH) is a rare disease of ectopic bone formation, and studies in different families have shown that POH is also caused by paternally inherited GNAS mutations.

Objective: Our purpose was to characterize parental origin of the mutated allele in de novo cases of POH and to draw phenotype/genotype correlations according to maternal or paternal transmission of a same GNAS mutation.

Design and Setting: We conducted a retrospective study on patients addressed to our referral center for the rare diseases of calcium and phosphorus metabolism.

Patients and Methods: We matched 10 cases of POH with cases of pseudohypoparathyroidism type 1a carrying the same GNAS mutations.

Main Outcome Measures: The parental origin of the mutated allele was studied using informative intragenic polymorphisms and subcloning of PCR products.

Results: Paternal origin of GNAS mutations was clearly demonstrated in eight POH cases including one patient with mutation in exon 1. Genotype/phenotype analyses suggest that there is no direct correlation between the ossifying process and the position of the inactivating GNAS mutation. It is, however, more severe in patients in whom origin of the mutation is paternal. Severe intrauterine growth retardation was clearly evidenced in paternally inherited mutations.

Conclusions: Clinical heterogeneity makes genetic counseling a delicate matter, especially in which paternal inheritance is concerned because it can lead to either a mild expression of pseudopseudohypoparathyroidism or a severe expression of POH. (J Clin Endocrinol Metab 95: 3028–3038, 2010)

Abbreviations: AHO, Albright’s hereditary osteodystrophy; CT, computed tomography; IUGR, intrauterine growth retardation; PHP1a, pseudohypoparathyroidism type 1a; POH, progressive osseous heteroplasia; PPHP, pseudopseudohypoparathyroidism; SNP, single-nucleotide polymorphism.
Progressive osseous heteroplasia (POH; online inheritance in man 166350) is characterized by progressive heterotropic ossification during infancy that initially occurs within the dermis and subcutaneous fat (osteoma cutis) and then extends progressively into deep connective tissue and skeletal muscle (1). It is a rare syndrome, with less than 100 cases described in the literature. POH was recently included in the phenotypic spectrum of GNAS inactivation mutations (2). GNAS encodes for a guanine nucleotide-binding protein (Gαs protein) and is mapped to locus 20q13.2-13.3, a complex locus subject to paternal imprinting. This locus gives rise to several different transcripts expressed from upstream promoters/first exons and is spliced onto shared downstream exons from exon 2 onward with different patterns of imprinted expression. Hence, they may be derived from the paternal allele (XLαs, NESPAS, and exon 1A), the maternal allele (NESP55), or both (Gαs) and can be either coding (Gαs and XLαs, NESP55) or nontranslated (exon 1A and NESPA) (3). Whereas NESP55, which encodes the chromogranin-like neuroendocrine secretory protein, is confined to the single first exon, XLαs has an open reading frame contiguous with that of Gαs such that it encodes a protein (XLαs) in which the N-terminal end of Gαs is replaced by a large acidic XL domain. As a consequence, GNAS mutations lead to a wide spectrum of phenotypes (4, 5). Heterozygous inactivating GNAS mutations are known to induce pseudohypoparathyroidism type 1a (PHP1a; online inheritance in man 103580) and pseudopseudohypoparathyroidism (PPHP; online inheritance in man 300800). PHP1a is characterized by Albright’s hereditary osteodystrophy (AHO) and is associated with multiple hormone resistance, particularly to PTH and TSH if the maternal allele is mutated. Indeed, this is the only allele expressed in the imprinted tissue (3, 5, 6), in particular in the proximal renal tubule (7), pituitary gland (8), thyroid (9, 10), and gonads (10). PPHP, on the other hand, is defined by isolated AHO clinical syndrome with no hormonal resistance (normal PTH level). Recently, in their analysis of cases of familial transmission, Shore et al. (2) provided evidence that POH is caused by paternally inherited GNAS mutations.

In this study, we conducted clinical, biological, and genetic studies of patients referred to our laboratory as having POH. Because only about 20 GNAS mutations (2, 4, 11–16) had previously been reported with POH, to our knowledge, our aim was to learn whether specific mutations were associated with POH phenotype. We also studied the pattern of inheritance and attempted to clarify how phenotype/genotype correlations might change according to maternal or paternal transmission of GNAS mutation to further understand the differences and similarities between these two conditions. For this purpose, patients diagnosed with PHP1a in whom the same mutation was found were selected from our database. Last but not least, this paper will for the first time put forward data from a human case study that is able to distinguish between XLαs functions and paternal Gαs haploinsufficiency in humans.

Patients and Methods

Patients
Ten patients from unrelated families (numbered 1–10), with clinical phenotypes evoking POH and carrying a GNAS mutation were studied. These patients were recruited through clinical geneticists or dermatologists. The clinical criteria used for diagnosis of POH are those described by Kaplan and colleagues (1, 17), i.e. the presence of heterotopic ossifications progressing from cutaneous and subcutaneous fat into deep connective and muscular tissue. In patients 1, 3, 5, 8, and 10, the tissue depth of the calcifications was ascertained by computed tomography (CT) imaging.

All of the evaluated subjects have no remarkable familial history, except for one family (family 7) with familial cases.

Nine patients from seven unrelated families (referenced A–G) carrying the same mutations as POH cases and diagnosed with PHP1a were selected from our database or the literature (patient D) (18). The clinical criteria used for AHO diagnosis included brachymetacarpia (of fourth and fifth ray), round-shaped face, short stature (below –2 SD), excess weight or obesity (body mass index >85% for age), and more or less associated with the following variable features: mental retardation, sc calcifications, and intracerebral calcifications.

Birth biometrics data were compared with the French reference chart AUDIPOG and results were expressed as centiles (19).

Laboratory investigations
Biochemical and endocrine analyses were performed using standard methods, with calcium, phosphate, and PTH measurements for all patients.

PTH resistance was defined by low calcium concentration (<2.2 mmol/liter), high serum phosphate concentration according to age, and increased serum level of PTH according to the normal values of the laboratory. The biological activity of Gαs protein (Gs bioactivity) was quantitatively evaluated in erythrocytes following the procedure described by Marguet et al. (20). Results were expressed in percentage of activity of normal adult controls. Gs bioactivity values less than 80% were considered to be reduced.

In two cases (patients 7 and 9), urinary cAMP response to synthetic human PTH administration was evaluated (Ellsworth-Howard test).

Statistical analysis
Gs activity between POH/PPHP and PHP1a patient was compared using the Student t test.

Molecular analysis
For each family, written informed consent was obtained from both patients and their parents for collection of DNA and molecular study. Genomic DNA was isolated from peripheral blood leukocytes using standard methods. We designed several sets of
primers for amplification of exons 1–13 of the GNAS gene and intron-exon junctions adapted from published sequences (21). PCRs were performed according to standard procedure except for exon 1 due to the highly GC-rich sequences. It may be summarized as follows: denaturation at 94°C for an initial phase of 5 min, followed by 10 cycles of 1 min at 94°C, 2 min at 72°C with a decrement of 1°C per cycle, 7 min at 72°C, followed by 30 cycles of 2 min at 94°C, 2 min at 68°C, 7 min at 72°C, and 10 min at 72°C. PCR products were purified and sequenced using the CEQ DTCS Quick Start kit (Beckman Coulter, Fullerton, CA) on the Beckman Coulter DNA sequencer.

To isolate the RNA of leukocytes, blood was collected into PAXgene tube (Beckton Dickinson, Lincoln Park, NJ). The RNA was purified using the PAXgene blood RNA kit (QIAGEN, Valencia, CA) with the deoxyribonuclease digestion step. Reverse transcription (RT-PCR) was performed on 1 μg of the total RNA, using 100 U of Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA), in accordance with manufacturer recommendations. PCR was then performed using 5 μl of the reverse transcriptase mixture, with the forward primer of exon 1 and reverse primer of exons 10–11 in a total reaction of 50 μl. Index cases’ parents were screened for the identified mutation by either DNA sequencing or, where possible, restriction analysis with the appropriate enzymes. When the mutation was identified as de novo; parental origin of the mutated allele was studied through familial informative intragenic polymorphisms [single nucleotide polymorphism (SNP)]: rs2295583 (GenBank accession number) in intron 3 (T/A), rs234629 in intron 6 (G/A), rs7121 in exon 5 (T/C), and rs919196 (T/C) in intron 6. The alleles from paternal and maternal origin were separated by subcloning, as described by Linglart et al. (6). After PCR, products were subcloned using the pGEM-T Easy Vectors kit (Promega, Madison, WI). Ten to 16 independent clones were chosen at random and sequenced. Each clone being differentially tagged by familial polymorphism, sequencing or restriction analysis allowed us to determine whether the mutation was on the maternal or paternal allele.

For patient 5 we used a specific strategy: the mutation in exon 1 eliminated the possibility of a PvuII restriction site. A large DNA fragment (from nucleotides 30516 to 32058), including the mutation and the informative SNP rs6123837 (G/A) in promoter was amplified using the following primers: GACGAGGAC-GAGCCTACTA and CTGCGGGGGCGCCCTTCGA. PCR product was digested with PvuII according to the manufacturer’s recommendations. The fragments were separated using gel electrophoresis and then extracted from the gel before finally being sequenced.

Methylation analysis
Exon A/B, NESP55, and XLas methylation was determined by quantitative PCR assay after digestion of genomic DNA with methylation-sensitive and methylation-insensitive restriction enzyme (22, 23).

Results
Clinical and biological phenotypes
Clinical phenotypes of POH subjects
Our study included 10 patients, five females and five males. The clinical and biological characteristics of the patients are described in Table 1. All patients had an early age of onset. Patients 3 and 5 developed ossifications at age 3 or 10 yr, respectively, whereas all the remaining cases displayed ossification during infancy. Degree of ossification varied: ossification was severe and impairing (Fig. 1), with extensive and invading lesions in four patients (1, 4, 6, and 10), whereas for four patients (2, 3, 5, 8, and 9), ossifications progressed into deeper tissues but had no impairing characteristics. Lastly, for patient 7 ossifications were superficial, with only cutaneous and sc lesions; they were, however, multiple or widespread. We were unable to give additional information on the evolution for this patient, who was classified as overlap POP/PPHP. Severe intrauterine growth retardation (IUGR; less than the third centile) was reported in eight patients (1, 2, 3, 4, 5, 8, 9, and 10) followed by feeding difficulties and growth retardation during prime infancy. With the exception of patient 6, none of the patients were overweight or showed signs of mental retardation.

Round faces and/or brachymetacarpia were observed in patients 2, 7, and 8. In patient 6 there is a persistent ambiguity between POH and PHP1a because this patient displayed severe, progressive, and impairing sc ossifications, which are associated with Albright’s phenotype.

Biological phenotypes of POH subjects
In all cases, again with the exception of case 6, there is an absence of hormonal resistance, with normal levels of calcium, phosphorus, and PTH and, when available, normal TSH value. An Ellsworth-Howard test was performed for patients 7 and 9 showing a normal increase in urinary cAMP. Gs bioactivity is normal or subnormal (between 70 and 93%) in all cases except in family 6 in which it is clearly reduced (52%).

Phenotypes of parents of index cases
For all patients but one (family 7), there were no affected parents or relatives and no family history of ectopic ossification. In family 7, mild sc calcifications were identified in the father of the affected case, as were mild AHO features in the sister and AHO phenotype in the sister’s two children. Their referral diagnoses were PPHP for the father and sister and PHP1a for the sister’s children (Fig. 2).

GNAS mutations analysis
Seven different heterozygous mutations were identified in POH cases (Table 1 and Fig. 3, bottom portion), with the same mutation in exon 1, c.85C>T (p.Q29X), being found in three unrelated families. Two had never previously been identified in either POH or PHP1a [frameshift mutations c.571_572delGT in exon 7 and c.623_624insT (p.E209X) in exon 8]. Three are new to POH (c.85C>T,
<table>
<thead>
<tr>
<th>Mutation</th>
<th>Subject (n)/sex</th>
<th>Age of first symptom/age at diagnosis</th>
<th>RF-BM-MR</th>
<th>Phenotype</th>
<th>Ca (mmol/liter)</th>
<th>Ph (mmol/liter)</th>
<th>PTH (pg/ml)</th>
<th>TSH (mUI/ml)</th>
<th>Gs-α activity</th>
<th>Familial transmission/mutated allele</th>
<th>Diagnosis</th>
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<tr>
<td>c.85C&gt;T (p.Q29X)</td>
<td>5/M</td>
<td>ND/11 yr</td>
<td>No-no-no</td>
<td>Multiple microSCO (knees, hands, feet) with some ossifications close to muscles</td>
<td>11 yr</td>
<td>2.31</td>
<td>1.65</td>
<td>55 (10–71)</td>
<td>3.2</td>
<td>73</td>
<td>De novo/paternal</td>
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<td></td>
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<td></td>
<td>Multiple, progressive, and impairing SCO (cervical, truncular, arms)</td>
<td>14 yr</td>
<td>2.3</td>
<td>1.8</td>
<td>88</td>
<td>5</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Multiple microSCO (knees, hands, wrist, buttocks, abdomen, feet) with some ossifications close to muscles</td>
<td>1 yr</td>
<td>2.2</td>
<td>2.1</td>
<td>178</td>
<td>ND</td>
<td>52</td>
<td>De novo/maternal</td>
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<td></td>
<td>6/M</td>
<td>PN/1 yr</td>
<td>Yes-yes-no</td>
<td>IUGR (10th centile)</td>
<td>22 yr</td>
<td>Ellsworth-Howard Test</td>
<td>Negative</td>
<td></td>
<td></td>
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<td></td>
<td>Multiple microSCO (knees, hands, wrist, buttocks, abdomen, feet) with some ossifications close to muscles</td>
<td>52 yr</td>
<td>2.2</td>
<td>0.6</td>
<td>Ni</td>
<td>74</td>
<td></td>
<td>DNA of parents not available</td>
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<td></td>
<td>9/F</td>
<td>2 yr/52 yr</td>
<td>No-no-no</td>
<td>IUGR (third centile)</td>
<td>2 yr</td>
<td>2.2</td>
<td>2.2</td>
<td>155</td>
<td>0.6</td>
<td>ND</td>
<td>Familial/maternal</td>
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<td></td>
<td>IUGR (10th centile)</td>
<td>4yr</td>
<td>2.2</td>
<td>2.2</td>
<td>155</td>
<td>0.6</td>
<td>ND</td>
<td>Familial/maternal</td>
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<td></td>
<td></td>
<td></td>
<td>IUGR (third centile)</td>
<td>7 yr</td>
<td>2.2</td>
<td>2.2</td>
<td>155</td>
<td>0.6</td>
<td>ND</td>
<td>Familial/maternal</td>
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<td></td>
<td></td>
<td>IUGR (third centile)</td>
<td>14 yr</td>
<td>2.45</td>
<td>0.98</td>
<td>36 (15–85)</td>
<td>1.7 (0.4–4.4)</td>
<td>70</td>
<td>De novo/paternal</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>IUGR (third centile)</td>
<td>7 months</td>
<td>2.4</td>
<td>2.1</td>
<td>25</td>
<td>9.6</td>
<td>60</td>
<td>Familial/maternal</td>
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<td>IUGR (third centile)</td>
<td>7 months</td>
<td>2.35</td>
<td>2</td>
<td>260</td>
<td>0.2</td>
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<td>2.35</td>
<td>2</td>
<td>260</td>
<td>0.2</td>
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<tr>
<th>Mutation</th>
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<th>Age of first symptom/age at diagnosis</th>
<th>RF-BM-MR</th>
<th>Phenotype</th>
<th>Biology</th>
<th>Familial transmission/mutated allele</th>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>c.345_346insT</td>
<td>1/F</td>
<td>At birth</td>
<td>No-no-no</td>
<td>B: 38/1880/4333.iUGR (third centile)</td>
<td>SCO and muscular severe, extensive, and impairing with hemihypopitrophy</td>
<td>10 months</td>
<td>2.6 1.5–2.2 63 (10–60) 4 (0.5–4) 82</td>
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<td></td>
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<td></td>
<td></td>
<td>8 months ¼ 4 sc/ 2 yr–4.5 scND growth retardation</td>
<td></td>
<td>ND</td>
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<td></td>
<td></td>
<td>22 months 2.5 1.42 (1–2.2) 46 (10–60)</td>
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<td>ND</td>
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<td></td>
<td></td>
<td></td>
<td>1/F At birth No-no-no</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>B: 38/1880/4333.iUGR (third centile)</td>
<td>SCO</td>
<td>1 yr 2.2 2.3 149 0.6° 55</td>
<td>De novo parental PHP1a</td>
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<td></td>
<td>8 months ¼ 4 sc/ 2 yr–4.5 scND growth retardation</td>
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<td>ND</td>
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<td></td>
<td>1/F At birth No-no-no</td>
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<td>ND</td>
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<td></td>
<td>1/F At birth No-no-no</td>
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<td></td>
<td>B: 38/1880/4333.iUGR (third centile)</td>
<td>SCO</td>
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<td></td>
<td>1/F At birth No-no-no</td>
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<td>B: 38/1880/4333.iUGR (third centile)</td>
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<td>ND</td>
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<td></td>
<td>1/F At birth No-no-no</td>
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</table>

(Continued)
c.139+1G>C, c.1039–1G>A), and the other had already been identified in both PHP1a and POH cases in either our laboratory or the literature (c.345_346insT, c.565_568delGACT). All the described mutations predict proteins that have no biological activity, if translated.

**Phenotype of matched PHP1a subjects**

Eight of the 10 POH patients were matched with PHP1a patients with the same GNAS mutations. Their clinical and biological features are displayed in Table 1. In each case (subjects A, B1, B2, C, D, E, F1, F2, G), the AHO phenotype is without ambiguity, with PTH resistance. Interestingly, all these PHP1a patients were described as having sc ossifications, sometimes multiple and extensive (patients B1, F1, and F2). We were unable to obtain sufficient birth biometric data for statistical analyses. However, obesity was a constant feature found in every case during childhood. Gs activity is decreased (range 52–67%) and close to that previously described in PHP1a patients (6).

**Intrafamilial transmission of the mutated allele**

In eight of the nine investigated POH cases, the affected patient had a de novo mutation (Table 1). For family 7, substitution c.1039-1G>A, carried by the proband, was found in the father, who also presented mild ossifications,

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**FIG. 1.** Whole-body CT of patient 1. Whole-body CT showing extensive ossification in lower limbs (left side; A) and the trunk (B).

**FIG. 2.** Pedigree of family 7 with familial case of POH. Squares and circles denote male and female family members, respectively. Solid black symbol indicates members with POH and gray symbol members with PPHP; hatched symbol indicates persons with PHP1a and symbol with N family members with no mutation. Affected patient is indicated by an arrow.

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**TABLE 1.**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Subject</th>
<th>Age of first symptom/age at diagnosis</th>
<th>Age</th>
<th>Phenotype</th>
<th>Biology</th>
<th>Diagnosis</th>
<th>RF-BM-MR</th>
<th>Familial transmission/mutated allele</th>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>c.623_624insT</td>
<td>4/M 3 months/5 yr</td>
<td>5 yr</td>
<td>Widespread dorsal SCO and muscular progressing to sacrum and coastal drill</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>c.1039–1G&gt;A</td>
<td>7/F 1 yr/28 yr</td>
<td>6 yr</td>
<td>Multiple and progressive micro SCO and micro SCO and bone and coastal drill</td>
<td>Yes, since 4 months</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>c.1039-1G&gt;A</td>
<td>4/F 4 months ND-ND-ND</td>
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</table>

Parentheses indicate normal values for age. RF, Round face; BM, brachymetacarpy; MR, mental retardation; B, birth; PN, postnatal; HC, head circumference; W, weight (SD score); H, height (SD score); SCO, subcutaneous ossifications; ND, not determined; BMI, body mass index; NL, normal.

* Delayed motor development secondary to high muscular tonus due to cervical calcifications;
  † determination of urinary excretion of cAMP after administration of PTH (Ellsworth-Howard test);
  ‡ Germain-Lee et al. (18); "microdactyly in the proband and her father, under treatment."

FIG. 2. Pedigree of family 7 with familial case of POH. Squares and circles denote male and female family members, respectively. Solid black symbol indicates members with POH and gray symbol members with PPHP; hatched symbol indicates persons with PHP1a and symbol with N family members with no mutation. Affected patient is indicated by an arrow.
and in the subject’s affected sister and the sister’s children, who were reported to have features of AHO (Fig. 2). In this case, transmission was paternal. In the seven PHP1a matched families, mutations were de novo in three cases (twins B1 and B2, C, and G) and were maternally inherited in four cases [A, E, F1, our laboratory results, and D, from the literature (18)].

Segregation analysis in de novo cases (Fig. 4)
Eight of the nine POH cases with de novo mutation had informative polymorphisms. The inheritance was paternal in families 1, 2, 3, 4, 5, 8, and 10 and maternal in family 6 (Fig. 4A). An example is given for patient 5 with c.85C>T mutation (Fig. 4B): DNA fragment of 1543 bp encompassing the informative SNP rs6123837 (G/A) and the mutation was amplified. PCR product was digested with PvuII. Only the normal allele was cut (1432 bp), whereas the mutant was not (1543 bp). The two fragments were sequenced: the normal allele of 1432 bp contains the maternal G polymorphism, whereas the uncut fragment contains the paternal A polymorphism.

In patient 6 we found expression of both normal (paternal) and c.85C>T (maternal) alleles, whereas only the normal maternal allele was expressed in patient 1 (c.623_624insT).

In the de novo PHP1a cases, we demonstrated that the mutated allele was maternally inherited.

Methylation pattern
We observed no abnormality in the methylation pattern at the four studied loci (data not shown).

Discussion
To date, about 20 GNAS mutations have been reported as associated with POH (2, 4, 11–16) to our knowledge (Fig. 3). All the described mutations predict truncated proteins that have no biological activity if translated, except for the missense mutation p.W281R, which is predicted to induce loss of function (14). They are spread out all along the gene including exon 1 according to the initial observations of Eddy et al. (15), who described a p.Q12X mutation in a girl with POH and no hormonal resistance.

We encountered a broad spectrum of heterotopic ossification in POH, ranging from multiple and invading sc lesions (patient 3) to severe hemicorporal ossification (patient 1). We also reported cases of POH/PPHP overlapping syndromes including mild calcifications and some features of Albright’s phenotype (patient 7), but these signs are not specific (round shaped face and brachydactyly). There is no specific genotype-phenotype correlation that distinguishes the more severe form from mild extraskeletal ossification. Such variability has already been observed in POH (2, 13, 16, 24). These two diseases may therefore be considered as extreme expressions of the same phenotype, as suggested by Adegbite et al. (13).

Five of the GNAS mutations identified in our POH patients had already been identified in patients with PHP1a. This raises the question of the parental origin of the mutation. The association between maternal inheritance and PHP1a is well established (5, 6, 25). Shore et al. (2), studying intrafamilial transmissions, concluded that
the inheritance of POH was paternal. In the present study, we have clearly demonstrated that in patients with de novo mutations, POH is due to inactivating GNAS mutations localized on the paternal allele. We compared the ossification process, hormonal resistance, and Gαs bioactivity in patients with the same mutation by matching POH/PHP1a cases (Table 1). Ossifications were observed in all cases but are more severe in patients in whom the origin of the mutation is paternal. The possibility remains that recruitment for this study was biased because only the most severe forms of ossification are diagnosed. Indeed, in patient 6, the first diagnosis was POH because of widespread ossifications, but further studies clearly demonstrated hormonal resistance and features of AHO and the final diagnosis was POH-like PHP1a. This patient was therefore included as a PHP1a patient for analyses and discussion. Similar cases have already been described in the literature (13, 15, 24, 26–28).
Gsα-dependent processes appear to be involved in the development of mineral deposition, which may not necessarily be linked to alterations in calcium and phosphate metabolism. Lietman et al. (29) links the reduction of Gα subunit expression to osteogenic differentiation of mesenchymal stem cells and shows that reduced expression of Gαs can induce an osteoblast-like phenotype as a result of the increased activity of runt-related transcription factor-2 (Runx2, an osteoblast-specific transcription factor). Mantovani et al. (30) provided evidence for the absence of Gsα imprinting in human bone and suggested that osteodystrophy in PHP1a and PPHP is due to Gsα haplo-insufficiency in bone.

The mutations in patients 5, 6, 9, and possibly 8 are all Gs specific, whereas the others affect also XLs expressed from the paternal allele.

In exon 1, the p.Q29X mutation was associated with the following phenotypes: PHP1a (16, 18), PHP1a-POH like (patient 6) when maternally inherited, PPHP (16), or POH (patients 5 and 9) when paternally inherited. In transgenic mice with disruption of exon 1, there is no evidence of heterotopic ossification at 3 months (31), whereas extensive but sc ossifications were evidenced at 12 months with no difference in mice for a maternally or paternally inherited mutation (32). Skeletal abnormalities, skin calcifications, and extraskeletal ossification were also observed in rCre-Gsα mice with homozygous (two alleles) restricted Gsα deletion in exon 1 (33).

The expression of the mutant GNAS or other imprinted transcripts from the GNAS locus and their mutated protein products or lack thereof could have an impact on the progress of the disease. However, this hypothesis is unlikely because previous studies have shown that mutant GNAS3 are seldom expressed (2). Epigenetic defects at the GNAS locus that could account for the asymmetric allelic expression of Gs as discussed by Michienzi et al. (34) in McCune-Albright syndrome are not identified in this study. The potential impact of an additional mutation in ectopic ossified tissue and the role of a modifier gene, unknown epigenetic modifications, or environmental factors have yet to be explored.

We have also shown that the same GNAS mutation can induce different levels of reduction in Gs activity, depending on whether the mutation is on the maternal or paternal allele (59 ± 2 and 80 ± 3%, respectively, P < 0.001, Fig. 5). A possible hypothesis is that POH cases with the highest Gs activity reflect the limitations of the erythrocyte complementation assay used (20, 35). A bias from the fact that adult reference values are used, whereas the subjects are evaluated during early childhood and so do not take into account a potential down-regulation as has been found in mice during the second half of the postnatal period (36) cannot be excluded. Another possibility is that in some tissues such as erythrocytes, XLαs has an inhibitory or interfering role in cAMP signaling as found in mice brown adipocytes (37), a role that is lost in patients in whom the XLαs mutation is paternally inherited. In contrast, Bastete et al. (38) and Linglart et al. (39) demonstrated a cAMP stimulating activity of this protein in another cell type. The possibility of tissue-specific Gsα imprinting in the erythroblasts should also be considered.

Despite a follow-up of several years in two cases (patients 5 and 9), we found that only a small subset of patients with POH shared multiple features with AHO patients, as observed previously (13–15). The fact that Gsα activity is subnormal in POH patients could explain why these patients have clinical findings of AHO that are limited to short stature and/or brachymetacarpia.

In this study we clearly demonstrated that severe IUGR is a property of the phenotype, regardless of which mutation is found (14, 15). Of the eight patients for whom this data are available, the observed rate (100%) of IUGR is significantly higher than what would be expected by chance (0.24%). Similar findings have been reported in patients with paternal deletion that include the GNAS locus (40). It has been hypothesized from mouse models (37, 41) that loss of XLαs is the main cause of IUGR. However, patients with specific exon 1 Gs mutations (patients 5, 6, 9) are reported with IUGR. We were unable to give additional birth biometric data in PHP1A patients to know whether IUGR is also associated with the presence of ossification.

Finally, our human case study is unable to distinguish between XLαs functions and paternal haploinsufficiency of Gαs, which suggests that in humans, loss of XLαs is not dominant over the simultaneous loss of Gαs derived from
the paternal allele. The key is perhaps neither Gs nor XLαs but their impact on other potential contributing genes. The data do nevertheless support further investigation of PPHP for postnatal symptoms insofar as this is feasible and the availability of records allows.

Cases of paternal familial transmission have been reported (2, 42, 43) for POH. For patients who show features of an ambiguous phenotype (extensive and invading ossifications characteristic of POH, associated with features of AHO and PTH resistance), determining the inheritance pattern, in particular in de novo cases, is helpful in establishing a more accurate diagnosis and preventing hypocalcemia and hypothyroidism. However, clinical heterogeneity makes genetic counseling extremely delicate, especially in cases of paternal inheritance, which can lead to either a mild expression of the phenotype in PPHP or a severe one in POH. Lietman et al. (44) reported one case of preimplantation genetic diagnosis in the context of paternal transmission of a severe case of AHO.

In conclusion, our data have, for the first time, brought forward a human case study that can distinguish between GNAS mutation on paternal or maternal allele. The loss of Gαs leads to ossification process because they can result from mutations on the maternal or paternal allele. Loss of paternally expressed XLαs as well as Gαs is associated with severe IUGR. Finally, we reported on a few clinical signs of AHO in POH in which erythrocyte Gs activity is higher than in PHP1a. This intriguing result requires further investigation, in particular to explore the effects of XLαs on cAMP signaling in imprinted tissues.

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