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Paternal Uniparental Isodisomy of the Entire Chromosome 20 as a Molecular Cause of Pseudohypoparathyroidism Type Ib (PHP-Ib)

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Abstract

Pseudohypoparathyroidism type Ib (PHP-Ib) typically defines the presence of end-organ resistance to parathyroid hormone in the absence of Albright's hereditary osteodystrophy. Patients affected by this disorder present with imprinting defects in the complex *GNAS* locus. Microdeletions within *STX16* or *GNAS* have been identified in familial cases with PHP-Ib, but the molecular cause of the *GNAS* imprinting defects in sporadic PHP-Ib cases remains poorly defined. We now report a case with sporadic PHP-Ib for whom a SNPlex analysis revealed loss of the maternal *GNAS* allele. Further analysis of the entire genome with a 100K SNP chip identified a paternal uniparental isodisomy affecting the entire chromosome 20 without evidence for another chromosomal abnormality. Our findings explain the observed *GNAS* methylation changes and the patient's hormone resistance, and furthermore suggest that chromosome 20 harbors, besides *GNAS*, no additional imprinted region that contributes to the clinical and laboratory phenotype.

Keywords

Pseudohypoparathyroidism; uniparental disomy; *GNAS*; imprinting; parathyroid hormone; stimulatory G protein

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INTRODUCTION

The transmission of both homologous chromosomes, or segments thereof, from a single parent to its offspring is described as uniparental disomy (UPD). UPDs are known to be responsible for a limited number of human diseases that often involve developmental defects [1,2], and some recent studies have also implicated acquired, somatic UPDs in tumorigenesis [3,4]. The clinical phenotypes associated with UPDs are caused by homozygosity of recessive disease-causing mutations or due to aberrant expression of an imprinted gene. In the latter case, the loss or duplication of one parental allele results in the deficiency or overexpression of the imprinted gene product(s), respectively.

GNAS is a complex imprinted locus on chromosome 20q13.3 that encodes the α -subunit of the stimulatory G protein ($G\alpha$) and several additional imprinted transcripts (reviewed in [5,6]). Derived exclusively from the paternal allele are *XL α s*, *A/B*, and antisense transcripts, and from the maternal allele is *NESP55*. Consistent with this imprinted expression pattern, the promoters of these imprinted transcripts are methylated on the silenced allele. In contrast, the $G\alpha$ promoter is not methylated, and accordingly, $G\alpha$ expression is biallelic in most tissues. However, it shows predominantly maternal expression in certain tissues, including the renal proximal tubules, thyroid, and pituitary.

Defects in *GNAS* imprinting are found in patients with PHP-Ib [7,8], a disorder of hormone resistance characterized by PTH- and, sometimes, TSH-resistance (reviewed in [9,10]). Unlike in patients with PHP-Ia, who carry loss-of-function mutations in $G\alpha$ -coding *GNAS* exons, PHP-Ib patients typically lack the features of AHO, although some PHP-Ib cases with *GNAS* imprinting defects have recently been shown to have AHO features [11-14]. Nearly all patients with autosomal dominant PHP-Ib show a loss of methylation at *GNAS* exon *A/B*, which is combined, in some patients, with loss of methylation at exon *XL* and the promoter of the antisense transcript, and with gain of methylation at exon *NESP55* [7,8]. Deletions involving the *NESP55* differentially methylated region or deletions in the gene encoding syntaxin-16 (*STX16*), which is located ~200 kb centromeric of *GNAS*, are responsible for the disease in patients with autosomal dominant PHP-Ib [15-17]

Most cases of PHP-Ib are sporadic and many such cases also demonstrate broad *GNAS* methylation defects [9,10]. However, in contrast to the autosomal dominant form of PHP-Ib, no specific gene mutation has been identified as a cause of the broad *GNAS* methylation defects associated with sporadic PHP-Ib. In fact, only two patients with this relatively frequent form of PHP-Ib have been defined at the molecular level thus far, both revealing a duplicated long arm of the paternal chromosome 20 (patUPD20q), i.e. the region which comprises the *GNAS* locus [18,19]. We now describe another patient with sporadic case of PHP-Ib who has paternal UPD affecting the entire chromosome 20.

MATERIALS AND METHODS

The study was approved by the Massachusetts General Hospital Institutional Review Board, and written informed consent was obtained from the patient and her parents. Genomic DNA was extracted from blood leukocytes, as described [20]. Multiplex analysis of all 23 SNPs was custom-designed and performed at Harvard-Partners Center for Genetics and Genomics (www.hpcgg.org). Microsatellites were analyzed as previously described [20]. Genotyping with 100K SNP chip analysis was performed as previously described [21].

RESULTS

Patient description and clinical course

The clinical and laboratory findings of the patient have been described previously [22,23]. Briefly, she presented with a gradually worsening limp, leg pain, and “knock knees” at the age of 3 years. No AHO features were evident, but initial lab data revealed hypocalcemia, hyperphosphatemia, and elevated serum PTH, which was consistent with the diagnosis of PHP1b. Treatment with calcium carbonate and increasing doses of calcitriol has been initiated, which reduced the bone pain, normalized serum calcium, and suppressed serum PTH. However, subsequent non-compliance to the treatment led to the recurrence of bone pain and radiological evidence of bilateral slipped capital femoral epiphysis.

She underwent bilateral percutaneous in-situ fixation of her slipped capital femoral epiphyses with two minor subsequent revisions and is maintained on medical management. Her regimen now consists of both Rocaltrol and calcium supplements. Her serum calcium levels have ranged from 10-11 mg/dl with phosphorous levels ranging from 4.4-5.7 mg/dl and PTH levels in the normal or somewhat above the normal range. Her urinary calcium to creatinine ratios have been normal with the most recent 5 years after diagnosis. Her 25-vitamin D level currently is 26 ng/ml with a 1,25 dihydroxy vitamin D of 38 pg/ml (Table 1). She has had no further bone pains or fractures, and has shown both clinical and biochemical improvement. She continues to grow just above the 97th percentile for height and her weight has stabilized at the 95th percentile for her age. She remains prepubertal and has demonstrated radiographical improvement in the appearance of her skeletal X-rays on follow-up studies.

Molecular findings

The frequent 3-kb deletion within *STX16* was previously ruled out in this patient [22], and analysis of *GNAS* methylation showed that the patient has loss of exon A/B methylation, combined with a loss of methylation at exon XL and the promoter of the antisense transcript and a gain of methylation at exon NESP55 [23]. To search for the genetic defect responsible for the hormone resistance and this methylation pattern, we examined genomic DNA from the patient and her mother. Analysis of SNPs between *GNAS* exon A/B and exon NESP55 and of microsatellites within the chromosomal region comprising *GNAS* revealed loss of heterozygosity and allelic discordance between the patient and her mother (Figure 1A,B). 100K SNP chip analysis of DNA from the patient and her parents showed that the loss of heterozygosity affects the entire chromosome 20, but it also indicated no changes in copy number (Figure 1C,D). These findings thus indicated that the patient has a paternal uniparental isodisomy of the entire chromosome 20.

DISCUSSION

The identified paternal UPD20 in our patient is consistent with the observed methylation defects at the *GNAS* locus and hormone resistance, and re-emphasizes the important role of *GNAS* methylation in silencing *Gsα* expression in the proximal renal tubules and thus the development of PTH-resistant hypocalcemia and hyperphosphatemia. One previously reported sporadic case of PHP-1b was shown to have paternal UPD involving chromosome 20 [18]. In addition, paternal UPD20 causing PHP-1b has also been identified in a family in which three additional members were coincidentally affected by PHP-1a [19]. In these two UPD cases, the long arm of chromosome 20 was duplicated, whereas the UPD in our patient involves the entire chromosome. Based on the similarity of the clinical and laboratory findings in the two patients with patUPD20q and the patient with patUPD20 described herein, it appears unlikely that duplication of the paternal short arm of chromosome 20

results in significant clinical phenotypes. Nonetheless, it remains conceivable that aberrant gene expression from as-yet-undefined imprinted loci and/or unmasked recessive traits located on this chromosomal region contributed to our patient's severe bone defects, which are predicted to be a consequence of secondary hyperparathyroidism associated with renal PTH-resistance [24], yet were not reported for both patients with patUPD20q [18,19].

Paternal UPD involving chromosome 20q and, particularly, the *GNAS* locus, appears to be a rare cause of PHP-Ib. Nevertheless, we suggest that this genetic abnormality should be considered as a possible molecular cause in sporadic PHP-Ib cases, particularly since it has important implications for genetic counseling. In fact, four patients with PHP-Ib due to partial paternal uniparental disomy of chromosome 20 were recently described [25], thus confirming our point that patients with broad *GNAS* methylation defects should be evaluated regarding this genetic defect.

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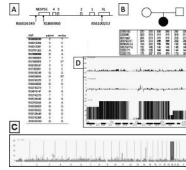


Figure 1. Identification of a paternal UPD of entire chromosome 20 in the index patient

A. Analysis of SNPs within the upstream region of *GNAS*. Genomic DNA from the patient and her mother were genotyped for 23 SNPs clustered within the centromeric portion of *GNAS* that includes exons NESP55, XL, and exons 1-4 of the antisense transcript, revealing two discordant SNPs (bold) located upstream or within NESP55.

B. Analysis of polymorphisms in the chromosomal region comprising *GNAS*. The patient and her parents were also genotyped for microsatellites within the telomeric end of chromosome 20q, demonstrating a loss of the maternal allele for five of eight informative markers (bold).

C. *100K SNP Chip analysis of leukocyte genomic DNA*. Genome-wide analysis with LOH probability (black) and the copy number (CN; grey) were plotted together.

D. *Analysis of chromosome 20*. The probability of LOH (JC LOH) is significantly elevated, while the copy number (JC GSA_CN) and the p-value (JC GSA_pVal) for the same chromosome are not different from other chromosomes. The gap represents the centromeric region. The cytobands of chromosome 20 are displayed below. Analysis was performed concomitantly on DNA samples from the patient and her parents, but the results shown here were obtained with DNA from the patient. The parental DNA did not reveal any abnormalities (data not shown).

Table 1

Follow-up Laboratory Course and Growth of Patient

	Pre-Treatment (months)	Post Treatment (months)	60	46	34	25	34	46	60	68
Chronological Age										
Calcium (mg/dl) (normal: 8.4-10.2)	3y3m	5y3m	8y3m	7y1m	6y2m	5y3m	6y2m	7y1m	8y3m	8y11m
Phosphate (mg/dl) (normal: 2.5-5.6)	7.2	10.2	11	10.9	10	10.2	10	10.9	11	10.6
Albumin (gm/dl) (normal: 3.0-5.0)	8.0	5.7	-	5.5	5.4	5.7	5.4	5.5	-	4.4
Alkaline Phosphatase (IU/L) (normal: 100-320)	4.3	-	4.6	4.5	-	-	-	4.5	4.6	4.6
Creatinine (mg/dL) (normal: 0.3-0.8)	643	258	147	120	170	258	170	120	147	121
25(OH)D3 (ng/mL) (normal: 9-52)	0.3	0.4	0.8	0.7	-	0.4	-	0.7	0.8	0.6
1,25(OH)D3 (pg/mL) (normal: 15-90)	20	30	19	25	15	30	15	25	19	26 (8y6m)
PTH (pg/mL) (normal: 10-65)	50	15	-	-	36	15	36	-	-	38 (8y6m)
Urine Ca/Urine Cr ratio (mg/mg)	3685	136	76	29	141	136	141	29	76	165
TSH (uIU/mL) (prepubertal normal: 0.6-5.5)	-	0.0157	0.099	0.201	0.039	0.0157	0.039	0.201	0.099	0.147
FT4	-	-	-	1.75	-	-	-	1.75	-	-
Height (cm) (%ile)	1	-	-	1.14	-	-	-	1.14	-	-
Weight (kg) (%ile)	107 (>97%ile)	125.2 (>97%ile)	142.3 (>97%ile)	135.4 (>97%ile)	129.4 (>97%ile)	125.2 (>97%ile)	129.4 (>97%ile)	135.4 (>97%ile)	142.3 (>97%ile)	144.3 (>97%ile)
BMI (kg/m ²)	26.2 (>97%ile)	38.1 (>97%ile)	42.4 (>97%ile)	36.2 (>97%ile)	40.7 (>97%ile)	38.1 (>97%ile)	40.7 (>97%ile)	36.2 (>97%ile)	42.4 (>97%ile)	41.6 (95th%ile)
Bone Age	22.9	24.3	20.9	19.7	24.3	24.3	24.3	19.7	20.9	20
Calcium Carbonate	-	-	-	-	10y	-	10y	-	-	10y
Calcitriol (daily)	0.1 mcg	0.8 mcg	1.0 mcg	1.0 mcg	1.0 mcg	0.8 mcg	1.0 mcg	1.0 mcg	1.0 mcg	0.5 mcg