

CLINICAL STUDY

New mechanisms involved in paternal 20q disomy associated with pseudohypoparathyroidism

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Abstract

Purpose: Type I pseudohypoparathyroidism (PHP-Ia) can be subclassified into Ia and Ib, depending on the presence or absence of Albright's hereditary osteodystrophy's phenotype, diminished α -subunit of the stimulatory G protein ($G_s\alpha$) activity and multihormonal resistance. Whereas PHP-Ia is mainly associated with heterozygous inactivating mutations in $G_s\alpha$ -coding exons of GNAS, PHP-Ib is caused by imprinting defects of GNAS. To date, just one patient with PHP and complete paternal uniparental disomy (UPD) has been described.

We sought to identify the molecular underlining defect in twenty patients with parathyroid hormone resistance, hypocalcemia and hyperphosphatemia, and abnormal methylation pattern at GNAS locus.

Methods: Microsatellite typing and comparative genome hybridization were performed for proband and parents.

Results: We describe four patients with partial paternal UPD of chromosome 20 involving pat20qUPD in one case, from 20q13.13-qter in two cases, and pat20p heterodisomy plus interstitial 20q isodisomy in one patient.

Conclusions: These observations demonstrate that mitotic recombination of chromosome 20 can also give rise to UPD and PHP, a situation similar to other imprinting disorders, such as Beckwith–Wiedemann syndrome or neonatal diabetes.

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Introduction

Uniparental disomy (UPD), a nonmendelian form of inheritance, is a condition in which a disomic individual has inherited both copies of a chromosome from one parent only (1) and is a consequence of at least two independent errors occurring during meiosis or immediately after fertilization (1, 2) (Figs 1 and 2 respectively). Depending on the identity of the two chromosome homologs, UPD is defined as either heterodisomy (inheritance of both homologs from one parent) or isodisomy (presence of two identical copies of one homolog), and even a mixture of both is possible, reflecting the number and location of meiotic recombinations. The origin of UPD lies in the meiotic nondisjunction events. Specifically, UPD can be due to nondisjunction during meiosis I or II in one parent, leading to a disomic gamete, followed by postzygotic loss of the other parent's chromosome (trisomy rescue), fertilization with a nullisomic gamete for that chromosome from the other parent (gamete

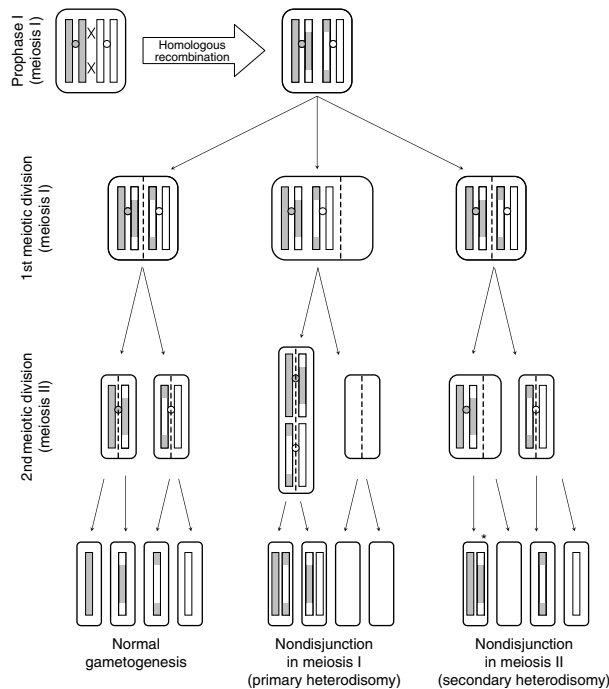


Figure 1 Schematic diagram of the events leading to primary and secondary heterodisomy, compared with normal gametogenesis, for a given pair of autosomes (Adapted from Rivolta *et al.* (2002) (54)). In regular meiosis, each pair of chromosomes is separated during the first meiotic division, whereas the sister chromatids of each chromosome detach during the second meiotic division. If nondisjunction occurs during the first meiotic division, both chromosomes of a pair (or none at all) can be transmitted to a gamete. In contrast, in a nondisjunction event occurring during the second meiotic division, a gamete can have two identical copies of the same chromosome formed from both of its sister chromatids. Vertical hatched lines indicate how chromosomes or chromatids are assorted during cell division. The asterisk represents the putative mechanism that leads the UPD in case 3.

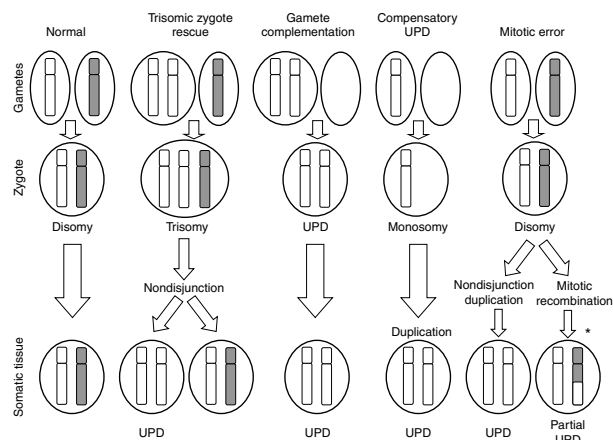


Figure 2 Schematic diagram of the different mechanisms leading to uniparental disomy (UPD) (Adapted from Kotzot (2001) (15)). The asterisk represents the putative mechanism that leads the UPD in cases 1, 2, and 4.

complementation), mitotic duplication, and post-fertilization error (1, 3–7).

Problems associated with UPD are i) placental or even fetal mosaicism normally resulting from trisomy rescue; ii) homozygosity of autosomal recessively inherited mutations; and iii) aberrant expression of imprinted genes. Well-known examples are maternal UPD (15) in ~25–30% of patients with Prader–Willi syndrome (PWS, MIM 176270); paternal UPD (15) in ~2–3% of cases with Angelman syndrome (AS, MIM 105830) (8); transient neonatal diabetes mellitus (paternal UPD6) (9); maternal UPD14 syndrome (10); paternal UPD14 syndrome (11); and Silver–Russell syndrome (maternal UPD7) (12). In addition to these syndromes, more than 100 cases of UPD of other chromosomes have been reported (2).

All these cases describe UPD of a whole chromosome or a whole arm. By contrast, apart from paternal UPD (11) (p15-pter) in 10–20% of cases with Beckwith–Wiedemann syndrome (BWS, MIM 130650) (13), UPD of segments of chromosomes or UPD associated with a complex chromosomal rearrangement have rarely been reported (<http://www.med.uni-jena.de/fish/sSMC/OOSTART-UPD.htm>). Segmental UPD has been defined as UPD of a part of one chromosome (interstitial or telomeric) together with biparental inheritance of the rest of this pair of chromosomes and a normal karyotype (14, 15).

Pseudohypoparathyroidism (PHP; MIM 103580) is a disorder of end-organ resistance that primarily affects the action of the parathyroid hormone (PTH) (16). Genetic defects associated with the various types of PHP involve the α -subunit of the stimulatory G protein ($G_s\alpha$), a signaling protein that is essential for the actions of PTH and many other hormones. Specifically, patients with PHP-Ia have heterozygous inactivating mutations in $G_s\alpha$ -encoding *GNAS* exons, and they also show

resistance to other hormones and a constellation of physical features characteristic of Albright's hereditary osteodystrophy (AHO) (17–19). Patients with AHO features but with no evidence for hormone resistance are said to have pseudopseudohypoparathyroidism (PPHP), and they also carry heterozygous inactivating $G_s\alpha$ mutations. Maternal inheritance of such a mutation leads to PHP-Ia, i.e. AHO plus hormone resistance, while paternal inheritance of the same mutation leads to PPHP, i.e. AHO only (18, 20, 21). This imprinted mode of inheritance for hormone resistance can be explained by the predominant maternal expression of $G_s\alpha$ in certain tissues, including renal proximal tubules (22). Unlike patients with PHP-Ia and PPHP, patients with PHP-Ib show normal $G_s\alpha$ activity in circulating blood cells and fibroblasts (21, 23, 24). Nevertheless, a loss of methylation at *GNAS* exon A/B, which is sometimes combined with epigenetic defects at other *GNAS* differentially methylated regions, is found in most patients with this disorder (25, 26). The familial form of PHP-Ib (AD-PHP-Ib) is typically associated with an isolated loss of methylation at the exon A/B DMR due to maternally inherited microdeletions within *STX16* (26–28). In addition, deletions removing the entire NESP55 DMR, located within *GNAS*, have been identified in some AD-PHP-Ib kindreds, and these affected individuals show loss of all the maternal *GNAS* imprints (29). Most sporadic PHP-Ib cases also have imprinting abnormalities of *GNAS* that involve multiple DMRs, but the genetic lesion(s) responsible, if any, for these imprinting abnormalities remains to be identified (Fig. 3). Paternal isodisomy of chromosome 20q and, thus, a paternal-specific methylation pattern on both alleles have also been shown to be associated with PHP-Ib in two patients (30, 31).

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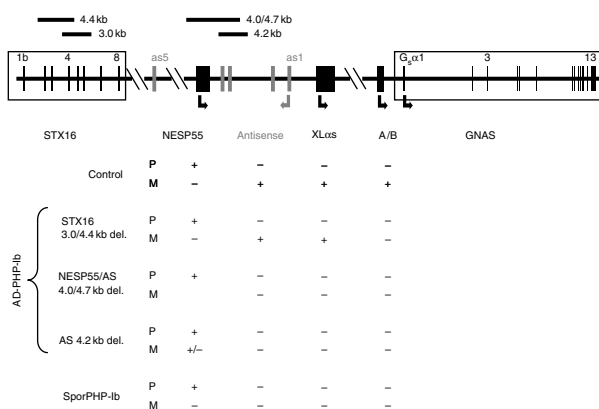


Figure 3 Schematic representation of the *GNAS* locus. Boxes and connecting lines indicate exons and introns respectively. Promoters of $XL\alpha s$, antisense (AS), A/B, and NESP55 transcripts are either maternally (M) or paternally (P) methylated, as indicated by + (methylated CpG islands) and - (unmethylated CpG islands). Arrows indicate origin and sense of transcription. Horizontal bars indicate the deletions identified in patients with AD-PHP-Ib.

Our genetic study was undertaken to identify possible chromosomal alterations in twenty affected patients. We describe new types of mutations consisting in segmental pat20qUPD and hetero/isodisomy for chromosome 20 associated with PHP.

Materials and methods

Case report

This study was approved by the appropriate Institutional Review Board, and informed consent was obtained from all human subjects.

Clinical features of each patient are summarized in Table 1 and Supplementary Table 1, see section on supplementary data given at the end of this article.

Case 1 (GS0060) A 32-year-old female patient was admitted to hospital for persistent paresthesia of the four limbs during the preceding week, together with hypertonia of both hands and palpitations. She had previously been diagnosed with extrinsic asthma and had a bone tumor in the left humerus that had been surgically treated. Biochemical studies revealed low calcium and potassium (2.5 mEq/l, normal values: 3.5–5.1 mEq/l) levels, so she was started on oral calcium, vitamin D, and potassium supplements and after discharge from hospital, the oral calcium dose was increased due to persistence of paresthesia and palpitations. At her last check-up, she remained asymptomatic with a normal thyroid function and PTH levels of 90.8 pg/ml.

Case 2 (GS0064) Clinical features of this patient were described in detail previously (31). Briefly, this was the case of a 25-year-old male patient diagnosed with PHP due to PTH and TSH resistance. He was first studied at the age of 10 months due to obesity, right cryptorchidism, and psychomotor retardation. At the age of 7 years, he was diagnosed with Wilkins epiphyseal dysgenesis and started on thyroxine (T_4) for his hypothyroidism. Two years later, biochemical tests revealed high serum PTH with hypocalcemia and hyperphosphatemia, leading to the diagnosis of PHP.

Q3 On physical examination, it was observed that he had a rounded face with an ogival palate, clear macrocephaly (+3 s.d.), and no subcutaneous ossifications. He had reached puberty, Tanner Stage 5, and his neurological examination was unremarkable with a negative Chvostek's sign, but mild to moderate cognitive impairment. A cranial CT scan showed many bilateral and symmetrical supratentorial calcifications in both the globus pallidus and the subcortical area of the frontal lobes, retraction of frontal cortical wrinkles, and a mega cisterna magna.

Table 1 Brief summary of clinical data of the index cases at diagnosis.

	Case 1 GS0060	Case 2 GS0064	Case 3 GS0082	Case 4 GS0090
Sex	Female	Male	Male	Male
Age at diagnosis (years)	26	9	5	46
Rounded face	No	Yes	Yes	No
Weight (kg)	57 (−0.1 s.d.)	57.3 (−0.92 s.d.)	37.5 (+5.5 s.d.)	84.8 (+1.5 s.d.)
Height (cm)	154 (−1 s.d.)	162 (−1.6 s.d.)	119.5 (p95)	160 (−2 s.d.)
BMI (kg/m ²)	24	21.8	26.5	33.12
Parental height	NA	F: 167 M: 144.5	F: 174 M: 170	NA
Parental age at gestation	F: 27 years M: 27 years	F: 31 years M: 32 years	F: 33 years M: 33 years	NA NA
Macrocephaly	No	Yes (> s.d. 3)	Yes (s.d. +3.3)	No
Q8 Subcutaneous ossification	No	No	No	NA
Intracranial calcifications	No	Yes	No	Yes
Brachydactyly	No	No	No	No
Cognitive retardation	No	Yes	No	No
Calcium (2.2–2.6 mmol/l)	1.3	2.2	1.2	1.6
Phosphate (1–1.5 mmol/l)	1.9	2.1	2.4	1.5
PTH (15–65 pg/ml)	601	940	292.4	127.4
Free T ₄ (0.7–1.9 ng/ml)	N	1.2 ^a	1.2	1.2
TSH (0.5–5 μU/ml)	N	2	2.8	2.9

F, father; M, mother; N, normal values; NA, not available.

^aThe patient was already on levothyroxine treatment at the time of PHP diagnosis.

Case 3 (GS0082) A 5-year-old male patient was admitted to Accident and Emergency due to a high fever that had begun 6 days earlier accompanied by frequent vomiting in the preceding 24 h. He also presented hyporexia, oliguria, malaise, cough and severe laryngeal stridor. At the age of 4 months, he had suffered bronchiolitis, and at the age of 10 months, he had chickenpox, both episodes requiring hospitalization. He was previously studied by his pediatric endocrinologist due to macrosomy (birth weight 4730 kg, s.d.: +3.34) and obesity. Physical examination on admission revealed a facial macular exanthema, bilateral erythematous tympanic membranes, and crepitations in the upper lobe of the left lung. Blood tests showed normal free T₄ and TSH, low serum calcium, hyperphosphatemia, magnesium 2.3 mmol/l (normal range 0.7–1.1 mmol/l), and elevated PTH. He was diagnosed with PHP and acute otitis media, treated with i.v. calcium for 3 days and oral calcium and calcitriol from then on, and remained asymptomatic until discharge 8 days later.

Case 4 (GS0090) A 46-year-old male was referred to the endocrinology clinic from A&E, where he had attended after a short tonic–clonic seizure. He had previously been studied in the Endocrinology Department for a history of syncopal episodes, with loss of consciousness and sphincter relaxation, tonic–clonic convulsions and sporadic seizures, and had been diagnosed with chronic hypocalcemia, hypertension, anxiety depression syndrome, and leukoencephalopathic changes associated with calcium deposits in the brain. He was started on antiepileptic (lamotrigine) and antidepressant (mianserine) drugs, and although he

was advised to take oral calcium and vitamin D treatment, he was not taking vitamin D at the time of his last convulsive episode. A cranial CT scan revealed dense symmetrical calcifications of the basal ganglia, cerebellum, and cerebral parenchyma. Blood tests showed hypocalcemia, hyperphosphatemia with elevated PTH and vitamin D (25OH vitamin D) 84.8 nmol/l (normal values: 20–100). Oral calcium doses were increased, and vitamin D treatment was reintroduced.

Molecular studies

GNAS sequence and methylation status analysis

The Institutional Review Board of Hospital de Cruces approved the study. Genetic analyses were performed after obtaining informed consent from the patient or parents. Genomic DNA was extracted from peripheral blood leukocytes using a commercial kit, following the manufacturer's instructions (QiaAmp Blood Mini, Qiagen). The thirteen coding exons of *GNAS* gene were amplified, and both strands of amplicons were sequenced as described previously (32).

Dosage and methylation analyses were carried out by multiplex ligation-dependent probe amplification (MS-MLPA) using the ME031A kit (MRC-Holland, Amsterdam, The Netherlands). The protocol was implemented following the manufacturer's recommendations. Analysis of the MS-MLPA PCR products was performed on an ABI3130XL genetic analyzer and using the GeneMapper software (Applied Biosystems). For the copy number analysis, the data generated were intra-normalized by dividing the peak area of each

amplification product by the total area of the reference probes only. The ratios were then obtained by dividing the intra-normalized probe ratio in a sample by the average intra-normalized probe ratio of all reference runs. For the methylation analysis, the intra-normalized peak area of each MS-MLPA probe from the digested sample was divided by the value obtained for the undigested sample.

When partial loss of methylation was identified, methylation quantification was performed by sequencing of subcloned PCR products after bisulfite modification as described (27).

To characterize the chromosome 20 haplotypes, eight polymorphic markers located at 20p and eighteen microsatellites at 20q were typed by fluorescent PCR.

Illumina A660 SNP array The Illumina Human660W-Quad BeadChip was used for all experiments, according to the methods described by the manufacturer. The array includes 657 366 markers distributed evenly across the genome, with 14 854 markers being located on chromosome 20. GenCall scores <0.15 at any locus were considered 'no calls'. Images were analyzed using the Chromosome Viewer tool contained in Beadstudio 3.2 (Illumina, San Diego, CA, USA). The metric used was the log *R* ratio which is the log (base 2) ratio of the observed normalized *R* value for a SNP divided by the expected normalized *R* value (33). In addition, an allele frequency analysis was carried out for all SNPs. All genomic positions were based upon NCBI Build 36 (dbSNP version 126).

Results and discussion

Since the first clinical case of UPD was reported, in 1988 (34), the presence, location, and extension of UPD have been associated with specific phenotypic features in BWS (OMIM #130650) (35), AS (OMIM #105830) (36), Silver–Russell (OMIM #180860) (37), matUPD14 (10), PWSs (OMIM #176270) (38), transient neonatal diabetes (OMIM #601410) (39), and patUPD14 (OMIM #608149) (40).

UPD for chromosome 20 has been reported previously in a very few cases, namely matUPD, usually associated with growth and psychomotor retardation and dysmorphic features (41–45), and patUPD, reported only in two cases with PHP-Ib (30, 31). Despite this, we analyzed many microsatellites across chromosome 20 in twenty patients with PHP due to lack of maternal methylation imprints at the *GNAS* locus, and in whom allelic loss had been ruled out by MS-MLPA, looking for disomy as a putative cause of the methylation defect (Supplementary Table 1). Four of the patients were suspected of having patUPD, and the characteristics of this disomy were investigated using SNP arrays.

The microsatellite analysis in cases 1 and 4 indicate that they inherited both copies of chromosome 20, one from each progenitor, except for the paternal isodisomic region found on the distal portion of the long arm (Table 2). The Illumina Human660W revealed that the 'breakpoint' for this region in case 1 is between the STRs *D20S887* and *D20S902* close to *D20S887* at the 20q13.13 band (the last heterozygous SNP was rs6020218 (48 083 796) and the first isodisomic one was rs4811002 (48 088 423)), and that in case 4 the breakpoint is between the STRs *D20S902* and *D20S1044* (the last heterozygous SNP was rs1052653 (49 438 061) and the first isodisomic was rs1569736 (49 439 729); Fig. 4A and B). Considering the patients previously reported with breakpoints in this region, we observed that Aldred *et al.* (46) described two patients with PHP-Ia in whom deletions were identified at 20q13 (46, XY, del (20) (q13.13q13.32) and 46, XX, del (20) (q13.31q13.33)). The male patient carried a deletion that started near the breakpoint of cases 1 and 4, so therefore this region could be considered a hotspot for homologous recombination for the long arm of chromosome 20.

On the other hand, the microsatellite markers for case 2 revealed the presence of a paternal isodisomy for the whole long arm of chromosome 20 (Table 2). Analysis of the results obtained in the Illumina Human660W confirmed this result (Fig. 4C). The genetic alteration in this patient is similar to the patient reported by Bastepe *et al.* (30) with pat20qUPD.

Regarding the putative mechanism leading to 20qUPD accompanied by the presence of both 20p homologs derived from both parents, a postzygotic mitotic recombination seems the most probable cause (Fig. 2). The presence of a pat20qUPD (whole long arm for case 2 and from 20q13.13-qter for cases 1 and 4) suggests that a somatic crossover between the two copies of chromosome 20q at mitosis, followed by random segregation of sister chromatids in embryo-derived cells (from the inner cell mass or later), is likely to have given rise to the segmental pat20qUPD observed in the patients, as described previously (15). Somatic recombination and random segregation can produce different cell types. This mechanism can result in somatic mosaicism, the extent of which will depend on the stage at which the postzygotic recombination event occurred (14). Somatic recombination at a very early stage, followed by a random loss of a particular cell type (in the extra-embryonic tissues, for instance), may be the reason for the lack of mosaicism observed in these patients. It is also possible that a low level of mosaicism in the patients may have gone undetected. The analysis of other tissues might have revealed a degree of mosaicism; however, only lymphocytes were available for the study, and the resulting phenotype strongly suggests that this mosaicism would be present at a low level.

Proof Only

Q9 **Table 2** Microsatellite analysis of cases at diagnosis.

	Case 1						Case 2						Case 3					Case 4								
	Father		Index		Mother		Father		Index		Mother		Father		Index	Mother	Brother	Father		Index		Mother				
D20S906	101	93	101	99	99	97	87	107	87	97	97	97	95	95	95	93	99	95	93	97	97	97	95	97	95	
D20S842	175	171	175	169	169	165	171	175	171	169	169	169	173	171	171	173	163	165	173	163	181	181	181	159	177	159
D20S882	83	83	83	83	83	77	83	79	83	77	77	79	87	83	83	87	77	83	87	77	83	77	83	83	83	83
D20S846	222	220	222	222	222	224	218	224	218	222	222	222	218	218	218	222	224	218	222	222	226	222	222	224	224	222
D20S851	66	76	66	82	82	78	84	78	84	66	66	70	82	70	70	82	66	84	82	66	84	86	84	70	70	70
D20S898	253	255	253	241	241	269	249	269	249	269	269	253	253	253	253	255	267	253	255	257	255	257	251	253	251	
D20S912	287	297	297	287	287	297	299	301	299	301	301	299	291	295	291	295	297	295	291	297	297	297	295	297	295	
D20S871	156	156	156	146	146	162	152	132	152	142	142	142	160	156	156	160	156	146	160	156	156	132	156	132	144	132
D20S195	261	263	261	251	251	251	245	245	245	253	255	251	245	251	251	259	255	251	259	251	239	251	251	251	251	251
D20S174	274	276	274	272	272	274	274	278	274	274	274	282	274	272	274	274	276	274	274	274	276	274	276	274	274	276
D20S861	127	127	127	129	129	123	125	127	125	125	127	135	127	129	129	129	127	137	129	127	127	127	127	127	127	127
D20S891	214	212	214	214	214	208	214	210	214	214	196	212	208	214	214	214	208	214	214	214	211	207	211	211	219	211
D20S887	252	260	252	264	264	266	266	266	266	266	250	252	264	264	264	264	252	264	252	266	262	266	252	260	252	252
D20S902	314	312	314	314	308	314	310	312	310	310	312	314	314	310	314	314	310	310	314	310	314	306	314	314	310	312
D20S1044	212	212	212	212	212	212	210	212	210	210	210	212	210	212	212	212	212	212	212	212	212	212	212	212	212	212
D20S468	192	202	192	192	192	208	208	202	208	208	208	202	202	202	202	208	208	202	208	207	207	207	207	201	207	207
D20S832	210	210	210	210	212	212	210	210	210	210	204	210	208	210	208	208	210	208	210	212	212	212	212	210	210	210
D20S746	254	254	254	254	254	254	250	250	250	250	254	254	254	254	254	254	254	254	254	254	250	250	250	250	250	250
D20S88	441	441	441	441	437	437	427	433	427	427	431	463	441	439	441	441	447	439	441	447	437	427	437	437	445	445
D20S102	177	173	177	177	173	177	181	177	181	181	177	177	177	177	177	179	177	179	177	179	173	173	173	177	177	177
D20S1063	200	202	200	200	200	202	202	200	202	202	200	202	200	200	200	202	202	200	202	200	202	200	200	200	200	200
D20S496	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205	205
D20S459	231	233	231	231	231	235	231	231	231	231	229	233	231	231	231	231	233	231	231	233	231	231	231	229	231	231
D20S443	144	144	144	144	144	144	138	144	138	138	138	144	138	144	138	144	142	138	144	144	144	144	144	138	142	142
D20S171	136	126	136	136	132	136	134	138	134	134	132	134	138	132	138	132	138	132	138	132	132	134	132	132	132	134
D20S173	182	180	182	182	182	184	134	184	134	134	184	184	184	192	184	184	184	184	184	180	184	180	180	182	182	182

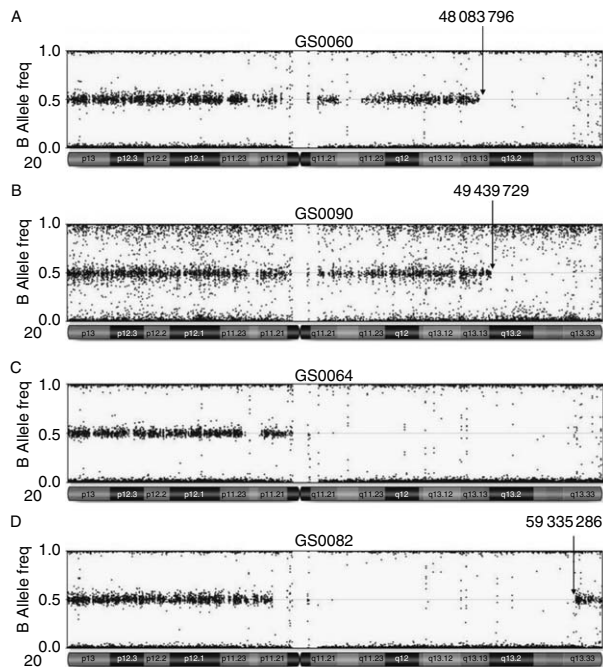


Figure 4 Array-CGH SNP genotyping of the patients analyzed. Chromosome 20 plot of case 1 (A), case 4 (B), case 2 (C), and case 3 (D). The panels show the homozygous/heterozygous distribution (B allele frequency) for all the SNPs genotyped. The genomic dosage panel (log *R* ratio) is not shown as it was normal for all the cases. The gray line in the center of the plot (0.5) represents heterozygosity at particular SNPs. The drastic reduction in heterozygous SNPs denotes the region of UPD. Disomy breakpoints at 20q are indicated by the arrows with the corresponding nucleotide position.

The molecular genetic findings in case 3 (GS0082) indicate that he did not inherit any copy of chromosome 20 from his mother. The microsatellite markers revealed the presence of a paternal heterodisomy for the short arm and a paternal isodisomy for the long arm of chromosome 20 (Table 2). The analysis of the results obtained by the Illumina Human660W array allowed us to identify a small homozygous region in 20p11.21 without informative microsatellite markers and a putative interstitial paternal heterodisomic region at the long arm (20q13.33–20-qter) (Fig. 4D). The 20q13.33 band has only been tested by the microsatellite marker *D20S173*, which begins at base 58 311 403, but the first heterozygous SNPs identified in this patient at rs6101320 is the nucleotide position 59 346 527. We also analyzed several extra microsatellite markers at the 20p11.21 (*D20S802*, *D20S566E*, and *D20S589E*) and 20q13.33 (*Z94413*, *D20S1024*, *D20S1106*, *G20531*, *RH25609*, and *D20S127E*) bands, but unfortunately they were non-informative, so it is also possible that the distal end of 20 does not have UPD.

The molecular findings could be explained by at least two homologous recombination events of chromosome

20 followed by nondisjunction during meiosis II (secondary heterodisomy) as shown in Fig. 1.

The interstitial deletion described by Aldred *et al.* (46) in the female patient includes a breakpoint at 20q13.31, coinciding with the position where case 3 has a homologous recombination between parental chromosomes. Therefore, it seems possible that in bands 20q13.13 (for the GS0060 and GS0090 patients) and 20q13.33 (for the GS0082 patient), there are regions of recombination that could lead to the deletions described by Aldred *et al.* and the partial isodisomies identified in these patients.

With respect to clinical implications, no significant differences were found in pat20qUPD compared with patients with methylation defect at *GNAS* locus of unknown origin; however, new genotype–phenotype correlations in PHP seem to emerge from our research. We observed that cases with longer pat20qUPDs (cases 2 and 3) were diagnosed with PHP at a younger age (9 and 5 years respectively) than those with shorter UPDs. Similarly, cases 2 and 3 both showed macrocephaly, rounded face, obesity/macrosomy in infancy, in contrast to cases 1 and 4 who lacked these prominent clinical features. Therefore, in our cases, the size of the underlying molecular defect correlated well with individual clinical and biochemical profiles as has been already reported in other imprinting disorders (49). In fact, when we looked at the imprinted genes located at 20q11.2–q13.13 (those which are shared by cases 2 and 3 but not by 1 and 4), we found that some of them could explain the different phenotypes. For example, *NNAT*, located at 20q11.2–q12 and coding for neuronatin, an imprinted gene actively transcribed only from the paternal allele (50) and whose putative role in human energy homeostasis has been pointed out (51), could explain the macrosomic appearance in the neonatal period of case 3 and the obesity in the infant period of case 2. Besides, it is well known that genes expressed from only one allele are often involved in regulation of growth and hence indirectly in the control of energy and glucose homeostasis (52). However, we should not forget the putative existence of a paternally inherited mutation that is inherited in a recessive manner and manifests as specific phenotype, as reported previously (53). Nevertheless, further cases are needed for specific genotype–phenotype correlations.

In summary, we describe four patients with PHP, out of twenty with methylation defects, who had a partial pat20qUPD involving distal 20q from 20q13.13–qter in two cases and interstitial pat20qUPD in one case. This is the first description of patients with PHP and segmental pat20qUPD. Our findings further highlight the complexity of the genetic basis of PHP and emphasize the importance of using polymorphic markers that map the whole of chromosome 20, as well as the SNPs-based arrays, for molecular diagnosis of UPD20 in PHP.

As the real prevalence of UPD in PHP patients might well be underestimated, we propose to perform a detailed molecular analysis of all patients diagnosed with PHP, including analysis for UPD in those with abnormal imprinting at all GNAS DMRs, and a further comparison of clinical and biochemical profiles of PHP patients with and without UPD, to improve our knowledge of the disease, and to provide patients and families with more accurate prognostic predictions and individualized clinical management and follow-up.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-10-0435>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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