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High frequency of paternal iso or heterodisomy at chromosome 20 associated with sporadic pseudohypoparathyroidism 1B★

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Abstract

Pseudohypoparathyroidism 1B (PHP1B) is caused by maternal epigenetic defects in the imprinted *GNAS* cluster. PHP1B can follow an autosomal dominant mode of inheritance or occur sporadically (spor-PHP1B). These latter patients present broad methylation changes of two or more differentially methylated regions (DMR) that, when mimicking the paternal allele, raises the suspicion of the occurrence of paternal uniparental disomy of chromosome 20 (upd(20)pat).

A cohort of 33 spor-PHP1B patients was screened for upd(20)pat using comparative genomic hybridization with SNP-chip. Methylation analyses were assessed by methylation specific-multiplex ligation-dependent probe amplification. Upd(20)pat was identified in 6 patients, all exhibiting typical paternal methylation pattern compared to normal controls, namely a complete loss of methylation of *GNAS* A/B:TSS-DMR, negligible methylation at *GNAS-AS1*:TSS-DMR and *GNAS-XL*:Ex1-DMR and complete gain of methylation at *GNAS-NESP*:TSS-DMR. The overall frequency of upd(20) is 18% in our cohort when searched considering both severe and partial loss of imprinting. However, twenty five patients displayed severe methylation pattern and the upd(20)pat frequency reaches 24% when searching in this group. Consequently, up to day, upd(20)pat is the most common anomaly than other genetic alterations in spor-PHP1B patients.

Upd(20)pat occurrence is not linked to the parental age in contrast to upd(20)mat, strongly associated with an advanced maternal childbearing age.

This study provides criteria to guide further investigations for upd(20)pat needed for an adequate genetic counseling.

★Conflict of interest: The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bone.2019.03.023>.

Keywords

PHP1B; Pseudohypoparathyroidism 1B; Paternal uniparental disomy of chromosome 20; Upd(20)pat; Upd(20)mat; GNAS; Methylation

1. Introduction

GNAS gene encodes the stimulatory guanine nucleotide-binding protein (Gas) and maps to 20q13.2–13.3, a complex locus subjected to parent-specific methylation. Molecular variant at *GNAS* lead to a wide spectrum of phenotypes depending on the parental localization of the genetic defect [1,2]. Heterozygous inactivating *GNAS* mutations are the cause of pseudohypoparathyroidism type 1a (PHP1A: *OMIM*N°103580), a syndrome characterized by multi-hormonal resistance and Albright Hereditary Osteodystrophy (AHO), when maternally inherited or PseudoPseudoHypoParathyroidism (PPHP: *OMIM*N°300800) or Progressive Osseous Heteroplasia (POH: *OMIM*N°166350) [3,4] when paternally inherited. As with PHP1A, patients affected by pseudohypoparathyroidism type 1B (PHP1B) develop resistance to PTH leading to hypocalcemia and hyperphosphatemia, which is often associated with resistance to TSH and occasionally with features of AHO. PHP1B patients don't carry variations in the *GNAS* exons coding Gas, but methylation changes at one or several differential methylated regions (DMRs) including at least *GNAS* A/B:TSS-DMR.

The *GNAS* locus gives rise to several different transcripts expressed from upstream promoters/first exons that show, with the exception of the Gas promoter, parent-specific methylation and are thus derived only from the non-methylated parental allele [5]. All alternative first exons are spliced onto the shared exons 2–13, giving rise to either coding (Gas, XLas, and NESP55), non-coding (AS), or presumably non-translated mRNAs (exon A/B).

The promoter region for the A/B transcript (*GNAS* A/B:TSS-DMR) is methylated on the maternal allele and its expression occurs only from the paternal allele. Likewise, the sense exon XL (*GNAS*-XL:Ex1-DMR) and the antisense exon AS (*GNAS*-AS1:TSS-DMR) are methylated on the maternal allele and transcribed only from the paternal allele. Conversely, the exon coding for NESP55 shows methylation on the paternal allele (*GNAS*-NESP:TSS-DMR) and its transcript is derived from the maternal allele.

PHP1B may follow an autosomal dominant pattern of inheritance (AD-PHP1B) or it can occur as a disorder that appears sporadically (spor-PHP1B). Loss-of-methylation (LoM) at *GNAS* A/B:TSS-DMR alone is found in AD-PHP1B cases caused either by microdeletions on the maternal allele comprising the gene encoding syntaxin 16 (*STX16*) [6–8], or by inversion of the region comprising exon A/B through exon 13 [9]. Copy numbers abnormalities encompassing one or several DMRs can affect the methylation patterns. For example maternal intragenic *GNAS* deletions including exon A/B to NESP55 DMR result in a paternal methylation pattern [10]. The four *GNAS* DMRs were also affected by deletions of AS exon 3–4 and/or NESP and AS 3–4 [6,8,11–13].

Genomic DNA from spor-PHP1B patients show broad *GNAS* methylation changes including LoM at *GNAS A/B:TSS-DMR*, *GNAS-XL:Ex1-DMR* and *GNAS-AS1:TSS-DMR* promoter as well as gain of methylation (GoM) at *GNAS-NESP:TSS-DMR* [14]. So far, no specific genetic mutation has been identified as a cause of these changes.

Uniparental disomy (UPD) is a condition in which individual inherited both copies of one chromosome from only a single parent. Consequently, the patient harbors the parent-specific methylation pattern. Since the first description by Bastepe et al. [15], thirteen patients have been defined at the molecular level revealing a paternal disomy of chromosome 20 (upd(20)pat) affecting either the entire chromosome or only the long arm (upd(20q)pat) [15–22].

We now describe six new patients harboring upd(20)pat from a cohort of 33 patients affected by spor-PHP1B. One patient presented with a particularly mixed pattern of paternal iso- and heterodisomy at chromosome 20. Taken previous reports into consideration, we estimated the frequency of upd(20)pat in spor-PHP1B patients and defined a specific methylation signature. Last, we evaluated the influence of the parents' childbearing ages on the occurrence of these epigenetic changes.

2. Subjects and methods

2.1. Patients

We studied 33 cases from Caucasian origin (17 males and 16 females) with the diagnosis of spor-PHP1B, who had been evaluated by our laboratory over the past 5 years. Data describing clinical symptoms were collected retrospectively using records from hospitals or primary care physicians. Routine biochemical assays were performed at the time of diagnosis, or during the follow up, in a variety of different clinical laboratories. This study analysis was conducted according to the Declaration of Helsinki with written consent for collection of DNA, clinical and laboratory data to conduct molecular studies obtained from the patient and/or their parents.

2.2. Molecular investigations

2.2.1. Methylation studies—Leukocyte genomic DNA was screened for *GNAS* mutations and *STX16* deletions using procedures routinely used in the laboratory [13]. Copy number variants (CNV, such as deletions and duplications) and methylation profiling were assessed by methylation specific-multiplex ligation-dependent probe amplification (MS-MLPA) assay using the ME031-A1 kit (MRC-Holland, Amsterdam, the Netherlands) [13,23] according to the manufacturer's instruction. PCR products were analyzed on an ABI3130 sequencer using GeneMapper (Applied Biosystems) and Coffalyser (MRC-Holland) softwares. The reproducibility of the assay was assessed by measuring the methylation at each DMR in a same control individual in eleven independent assays (Table S1), even if the reproducibility and reliability of the MS-MLPA assay were previously described by Elli et al. [24].

The diagnosis of spor-PHP1B was established on the basis of lack of a positive familial history, absence of CNV within *STX16* or *NESP*, LoM at *GNAS A/B:TSS-DMR*, associated

to epigenetic changes at two or more DMRs including GNAS-XL:Ex1-DMR, *GNAS-AS1*:TSS-DMR as well as GoM at GNAS-NESP:TSS-DMR.

2.2.2. Uniparental disomy—Uniparental disomy was searched on DNA using two different procedures: array CGH and SNP genotyping. CGH/SNP array analysis (Agilent SurePrint G3 Cancer CGH + SNP 4×180K) was processed according to the manufacturer's instruction (Agilent). One patient was also studied using a 300K SNP chip (CytoSNP-12 Illumina®) which included 7614 markers (7598 SNPs and 16 CNVs) located on chromosome 20.

Thirty six specific SNP primers (listed in Fig. S2) were designed across chromosome 20 to build the chromosome 20 haplotypes. Briefly, the amplification refractory mutation system (ARMS)-PCR technique [25] was used to amplify the 36 SNPs using 7500 Fast Real-Time PCR System (Thermo Fisher Scientific) which allows single-plex SNP interrogation through fluorescence detection. Analysis of microsatellite markers across the 20q13.2 region was performed by the center of Human Genetic Research of the Massachusetts General Hospital as previously described [26]. Loss of heterozygosity (LoH) at each SNP indicates inheritance of two copies of the chromosome from one parent (isodisomy). Heterodisomy of chromosome 20 (hUPD) correspond to the inheritance of the two chromosomal homologs from one parent. It was searched in trio including the patient and both parental DNAs or in duo with only one parental DNA. Heterodisomy is inferred from allelic discordance between the patient and his/her mother or allelic concordance with his/her father. All genomic positions were based upon hg19/GRCh37 assembly.

2.3. Statistical analysis

For each biochemical parameter, differences between groups were calculated using the Mann and Whitney nonparametric *U* test for unpaired samples using GraphPadPrism software. A two-tailed *p* value < .05 was considered statistically significant.

3. Results

Clinical and biochemical data of the 33 patients diagnosed as spor-PHP1B are shown in Table S3. Depending of the degree of methylation defect at GNAS-XL:Ex1-DMR, patients can be subdivided in two major groups, one with a severe methylation defect ($n = 25$; < 10%) and another with a partial methylation defect ($n = 8$; > 10%). The main clinical findings at presentation were convulsions associated with hypocalcemia and an elevated PTH level. Ten patients also had elevated TSH levels. Fifteen patients presented with mild AHO features as suggested by round face and slight metacarpal shortening. One patient (patient 6) for whom data were available exhibited a significant early-onset weight gain; the BMI had returned to normal before puberty (Fig. S4).

3.1. Upd(20)pat was identified in 6 patients (Figs. 1, 2 and Table S3, patients 1–6)

Four patients displayed complete upd(20)pat, one patient a upd (20q)pat (Fig. 1, patients 1 and 2). Patient 6 harbors two paternal chromosomes 20, one of which had undergone mixed upd(20) rearrangement (Fig. 2). Analysis of DNA from this patient and his parents using a

300K SNP chip showed a LoH (paternal isodisomy, iUPD) extending from 20p13 to 20q13.2 with telomeric paternal heterodisomy (hUPD). The SNP analysis indicated that the hUPD overlaps the *GNAS* locus at position 20q13.3. Finally, on 6 patients with upd(20) identified in this study, origin of parental disomy was determined only for two patients (paternal DNAs were available). Others upd(20)pat were inferred on the phenotype patient.

3.2. Percent of methylation at each *GNAS* DMRs was compared between spor-PHP1B patients with or without upd(20)pat (Fig. 3)

All patients with upd(20)pat display a complete loss of methylation of *GNAS A/B:TSS-DMR* ($0 \pm 0\%$), negligible methylation at *GNAS-ASI:TSS-DMR* and *GNAS-XL:Ex1-DMR* ($1.1 \pm 1.6\%$ and $0.77 \pm 0.9\%$, respectively) and complete methylation at *GNAS-NESP:TSS-DMR* ($97 \pm 3.3\%$) compare to normal control.

No statistical differences in the degree of methylation at *GNAS-ASI:TSS-DMR* and *GNAS A/B:TSS-DMR* were observed between patients with or without upd(20)pat. Interestingly, we observed that spor-PHP1B patients without upd(20)pat displayed a broader range in the degree of methylation at *GNAS-XL:Ex1-DMR* [0–44%] compared to spor-PHP1B with upd(20)pat [0–2%], even if the threshold of significance is not reached ($p = .11$).

3.3. Parental age at conception in upd(20) (Fig. 4)

We compared parental ages at conception between spor-PHP1B patients with or without upd(20)pat and used as control AD-PHP1B patients from our laboratory ($n = 17$). We added to our data patients previously described with paternal and maternal upd(20) [15,18,21,27–29]. The maternal age at conception was similar for AD-PHP1B and spor-PHP1B patients without upd(20)pat (26.7 ± 6.25 years, $n = 17$ vs. 26.7 ± 4.98 , $n = 20$; $p = .861$). We observed a slightly higher in maternal age of conception in upd(20)pat patients (30.1 ± 6.24 years, $n = 8$) even it is not statistically different from the other two groups (spor-PHP1B, $p = .125$ or AD-PHP1B, $p = .186$). By contrast, in upd(20)mat patients, maternal age at conception is statistically advanced compared to others groups and particularly to AD-PHP1B group (38.1 ± 4.69 years; $n = 15$ vs. 26.7 ± 6.25 , $n = 17$, $p < .0001$) [27–29]. The paternal age at conception was higher in upd(20)pat patients, but the difference is not statistically significant (30.9 ± 6.84 years, $n = 7$ vs. 28.7 ± 6.4 , $n = 15$).

4. Discussion

This report presents clinical and biochemical data on a large cohort of patients with an imprinted disorder known as spor-PHP1B caused by methylation abnormalities at *GNAS* locus.

With respect to clinical implications, no significant differences were found in upd(20)pat patients compared with patients with methylation defect at *GNAS* locus of unknown origin. Clinical symptoms, mainly convulsion or seizure, develop at an age similar to other PHP1B patients [30]. Out of 17 patients with upd(20)pat in whom the data were available, six (35%) presented advanced weight ($> +2$ SD) for age at the time of diagnosis [15–18]. An intriguing feature remains the normalization of the weight after six years and indeed, Takatani et al. [21] reported that none other patients with upd(20)pat are obese at the age of 7 and 12,

respectively (Table 1). Patients with AD-PHP1B due to *STX16* deletions and spor-PHP1B display very similar early overgrowth [31]. Recently, Gruters-Kieslich et al. [32] found obesity during the first year of life being the first clinical evidence for PHP1B suggesting that epigenetic *GNAS* analyses should be considered for unexplained obesity. Patients with upd(20) are therefore a good model to better understand the pathological mechanism responsible for the much-enhanced growth. With the exception of A/B transcript, our study brings arguments suggesting that the paternal transcripts which are biallelically expressed in spor-PHP1B with upd(20)pat and mono-allelically expressed in AD-PHP1B, cannot be responsible for the overgrowth in all forms of PHP1B patients. That restrains further investigations to the role of the defect of Gas expression and/or the biallelic expression of A/B.

UPD can involve either the whole chromosome and reflects complete isodisomy or affect the long arm of the chromosome 20 or be distal, from 20q13.13-qter [16,18]. It can be segmental, interspersed with regions of heterodisomy. In this study, we described a rare mixed chromosomal rearrangement associating paternal telomeric heterodisomy and paternal central isodisomy different from that previously described with different disomy breakpoint in the long arm [18]. The co-occurrence of meiotic recombination, abnormal segregation and subsequent correction are implicated [33].

We found that 18% of spor-PHP1B had inherited two copies of the paternal alleles at *GNAS* locus, a frequency closed to that reported by Fernandez-Rebollo et al. [18]. When combined with data from previous cohort reports [16,18,19,21], the frequency decreased to 13% (14 with upd(20)pat among 105 spor-PHP1B, Table 1). In this study, we used SNP genotyping with high density arrays that detect blocks of homozygosity which offers a more reliable procedure than microsatellite markers with high informativity but limited density that can miss a small segmental UPD [17,18]. We have however to point out that the number of reported UPD cases could be a slight underestimate because heterodisomy could be missed in absence of parental samples.

Different technologies have been used to study the methylation at *GNAS* locus. Maupetit-Mehouas et al. [34], Elli et al. [35] and Elli et al. [24] described complex LoM and GoM associations with complete and partial defects (pLoM) in spor-PHP1B. The overall frequency of upd(20) was low (8%) when searched considering both severe and partial loss of imprinting (pLoI) [35]. In the present paper we studied a cohort with the same MS-MLPA protocol which is currently used as a routine procedure for the quantification of the methylation defect at *GNAS* locus. In our cohort, severe methylation defects at all *GNAS* TSS-DMRs were only detected in upd(20)pat. Indeed, a broader range in the degree of methylation at *GNAS-XL:Ex1-DMR* is not in favour of upd(20). UPD might only be suspected in the presence of severe LoI especially *GNAS-XL:Ex1-DMR*. So, twenty five patients displayed severe methylation pattern (Table S3) and the upd(20)pat frequency reaches 24% when searching in this group.

The variation of methylation at *GNAS-XL:Ex1-DMR* remains without underlying genetic cause. A *de novo* mutation somewhere, within or not the *GNAS* domain that impacts on methylation establishment would not be excluded. In fact, mixed pattern of methylation

defects and pLoM at *GNAS-XL:Ex1-DMR* appear to be the most prevalent methylation pattern defect in spor-PHP1B [35]. Susceptibility to methylation defects has been described in multilocus imprinted disorders (MLID) [36,37] leading to the hypothesis of an imprinted gene network. Methylation defects in *GNAS* have been found in a subset of Beckwith-Wiedemann syndrome (BWS) cases [38], and the presence of PHP1B in BWS patients has been described, at least, once [39]. Finally, sporadic cases of PHP1B with pLoI could represent true stochastic errors in early post-zygotic phases and embryonic maintenance of methylation [40] and might correspond to mosaicism [24].

The identification of a genetic cause allows adapted genetic counseling for the affected patient and their families. With a prevalence estimated to be approximately 1.65/10000 [41], UPD is a rare condition resulting from meiotic nondisjunction followed by compensatory mechanisms including trisomic rescue, monosomic rescue, compensatory UPD and/or somatic recombination errors [42]. No case of recurrence has been reported until now. A reassuring genetic counseling can be brought to the family for prenatal diagnosis.

Last, our study does not show a correlation between paternal or maternal ages at the conception and the occurrence of upd(20)pat in their child. In contrast an advanced maternal age is clearly associated to upd(20)mat [27–29,43,44]. Incidence of aneuploidie in oocytes increased with maternal age at childbirth and would contribute to the development of trisomy rescue type maternal UPD or monosomy rescue type paternal UPD by replication of a single paternally derived chromosome [45]. The lack of connection with parental age suggests upd (pat) to be a random event during the formation of reproductive cells (eggs and sperm).

In conclusion, our study emphasizes the high frequency of upd(20) pat in spor-PHP1B patients. Methylation profiling using MS-MLPA is a convenient tool for evaluating the methylation pattern at *GNAS* locus to better define the phenotype of spor-PHP1B patients who should benefit of genetic screening for upd(20)pat. While upd(20)mat is strongly associated with an advanced maternal childbearing age, the upd(20)pat occurrence is not linked to the parental age at the conception.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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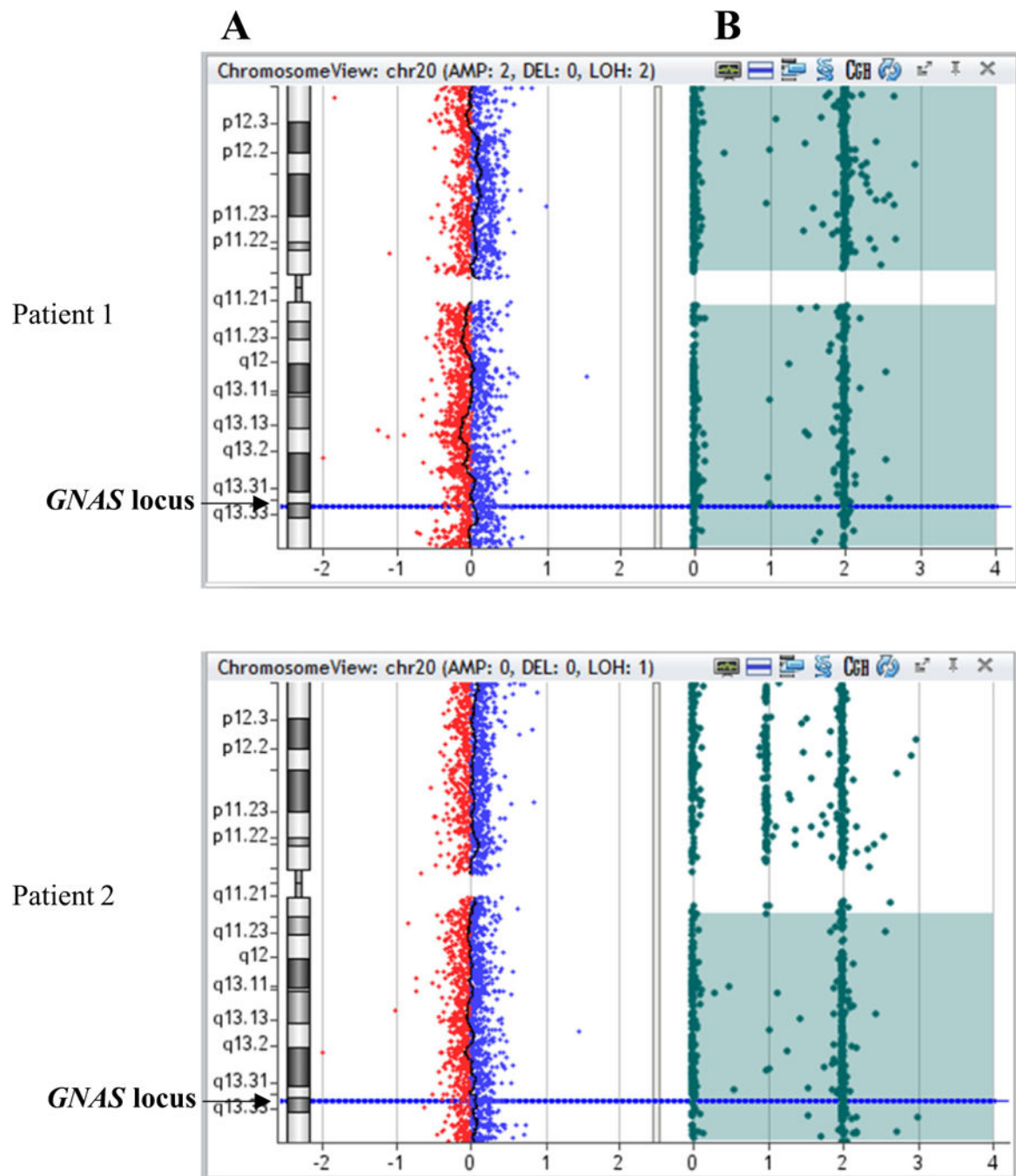
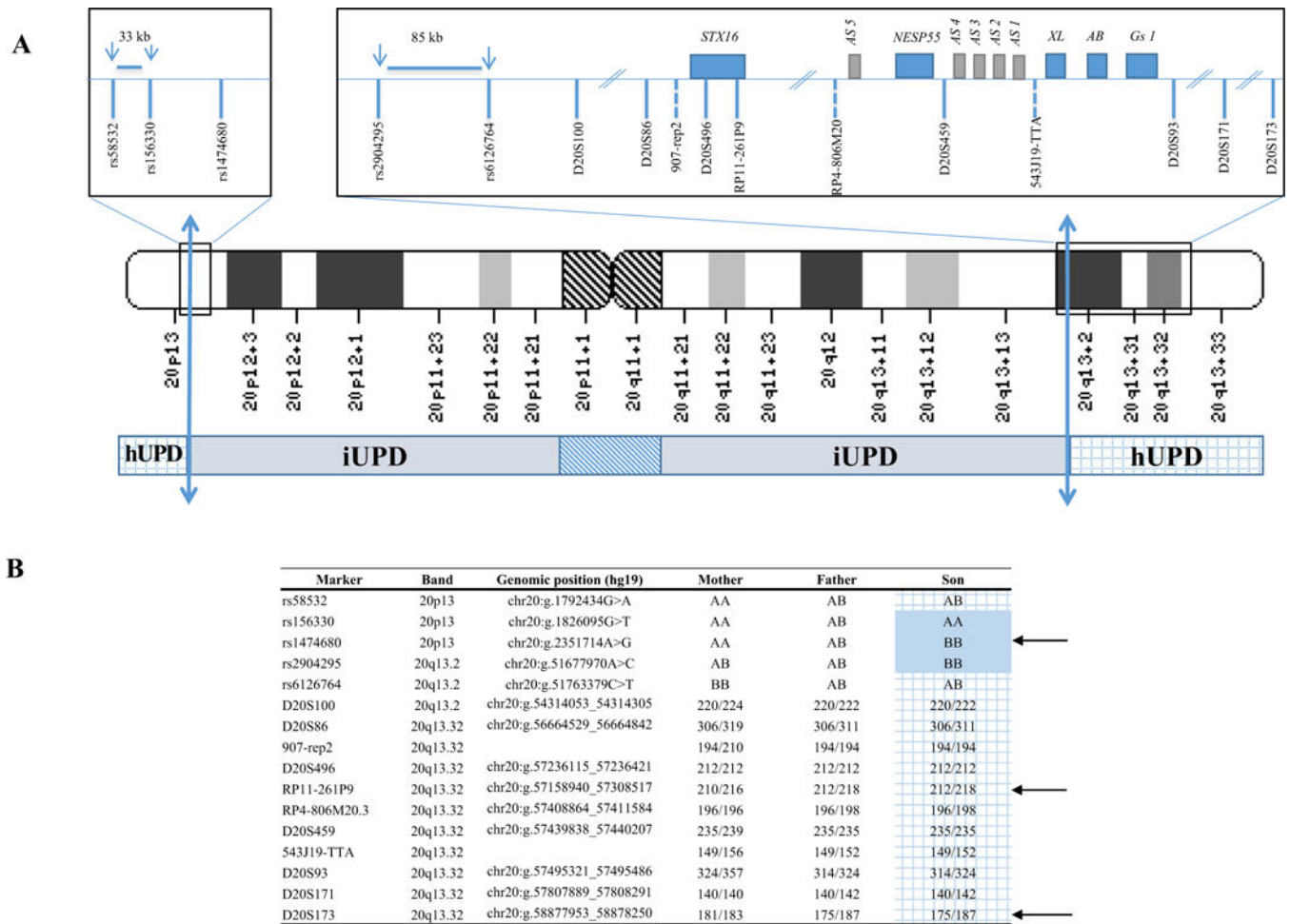


Fig. 1.

CGH/SNP-array in two patients presenting upd(20)pat (patient 1) or upd(20q)pat (patient 2). A: copy number, the X-axis represents the normalized log₂ ratio Cy5(patient)/Cy3(healthy control) fluorescence intensity thresholds - 1 (loss) and 1 (gain), while the Y-axis represents the chromosome 20; B: SNP array; each SNP probe (represented by a filled circle) at a given locus can exist either in homozygous state (a single allele at 0 or 2) or in heterozygous state (at 1). Homozygosity is demonstrated by probes present at either 0 or 2 and the absence of probes at 1.

**Fig. 2.**

Mixed rearrangement in chromosome 20 (patient 6).

upd(20)pat.arr[GRCh37] 20p13(63244_1792434)x2 htz,20p13q13.2(1800783_51758074)x2 hmz, 20q13.2q13.33(51763379_62909908)x2 htz. A. The inferred breakpoints between paternal isodisomy (iUPD) and paternal heterodisomy (hUPD) are indicated by the two-way arrows: in the short arm, they are located between the last heterozygous SNP (rs58532) (chr20:g.1792434) and the first homozygous one rs156330 (chr20:g.1826095) and in the long arm between rs2904295 (chr20:g.51677970) and rs6126764 (chr20:g.51763379). B. SNP (single nucleotide polymorphism) and microsatellite markers analysis in patient 6 confirmed mixed rearrangement with paternal isodisomy (iUPD) extending from 20p13 to 20q13.2 with telomeric paternal heterodisomy (hUPD)(grid). Arrows indicate informative SNPs or microsatellite markers. The hUPD overlaps the *GNAS* locus at position 20q13.3.

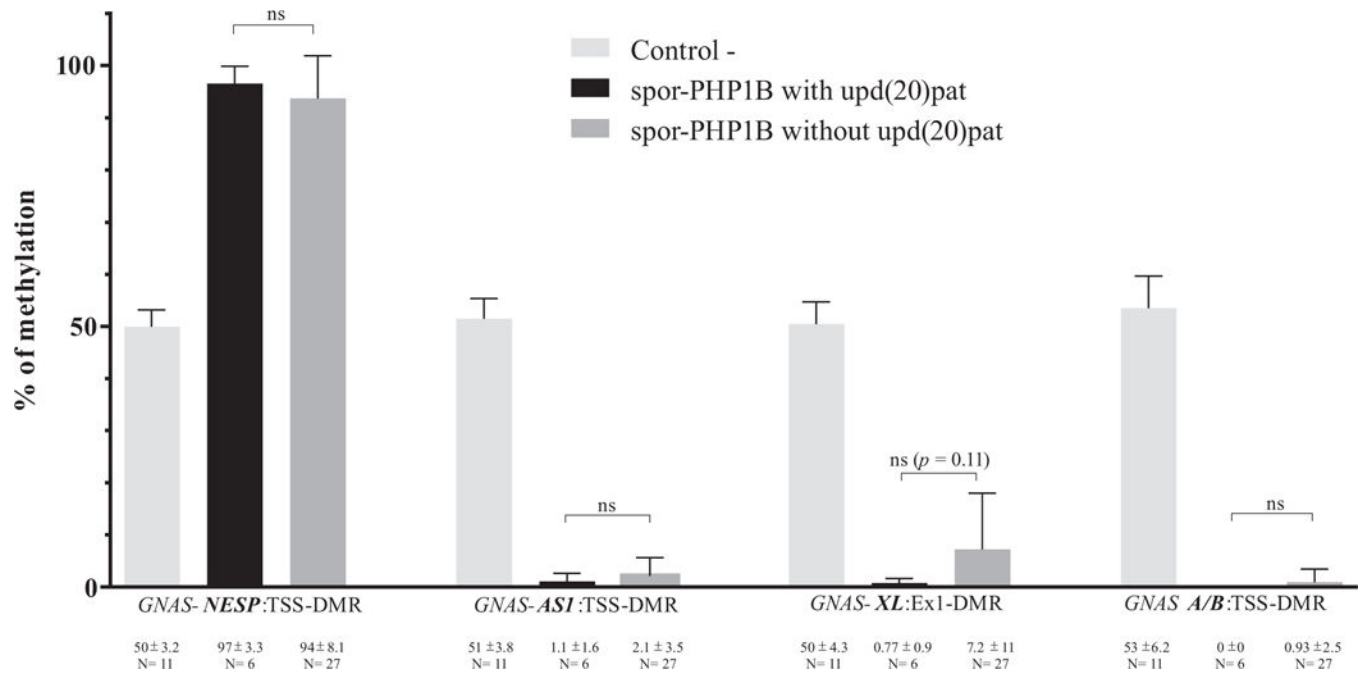


Fig. 3. Methylation pattern (%) at *GNAS* locus in patients with spor-PHP1B with or without upd(20)pat.

Dosage and differential methylation analyses were assessed by multiplex ligation-dependent probe amplification and methylation specific-MLPA (MS-MLPA). The relative peak area of each target probe from the ligation-only reaction of the patient DNA was compared to that obtained from the controls. Data are expressed as percentage of methylation (mean ± SD) for each DMRs. The percentage was 50 in the control for the DMRs in which allele-specific methylation was observed. A percentage closed to 100 suggests biallelic methylation. In contrast, a value closed to 0 suggests biallelic demethylation at these DMRs. *p* values for comparison between groups are indicated. ns: no significance.

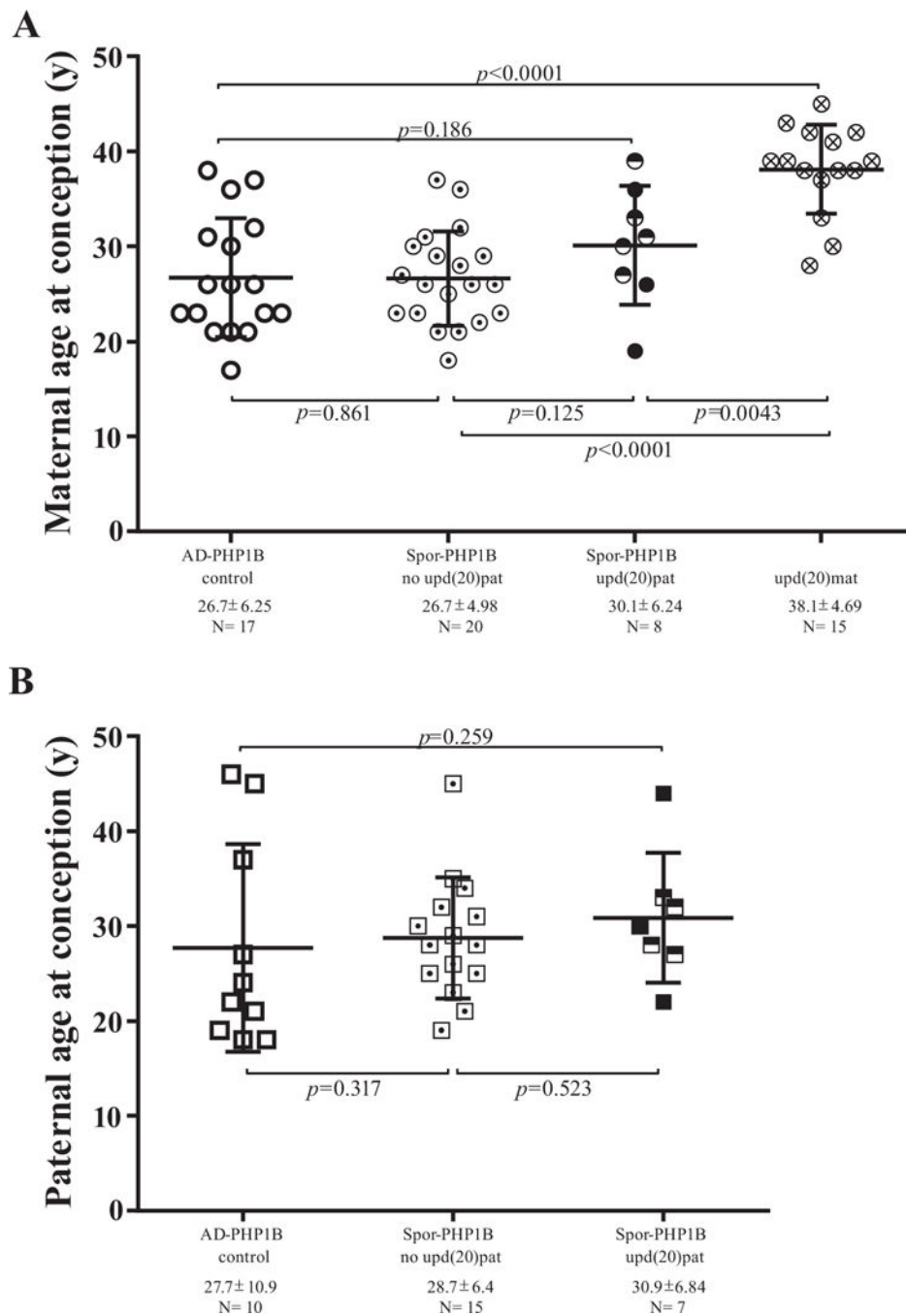


Fig. 4. Maternal and paternal age at conception in patients with spor-PHP1B with and without UPD. Circles display maternal age (A) and squares paternal age (B) respectively. Empty form: control patient (AD-PHP1B); form with a point: spor-PHP1B without upd(20) pat; half full form (literature [15,18,21]) and full form (this study): spor-PHP1B due to upd(20)pat; form with a cross: upd(20)mat (literature [24,27]). N = number of subjects per group. The

horizontal and vertical bars for each group represent the mean \pm SD. *p* values for comparison between groups are indicated; y: years.

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Table 1 Clinical and epigenetics features with parental age at conception of patients with spor-PHP1B due to upd(20)pat reported in the literature and comparison with our patients. upd(20)pat: paternal disomy at chromosome 20; ND: not determined.

Authors	Bastepe et al. [15]		Lecumberri et al. [22]		Bastepe et al. [16]		Fernandez-Rebollo et al. [18]		Dixit et al. [17]	
	Case report	Case report	Case report	Case report	Patient 1	Patient 2	Patient 3	Patient 4	Patient 1	Patient 2
Frequency	Upd(20q)	Upd(20q)	Upd(20q)	1/22 (4.5%)	Upd (20q13.13-qter)	iUPD(20q)	HUPD(20p).iUPD+putative HUPD (20)(q13.33q23)	Upd(20q13.13-qter)	Upd(20q12-q13.33-qter)	Upd(20q13.31-q13.32)
Method	STR	STR	STR	SNPs + 100K SNP array	STR	MS-MLPA	STR, SNP array	MS-MLPA	STR, SNP array	Methylation-specific PCR
<i>GNAS</i> -TSS-DMRs methylation analysis	Methylation-sensitive restriction enzyme	MS-MLPA	MS-MLPA	ND	ND	GoM	MS-MLPA	MS-MLPA	MS-MLPA	Methylation-specific PCR
<i>GNAS</i> -NESP-TSS-DMR						GoM				
<i>GNAS</i> -AS /-TSS-DMR						LoM				
<i>GNAS</i> -XL-Ex1-DMR						LoM				
<i>GNAS</i> A/B-TSS-DMR						LoM				
Sex	M	M	F	F	F	M	M	M	M	M
Age at diagnosis (years)	5	9	3.5	3.5	26	9	5	46	14.5	5.5
Weight at birth (percentile)	+3.4	-1	+2	+2	ND	-0.92	+5.5	+1.5	50-75	25-50
Weight at diagnosis (SD)	+3.6	-2	>+2	>+2	-1	-1.6	+2	-2	0	+2
Height at diagnosis (SD)	-	+	-	-	-	+	-	ND	-	ND
AHO signs										
Maternal age at conception (years)	39	ND	ND	ND	27	31	33	33	ND	ND
Paternal age at conception (years)		ND	ND	ND	27	32	33	33	ND	ND
Authors	Takatani et al. [21]		Park et al. [20]		Jin et al. [19]		This paper			
	Patient 1		Patient 2		Patient 1		Patient 2		Patient 3	
Frequency	2/23 (8.7%)		Case report osteosarcoma		1/7 (14%)		6/33 (18.2%)		6	
Upd(20)pat type	Upd(20q)		Upd(20q)		Upd(20q)		Upd(20q)		Upd(20)	
Method	SNPs GNAS + STR		STR		STR		CGH/SNP array		STR + SNP array	
<i>GNAS</i> -TSS DMRs methylation analysis					MS-MLPA					
<i>GNAS</i> -NESP-TSS-DMR			GoM		97%		101%		98%	
<i>GNAS</i> -AS /-TSS-DMR			LoM		0%		0%		3%	
<i>GNAS</i> -XL-Ex1-DMR			LoM		0%		1%		2%	
					0%		0%		0%	

