Paternal isodisomy is a frequent cause of sporadic pseuhypoparathyroidism IB

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Abstract

Pseudohypoparathyroidism type IB (PHP1B) is an imprinting disorder characterized by end-organ resistance to parathyroid hormone leading hypocalcemia and hyperphosphatemia and, frequently, to thyroid-stimulating hormone. PHP1B is associated with methylation changes at one or several differentially methylated regions (DMRs) within the complex *GNAS* locus, (20q13.2-13.3). While the PHP1B familial form (AD-PHP1B) associated to an isolated loss of methylation at the *GNAS* A/B DMR, is due to a deletion in the *STX16* gene, the cause of the *GNAS* diffuse imprinting defects observed in sporadic PHP1B (spor-PHP-1b) remains understood. Uniparental disomy (UPD) is when both copies of a chromosome are from one parent only. The epigenetic changes observed in spor-PHP1B mimic the paternal-specific methylation pattern. Thus, paternal UPD of chromosome 20 (patUPD20) without maternal contribution is a plausible cause of PHP1B.

We screened a cohort of 54 patients presenting spor-PHP1B to evaluate the frequency of patUPD20. Comparative Genomic Hybridization combined with Single Nucleotide Polymorphism arrays (CGH+SNP-array) was used to identify the absence of copy number variant (CNV) with loss of heterozygosity (LOH). Comparison of short tandem repeats (STR) along chromosome 20 between the proband and his parents was used to confirm CNV or LOH.

Because CGH+SNP-array required high quality DNA, only 21 samples were tested and 18 analysed. Four patients (22%) were found with patUPD20 : three with complete patUPD20, one with patUPD of the long arm of chromosome 20. In addition, we described an interstitial UPD including *GNAS* locus.

Our study suggests that patUPD20 is a frequent cause of PHP1B that is important for genetic counseling and should be tested in evaluation of patients with sporadic PHP1B.

Objectives

The first objective of the research project is to confirm patUPD (isodisomie) of chromosome 20 as a mechanism responsible for sporadic PHP1B and second, to assess the frequency of this mechanism in patients with sporadic PHP1B.

Methods and results

Using the MS-MLPA ME031-A1 kit (MRC-Holland, Amsterdam, the Netherlands) and quantification of the methylation after bisulfite treatment and pyrosequencing we identified methylation changes at *GNAS* locus in a cohort of 54 patients : loss of methylation at AS, XL and A/B DMRs and gain of methylation at Nesp55 on the maternal allele suggesting two copies of the paternal pattern without maternal contribution.

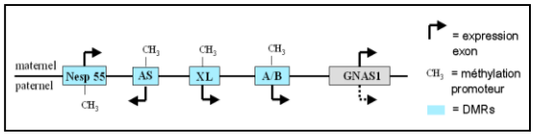
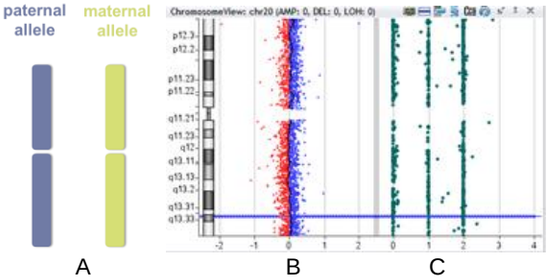


Fig 1 : organisation of the *GNAS* locus indicating the different DMRs on the paternal and maternal alleles. (see ref 1).
Alternative first exons which splice into exon 2 to generate alternative mRNAs encoding NESP55, XLαs and GSα are shown as boxes labeled NESP, XLαs, A/B, and 1, respectively.

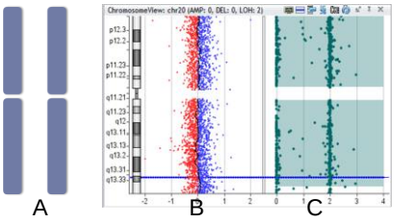
a-CGH-SNP genotyping (Fig 2) .
(pangenomics array 4x180K SurePrint G3 Cancer CGH+SNP Agilent)
A : paternal and maternal contribution
B : genomic dosage using CGH-array : 0 denoted two copies (copy neutral); number <-0.5 or > 0.5 denotes deletion or amplification respectively.
C : SNP-array : Lines 0 and 2 refer to homozygosity and the line 1 in the center of the plot represents heterozygosity at particular SNPs at chromosome 20.

Fig 2 : control patient : A : paternal (blue) and maternal (yellow) chromosomes; B: no CNV; C no LOH.



We found 5 patients (22%) with patUPD20 : 3 complete patUPD20 (fig. 3), 1 patUPD of the long arm of chromosome 20 (fig. 4) and 1 with an intertitial UPD including GNAS locus (fig. 5).

Fig 3 : complete patUPD20
A : (two paternal copies in blue)
The drastic reduction in heterozygous SNPs with normal CNV (B) denotes the region of uniparental isodisomy (C).



The fully informative markers on 20q reveal LOH with a lack of maternal contribution

Fig 4 : patUPD of the long arm of chromosome 20
B : no CNV
C : LOH is limited to the long arm of the chromosome

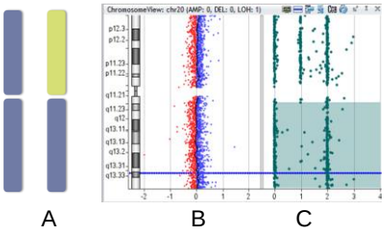
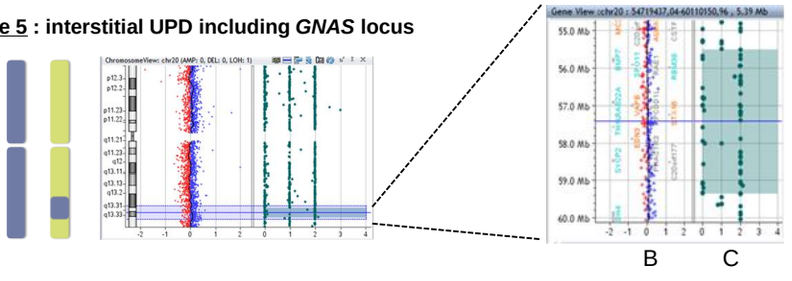


Figure 5 : interstitial UPD including *GNAS* locus



B : No CNV
C: interstitial LOH

Conclusions

With a frequency up to 20%, our study suggests that patUPD20 is a frequent cause of PHP1B as it was found in other imprinting disorders.
UPD should be tested in evaluation of patients with sporadic PHP1B that is important for genetic counseling.

References

1-J Liu, S Yu, D Litman, W Chen and L S. Weinstein *Mol. Cell. Biol.* 2000, 20(16):5808.
2- Dixit A, Chandler KE, Suri M 2013 *J Clin Endocrinol Metab* 98:E103-108