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-PHP1B) 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.

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.

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.

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 interstitial UPD including GNAS locus (fig. 5).

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

2- Dixit A, Chandler KE, Suri M 2013 J Clin Endocrinol Metab 98:E103-108