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► **To cite this version:**

Andreea Apetrei, Arnaud Molin, Cindy Colson, H. Mittre, Matthieu Decamp, et al.. Identification by whole genome sequencing of a 140Kb inversion in GNAS locus involving NPELP1 and Xlas gene in a family presenting an autosomal dominant pseudohypoparathyroidism type 1b. Assise de génétique Humaine et Médicale 2020, Jan 2020, Tours, France. hal-03283983

HAL Id: hal-03283983

<https://hal.science/hal-03283983v1>

Submitted on 12 Jul 2021

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Identification by whole genome sequencing of a 140Kb inversion in *GNAS* locus involving *NPELP1* and *Xlas* gene in a family presenting an autosomal dominant pseudohypoparathyroidism type 1b

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Background :

The *GNAS* complex locus encodes the alpha-subunit of the stimulatory G protein (G α) and additional imprinted transcripts. Pseudohypoparathyroidism type 1b (PHP1b) is characterized by renal resistance to PTH (and, sometimes, a mild resistance to TSH), which occurs typically in the absence of any features of Albright's hereditary osteodystrophy. PHP1b is caused by epigenetic modifications at one or several differentially methylated regions (DMR) within *GNAS* locus that reduce transcription of G α from the maternal allele, resulting in diminished G α expression in the tissues in which the paternal G α allele is normally silenced. The most frequent genetic cause for the autosomal dominant PHP1b (AD-PHP1b) is the maternally inherited *STX16* deletion, which leads to isolated loss-of-methylation (LOM) at exon A/B alone. Other rare molecular mechanisms are described, especially copy numbers abnormalities encompassing one or several DMRs (NEPS55, NESPAS, XLas).

Patients & Methods :

In this study we investigated a family with two affected individuals, the proband and his sister, both presenting a typical phenotype of PHP1b (Table 1).

| | I.1 | II.1 | II.2 | II.3 | I.2 |
|---------------------------|-------|-------|------|------|--------|
| Adult height (cm) | 172 | 168 | - | 157 | 165 |
| Age at laboratory testing | 43y9m | 12y7m | - | 6a4m | 37y11m |
| PTH (pg/mL) | 37,9 | 1007 | 17 | 682 | 44,6 |
| Calcium (mmol/L) | 2,35 | 1,93 | - | 2,18 | 2,4 |
| Ionised Calcium (mmol/L) | 1,15 | 0,93 | - | 2,34 | - |
| Phosphate (mmol/L) | 1,44 | 2,24 | - | 1,76 | 1,09 |
| 25D (ng/mL) | 9 | 10 | 8 | 8 | 14 |
| 1,25D (pg/mL) | 50 | 43 | 62 | 56 | 41 |
| TSH (mU/L) | - | 4 | - | 4,62 | - |
| FT4 (ng/dL) | - | 1,15 | - | 0,66 | - |

Table 1 : Laboratory analysis of a family with AD-PHP1b. Pedigree of the investigated family, consistent with the mode of inheritance of PHP1b. Birth parameters, age at diagnosis and laboratory findings at presentation are provided.

A Sanger sequencing of the *GNAS* locus and a methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) analysis were realized for the 2 affected siblings (II.1 and II.3) and their parents (I.1 and I.2). A whole genome sequencing (WGS) analysis was performed for the proband (I.1) : the library preparation (350pb fragments with paired-end 2x150pb) used the NEBNext DNA Library Prep Kit, the sequencing was performed using an Illumina platform, the sequences were aligned at the reference human genome GRCh38 and the data was processed using the GATK pipeline. The inversion was confirmed by a Sanger analysis of the break-points and by a PCR analysis of the genomic DNA using primers designed to amplify across both breakpoints of the mutant allele. A quantitative real-time polymerase chain reaction (qPCR) was realized for the index case and his parents.

Results :

There was no gain, deletion or mutation in the *GNAS* locus.

The epigenetic analysis by MS-MLPA showed a complete LOM at A/B-DMR, a partial LOM at XLas-DMR and normal methylation for AS-DMR and NESP-DMR for the two patients.

There was no LOM for their parents (Table 2).

| % Méthylation | I.1 | II.1 | II.3 | I.2 |
|---------------|-----|------|------|-----|
| % NESP | 50 | 49 | 49 | 49 |
| % AS | 47 | 49 | 52 | 65 |
| %XLa | 48 | 21 | 22 | 56 |
| %A/B | 47 | 0 | 0 | 44 |

Table 2 : MS-MLPA results as mean values of the percentages of all studied DMRs.

The WGS analysis revealed a paracentric inversion of 140 Kb with the breakpoint localized at 5': intron *NPELP1* and 3': exon *XLas* (Figure 1).

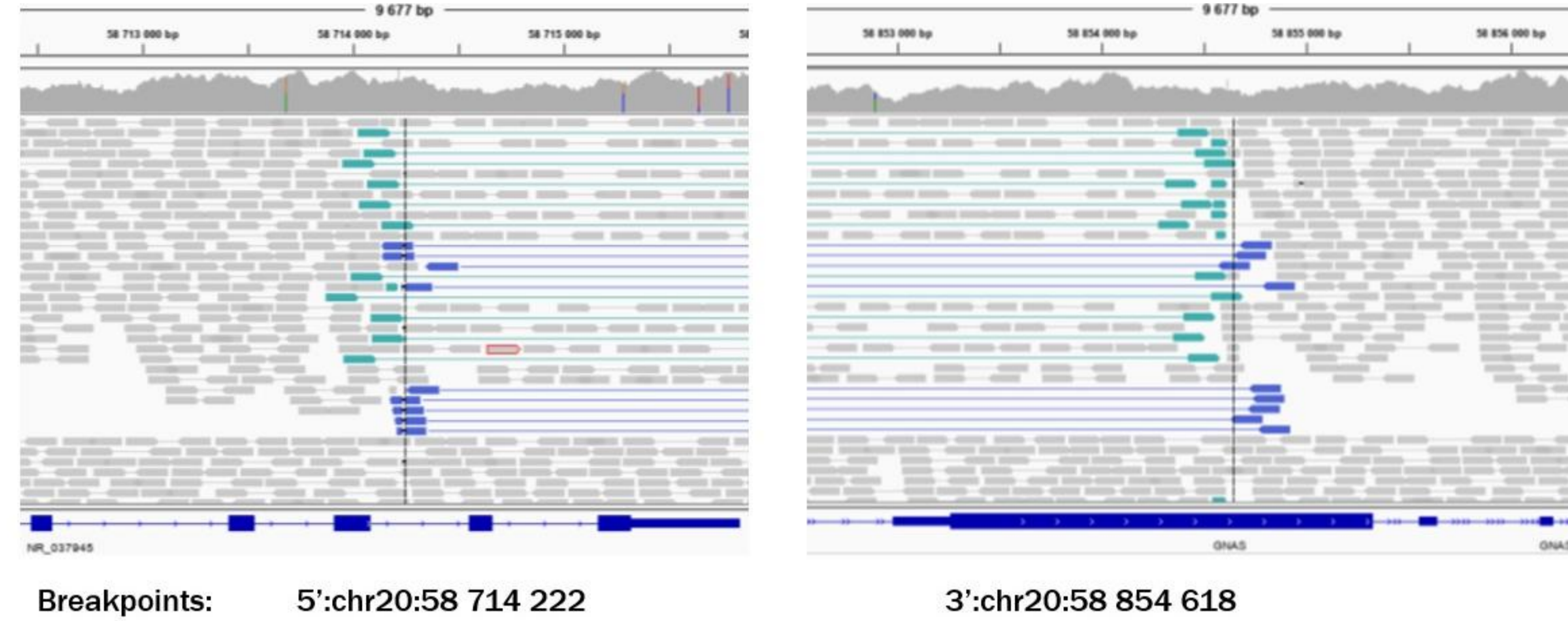


Figure 1 : Use of the Integrative Genome Viewer to show WGS data of the centromeric and telomeric breakpoints of the inversion. The blue and the green bars are indicative of nucleotide sequences that match only partially with these regions of chromosome 20.

A PCR analysis of the genomic DNA using primers designed to amplify across both breakpoints for the wild-type and mutant allele was realized (Figure 2A). Amplicons were obtained for the index case, his sister and his mother, while no PCR product was generated for DNA from the father (Figure 2B). The qPCR analysis showed a 50% augmentation for the father and no significant difference for the mother in the WT allele DNA quantification compared to the index case (Figure 2C). The breakpoints were confirmed by Sanger analysis for both patients and their mother (Figure 3).

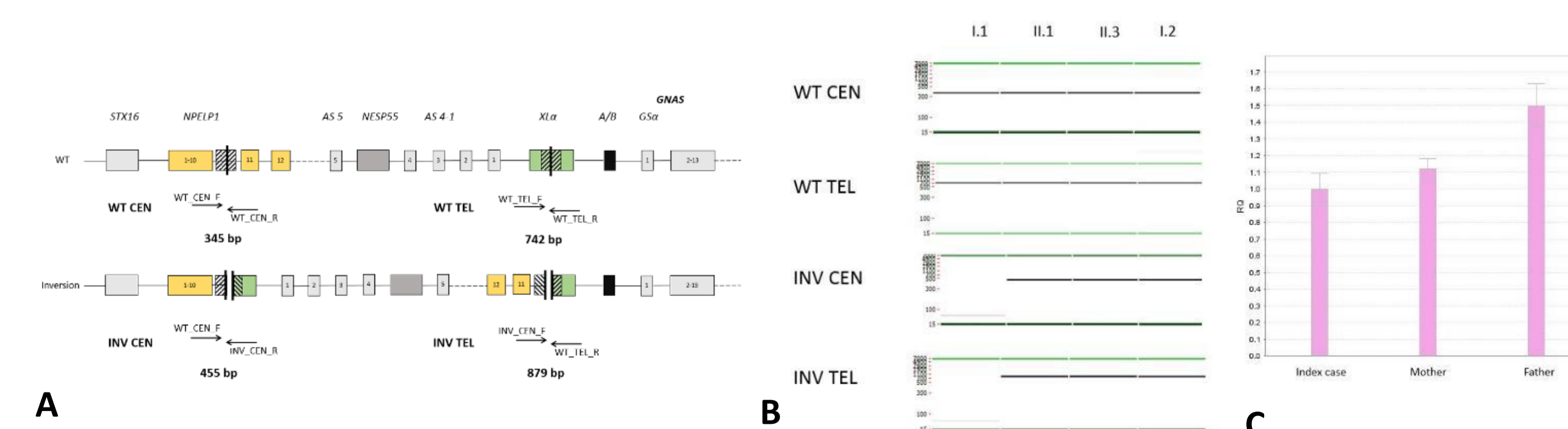


Figure 2 : A. Schematic representation of PCR primers and their approximate locations in the wild-type and mutant alleles comprising the *GNAS* locus. B. Presence of the inversion detected on II.1, II.3 and I.2's DNAs using PCR across both breakpoints for the wild-type and mutant allele. C. qPCR results: DNA quantification for the WT centromeric region compared to the index case (used as a control).

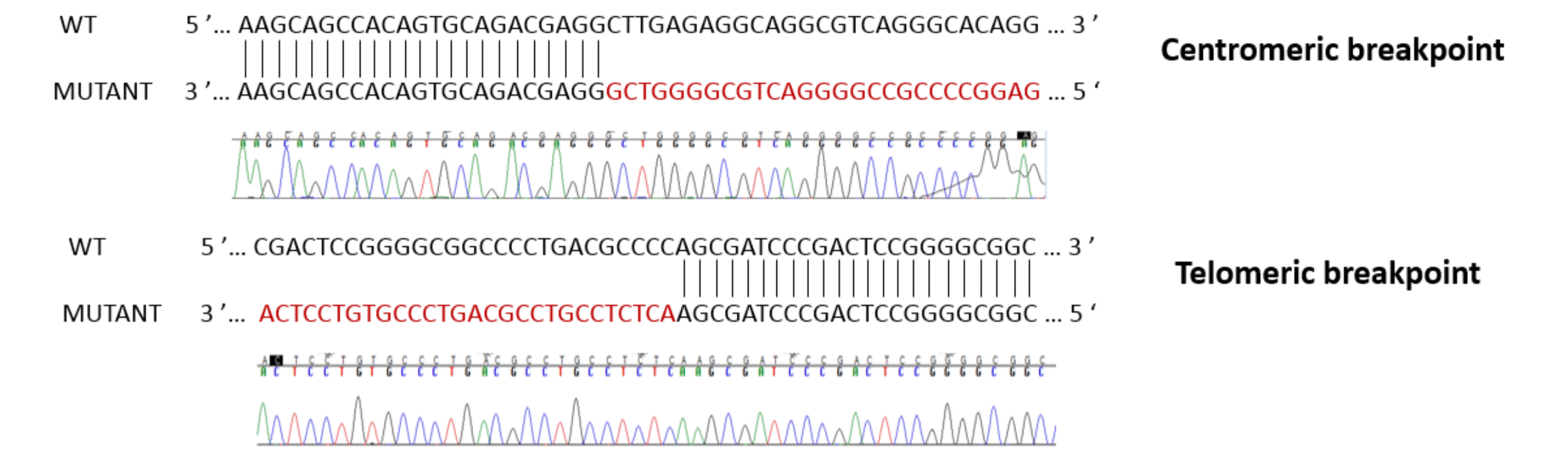


Figure 3 : Nucleotide sequence analysis of the mutant allele across the centromeric and the telomeric breakpoints using genomic DNA of patient II.1. **Upper panel:** centromeric breakpoint : identical nucleotide sequence up to the breakpoint followed by nucleotide sequence from the WT (black) or the mutant allele (red). Below are the representative nucleotide sequences of the mutant allele. **Lower panel:** telomeric breakpoint : nucleotide sequence from the WT (black) or mutant allele (red) followed by identical nucleotide sequence after the breakpoint. Below are the representative nucleotide sequences of the mutant allele.

Discussion :

We detected a novel genetic rearrangement in a family with AD-PHP1B, an inversion 20q13.3 which includes the region between *NPELP1* and *XLas*. DNA methylation analysis showed a complete LOM for the DMRs situated outside the inversion for the two patients and a significant increase of methylation (~20%) for the DMRs situated in the inversed region for their mother (Figure 4).

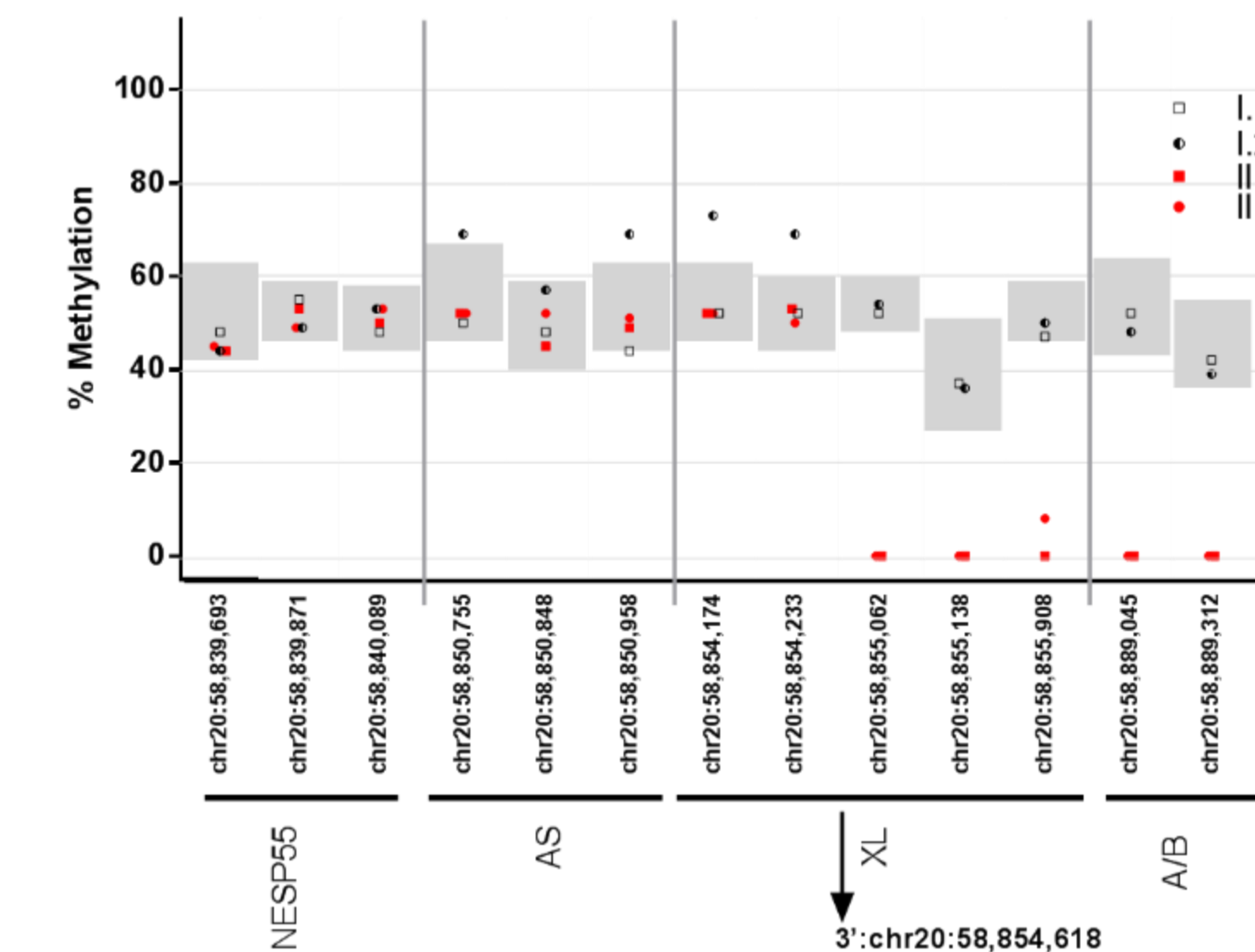


Figure 4 : Methylation percentage obtained by MS-MLPA for 5 DMRs. Gray vertical bar indicate the 95% confidence interval in 12 control subjects. The chromosomal position of the start of each CpG site whose methylation level was assessed by MS-MLPA is written underneath.

To our knowledge, only one large inversion has been described in AD-PHP1b which included the entire A/B DMR and *GNAS* gene¹. The cause of this methylation profile remains unknown, but these results are raising the question of a new imprinted locus in the inversed region. Further studies are necessary in order to verify this hypothesis. The WGS is useful in a diagnosis purpose in the cases in which the recurrent genetic etiologies are not found.

References :

- 1 Grigelioniene et al, *JBMR*, 2017, PMID : 28084650

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