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CAUSE OF PSEUDOHYPOPARATHYROIDISM TYPE 1A

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INTRODUCTION : *GNAS* LOCUS & PHP1A

The complex *GNAS* locus includes various transcripts of monoallelic expressions under control of differentially methylated regions according to the parental origin.

The $G\alpha_s$ protein, predominantly expressed from the maternal allele is coded by exons 1 to 13 of the *GNAS* locus, is essential to activate the signalling path of receptors coupled with the G proteins alike the receptor of parathyroid hormone (PTH), thyroid-stimulating hormone (TSH), gonadotropins (LH and FSH), growth hormone-releasing hormone (GHRH), and central nervous system neurotransmitters.

Disorder of maternal *GNAS* locus complex (**Figure 1**) are causes of pseudohypoparathyroidism type Ia, an autosomal dominant disease, characterized by organ resistance to these endocrine hormones, variable degrees of intellectual disability and the Albright hereditary osteodystrophy (AHO) phenotype (short stature, round facies, obesity, subcutaneous ossifications and brachydactyly type E).

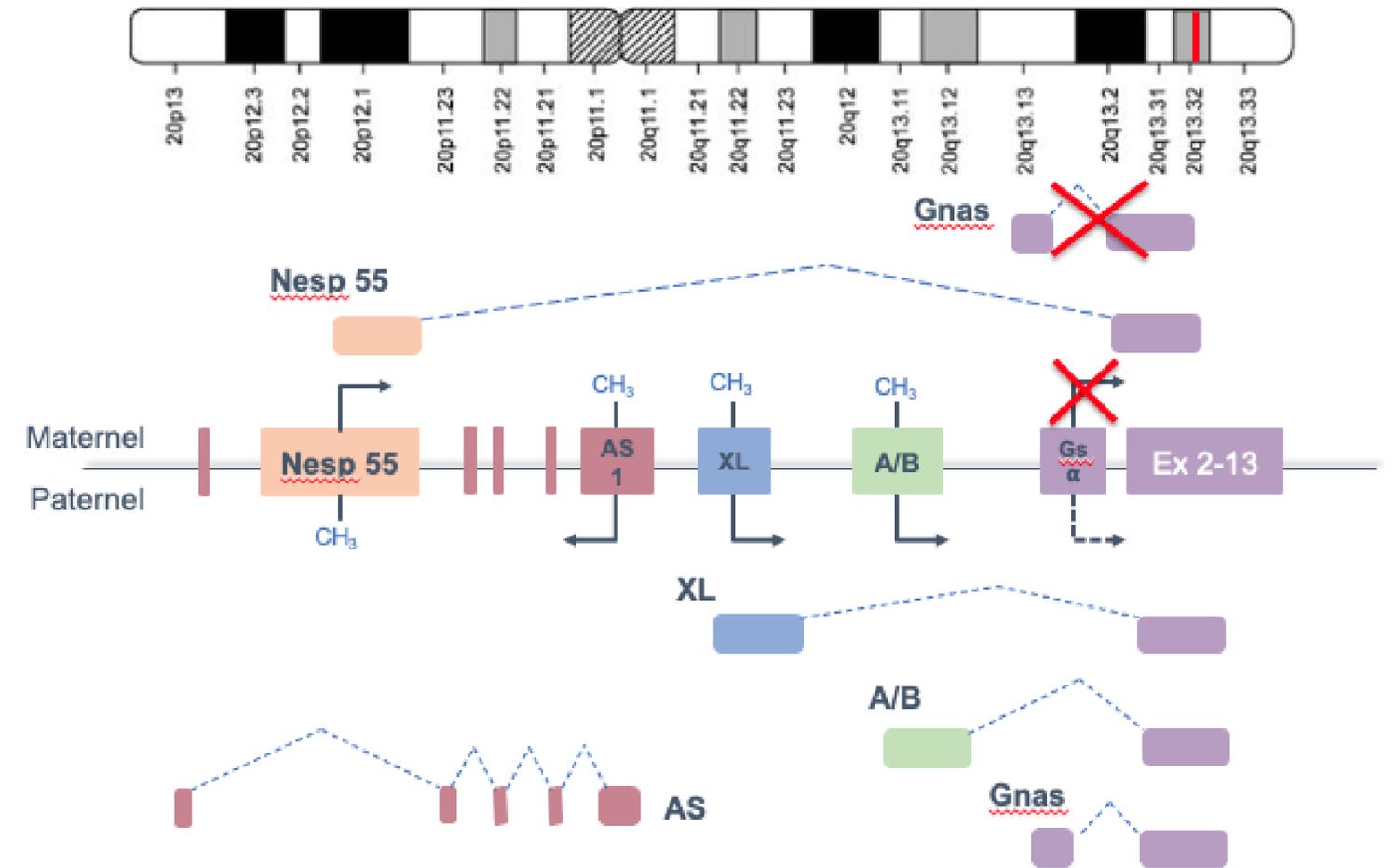
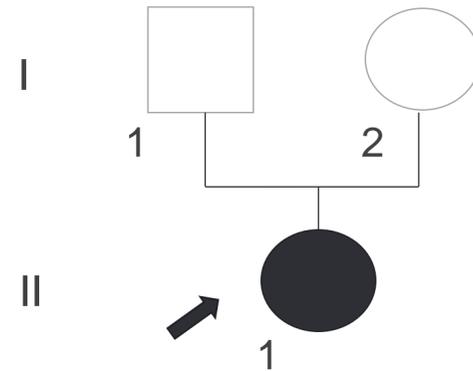


Figure 1 : Schematic representation of *GNAS* complex locus, the various transcripts by parental origin and the defect of maternal $G\alpha_s$ allele (adapted from Pulix M. et al, 2020).

PATIENTS & METHODS

In this study, we investigated two affected individuals, unrelated and without relevant family history, both presenting a typical phenotype of PHP1a (figure 2, table 1).

Family A



Family B

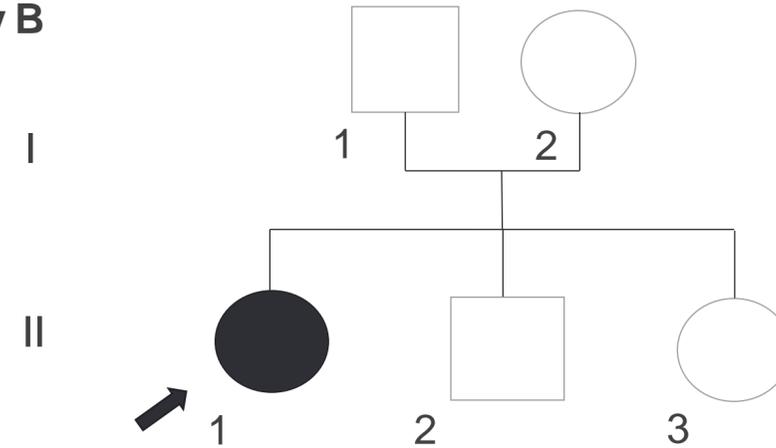


Figure 2 : Pedigrees of the two investigated families.

GNAS locus analysis

A Sanger sequencing of the *GNAS* locus and a methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) analysis were performed for the 2 affected subjects.

A whole genome sequencing (WGS) analysis was performed for the 2 affected patients : the library preparation used a paired-end (PE) with 2x150 bp method.

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.

In order to analyse *GNAS* expression, a qRT-PCR was carried out from RNAs of the probands and one healthy, sex and age paired control.

Primers spanned the coding region of exons 1 and 2.

	A II.1	B II.1
AHO features	Yes	Yes
Serum calcium	↓	↓
Serum phosphate	↑	↑
Serum PTH	↑	↑
Serum TSH	↑	↑
Serum FSH/LH	↑	*
Clinical phenotype	PHP1a	PHP1a

Table 1 : Clinical features of the two patients.

* Not documented.

RESULTS

There was no gain, deletion or mutation in the *GNAS* locus detected by the routine analysis.

The epigenetic analysis by MS-MLPA showed normal methylation for the two patients.

The WGS analysis revealed on the two probands two deletions of 466 bp and 1439 bp respectively with both distal breakpoints localized in 5'UTR of *GNAS* (**Figure 3**).

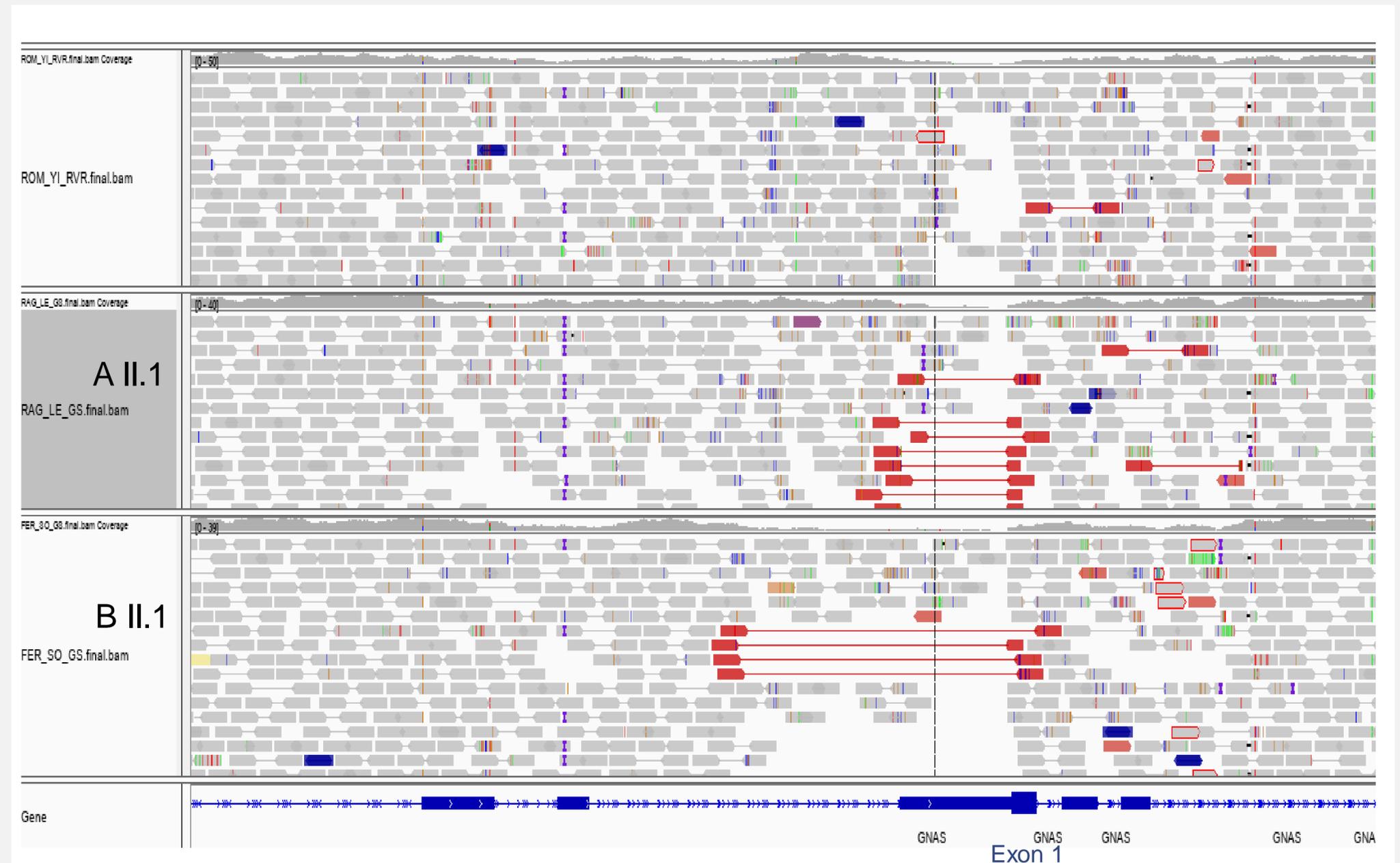


Figure 3 : Use of the Integrative Genome Viewer to show WGS (2 x 150 PE) data of the two deletions found in the same 5'UTR region of *GNAS* gene.

RESULTS

The deletion was confirmed by a PCR analysis of the genomic DNA using primers designed outside of the deleted regions (**Figure 4, Figure 5**) and by a Sanger analysis.

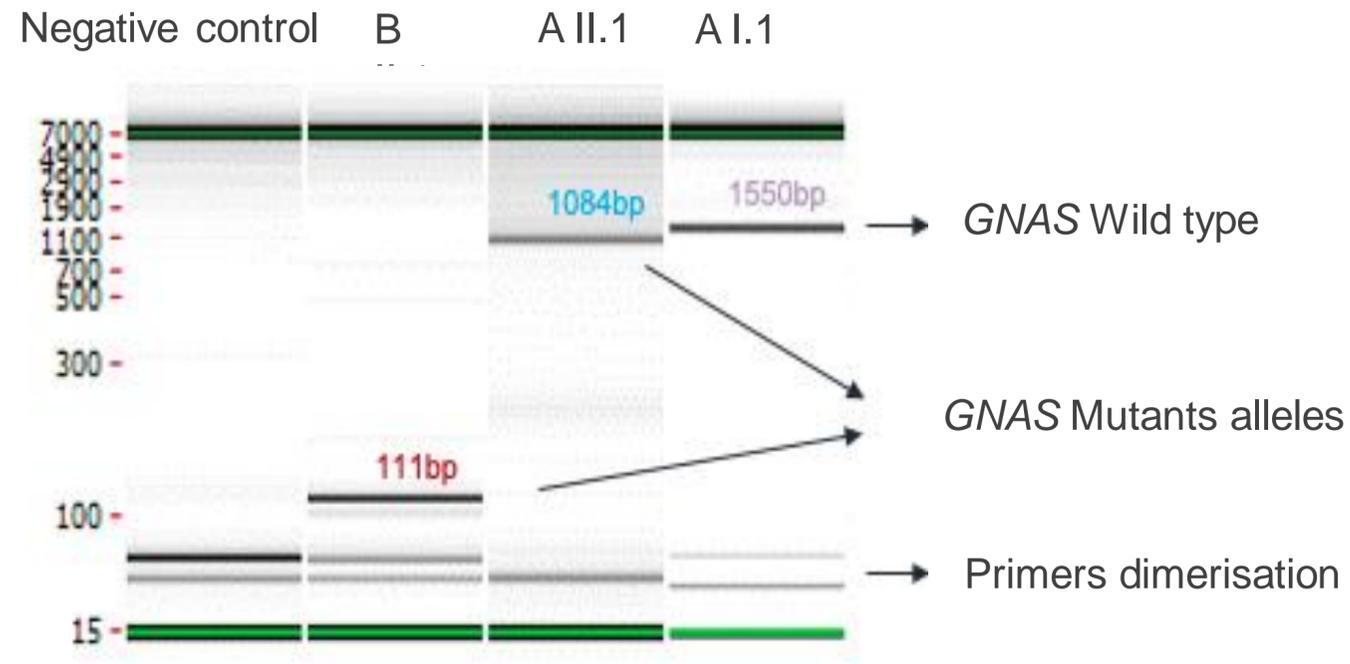


Figure 4 : Capillary electrophoresis of PCR products.

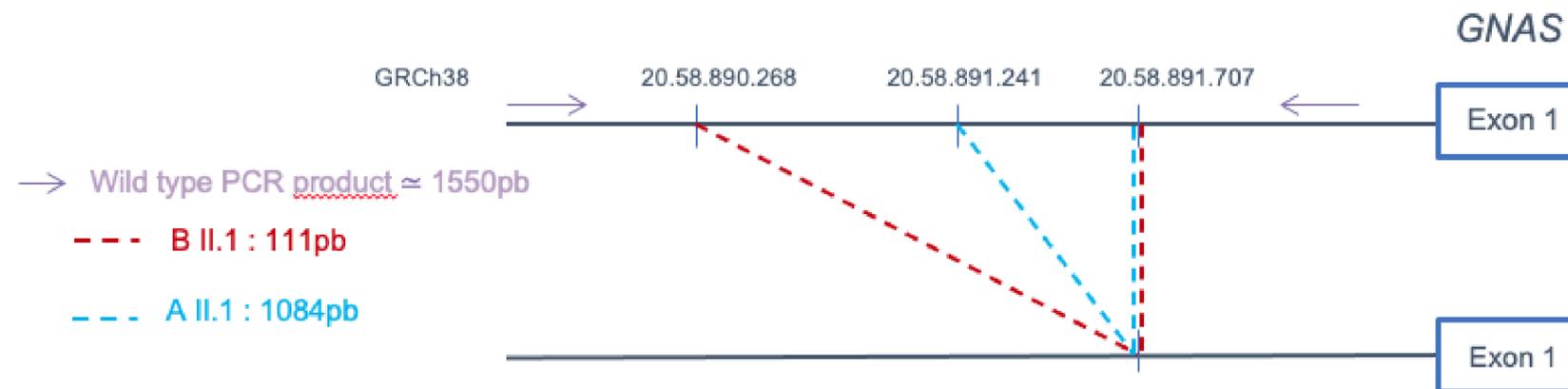


Figure 5 : Schematic representation of PCR primers, their approximate locations in the wild type allele and the expected size of PCR products for the wild type and the mutants alleles.

The qRT-PCR analysis showed a significant reduction of *GNAS* expression by $\approx 50\%$ and $\approx 90\%$ respectively in comparison with a normal control (**Figure 6**).

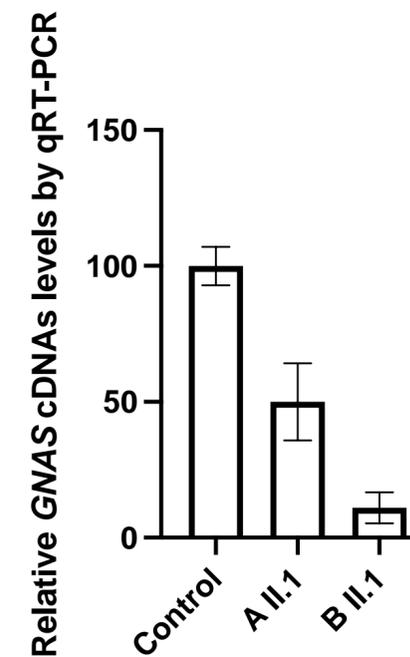


Figure 6 : Relative *GNAS* expression level measured by qRT-PCR, normalized to GAPDH expression level in the probands and a healthy control.

DISCUSSION & CONCLUSION

We detected two new deletions in the 5'UTR region of the *GNAS* gene, of 466 bp and 1439 bp respectively, onto two female patients with a typical phenotype of PHP1a using WGS technology.

These two patients had previously benefited from sequencing of a targeted panel of genes involved in phospho-calcium metabolism disorders, carried out in our laboratory and including the *GNAS* gene, as well as an MS-MLPA in search of rearrangements and/or methylation abnormality at the *GNAS* locus, all non-contributory.

As this *GNAS* gene region is highly repeated and particularly difficult to sequence, we report here the usefulness of WGS in these cases of undocumented rare diseases.

These observations suggest the possibility that the 5'UTR common distal breakpoint could be a recurrent hot spot site.

Targeted analysis of this region could be implemented in routine practice in order to detect such type of rearrangement.