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## Quantitative expression assay of *GNAS* gene: a reliable functional analysis in various clinical and molecular situations

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### Introduction

The complex *GNAS* locus encodes the  $\alpha$  subunit of the stimulatory G protein and additional imprinted transcripts. Three main phenotypes are associated with genetic anomalies in this locus: Pseudohypoparathyroidism type 1a (PHP1a), type 1b (PHP1b) and pseudopseudohypoparathyroidism (PPHP). Identification and functional analysis of genetic modifications can be challenging in some cases. Most splicing variants trigger the nonsense-mediated mRNA decay (NMD), a mRNA quality-control mechanism that eliminates mRNA premature translation-termination codons.

### Material and methods

*GNAS* gene sequencing, methylation and CNV analysis were realised following the routine diagnosis protocol. Whole genome sequencing was performed in three cases. A quantitative expression analysis of *GNAS* gene using quantitative Real-Time PCR and/or droplet digital PCR was performed on whole blood RNA in comparison with a sex and age matched control, using *GAPDH* gene expression for normalisation. The retrotranscription was performed using a oligoT primer for the quantitative analysis and a *GNAS* - gene specific primer for the qualitative analysis.

### Results

- We investigated eight French families presenting various *GNAS*-associated phenotypes: 5 PHP1a, 1 PHP1b, 1 PPHP and 1 partial PPHP phenotype (Table 1)
- We identified 5 novel variants: 2 synonymous, 1 missense, 2 intronic and 3 novel chromosomal rearrangements: 1 inversion in the regulatory region upstream the *GNAS* gene and two different deletions localised in 5'UTR (Figure 1). The qualitative RNA study was relevant in 4/8 cases (Table 1).
- In 6/8 of cases (75%) a significant reduction of *GNAS* expression was revealed. Case C presented a severe PHP1a phenotype and only 10% of normal cDNA. Case G, with partial PPHP phenotype presents a synonymous *GNAS* variant with no impact on RNA splicing. Case H has an abnormal RNA present, without haploinsufficiency (Figure 2).

Case	Phenotype	Variant	Mechanism
A	PHP1b	140 kb Inversion	Unknown
B	PHP1a (severe)	1439 bp 5'UTR Deletion	Unknown
C	PHP1a	466 bp 5'UTR Deletion	Unknown
D	PPHP	c.108C>A p.(Val36=)	New acceptor splice site (36 bp deletion)*
E	PHP1a	c.139G>T p.(Gly47Cys)	Intron retention
F	PHP1a	c.258-1G>A	No qualitative analysis
G	Partial PPHP	c.654C>T p.(Val217=)	No consequence, normal splicing
H	PHP1a	c.971-2A>G	Complex splice impact, partial exon 12 deletion and intron retention

Table 1. Clinical and genetic data for eight individuals from eight different families. PHP1a/b = pseudohypoparathyroidism type 1a/1b; PPHP = pseudopseudohypoparathyroidism \*Apetrei et al, 2021, PMID: 33997150

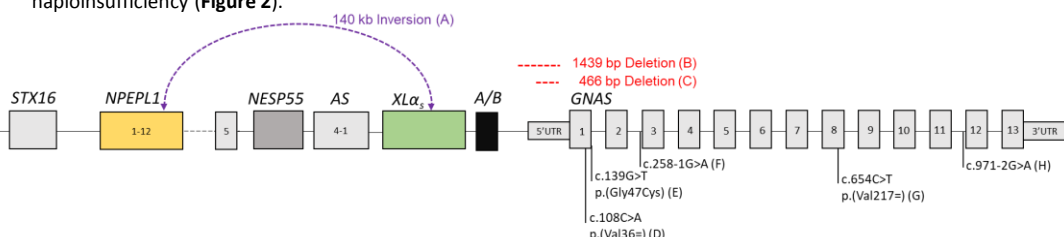


Figure 1. Schematic representation of the *GNAS* locus and localisation of the variants. *GNAS* locus contains *GNAS* gene with 13 exons, a regulatory region upward containing 4 additional imprinted transcripts. *STX16* gene contains an imprinting control region. *NPEPL1* gene has no known function. The localisation of the three rearrangements is indicated in violet (inversion) and red (two 5'UTR deletions) and with a black line for the 5 punctual variants. The codon and the predicted proteic nomenclature is indicated below each variant when known. The affected individual is indicated in parenthesis for each variant.

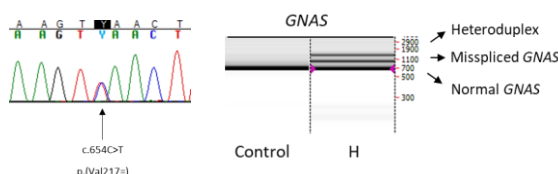


Figure 2. *GNAS* main transcript mRNA analysis in eight families on cDNA obtained by RT-PCR using oligo(dT)18 primer. A. Relative *GNAS* gene expression level (mean  $\pm$  SEM) measured by quantitative real-time PCR or droplet-digital PCR, normalised to *GAPDH* expression level in each proband and a healthy sex and age matched control. At least two replicates were performed for each cDNA. In blue - significant reduction of *GNAS* expression level, in grey - normal *GNAS* expression level. B. Chromatogram from cDNA sequencing of a nucleotide change from C to T in the individual G. C. Capillary electrophoresis performed on the cDNA in the individual H and a healthy sex and age matched control. On the first lane: *GNAS* PCR product in the control, on the second lane: 3 *GNAS* PCR products in the affected G individual, corresponding to the wild-type allele, the mutant, longer allele and the heteroduplex (a mixture of wild-type and mutant allele) product.

### Discussion and Conclusions

- Most regulatory and splicing – impacting variants are responsible of a significant reduction of *GNAS* mRNA level.
- Some variants can escape the NMD and produce an abnormal RNA without diminishing the overall mRNA quantity.
- Quantitative whole blood expression assay of *GNAS* gene is a reliable functional analysis that should be considered even without knowing the exact molecular cause whenever the patient has a *GNAS*-associated phenotype.