



HAL
open science

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

Andreea Apetrei, Arnaud Molin, Louise-May Thibaut, Jérémy Danguy, Bertrand de L'isle, Isabelle Durand, Antoine Resbeut, Magali Demoor, Nicolas Gruchy, Nicolas Richard

► To cite this version:

Andreea Apetrei, Arnaud Molin, Louise-May Thibaut, Jérémy Danguy, Bertrand de L'isle, et al.. Quantitative expression assay of GNAS gene: a reliable functional analysis in various clinical and molecular situations. European Human Genetics Conference 2023, Jun 2023, Glasgow, United Kingdom. hal-04345466

HAL Id: hal-04345466

<https://hal.science/hal-04345466v1>

Submitted on 14 Dec 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

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

Apetrei A^{1,2}, Molin A^{1,2}, Thibaut LM¹, Danguy J¹, De l'Isle B², Durand I¹, Resbeut A¹, Demoor M², Gruchy N^{1,2}, Richard N^{1,2}

¹ Département de Génétique, Caen University Hospital, Caen, France; ² RU7450 BioTARGen, Normandy University, Caen, France. The authors declare no conflict of interest.

Introduction

The complex *GNAS* locus encodes the α 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

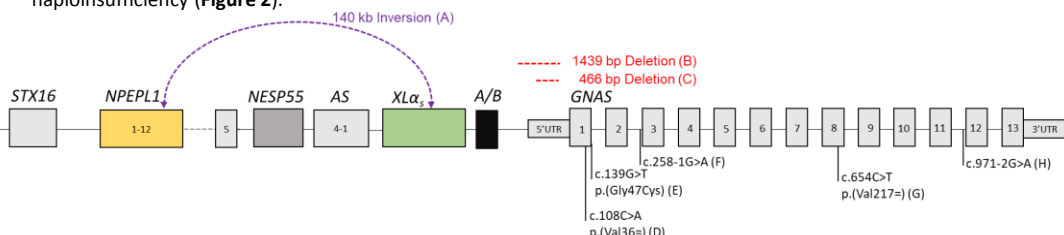


Figure 1. Schematic representation of the *GNAS* locus and localisation of the variants. *GNAS* locus contains *GNAS* gene with 13 exons, a regulatory region upward containing 4 additional imprinted transcripts. *STX16* gene contains an imprinting control region. *NPEPL1* gene has no known function. 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.

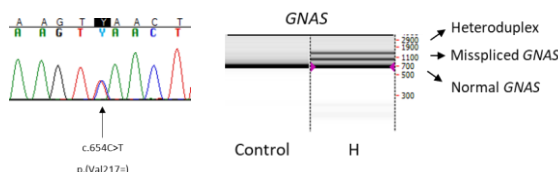


Figure 2. *GNAS* main transcript mRNA analysis in eight families on cDNA obtained by RT-PCR using oligo(dT)18 primer. A. Relative *GNAS* gene expression level (mean \pm SEM) measured by quantitative real-time PCR or droplet-digital PCR, normalised to *GAPDH* expression level in each proband and a healthy 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.