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A novel synonymous variant in exon 1 of *GNAS* gene results in a cryptic splice site and causes pseudohypoparathyroidism type 1A and pseudo-pseudohypoparathyroidism in a French family

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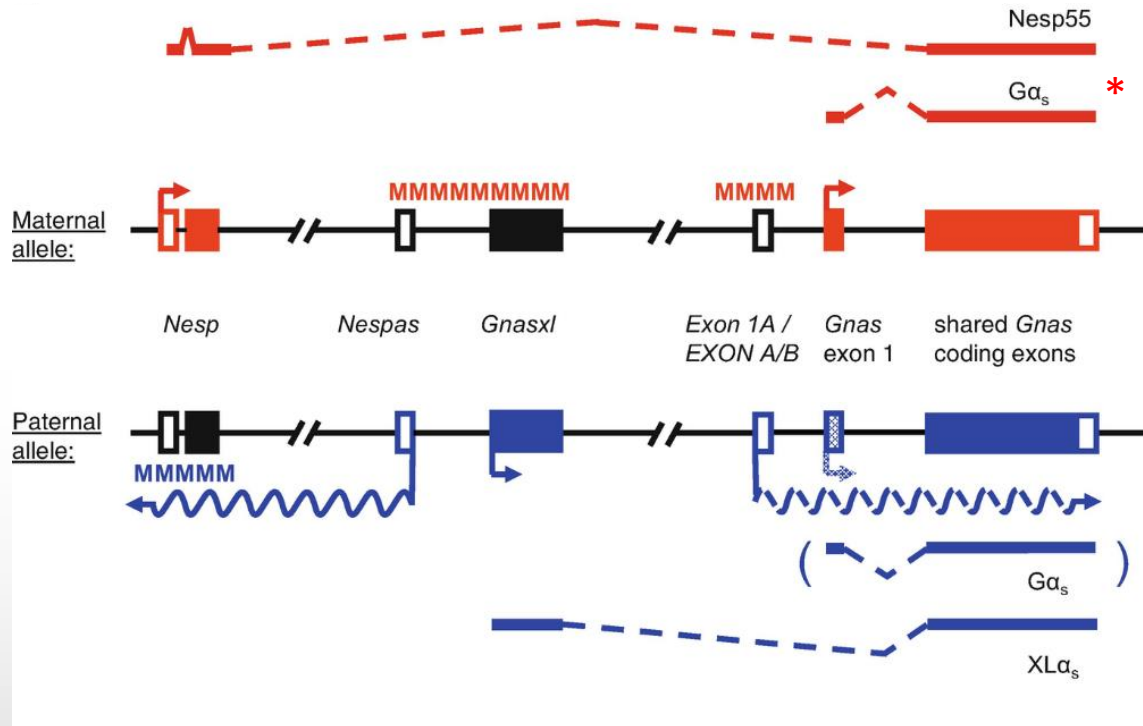
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Apetrei et al, 2021, Bone Reports



GNAS complex locus



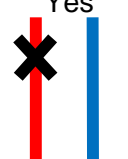
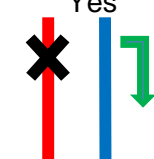
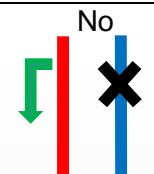
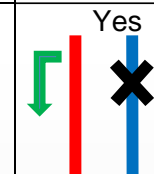
The main imprinted sense and antisense transcripts from the *GNAS* complex locus.

* $G\alpha_s$ is predominantly expressed from the maternal allele in the renal proximal tubules, parathyroid, thyroid, pituitary gland and ovaries and has a biallelic expression in the other tissues.

M – methylated region

Adapted from Pulix M., Plagge A. (2020) *Imprinted Genes and Hypothalamic Function*

GNAS – associated phenotype

	Genetic defect	Parental origin	Hormonal resistance (PTH, TSH) [§]	AHO Phénotype [#]
PHP1A	Coding sequence variant	Maternal	Yes 	Yes 
PPHP	Coding sequence variant	Paternal	No 	Yes 

The main disease subtypes caused by inactivating *GNAS* gene mutations.

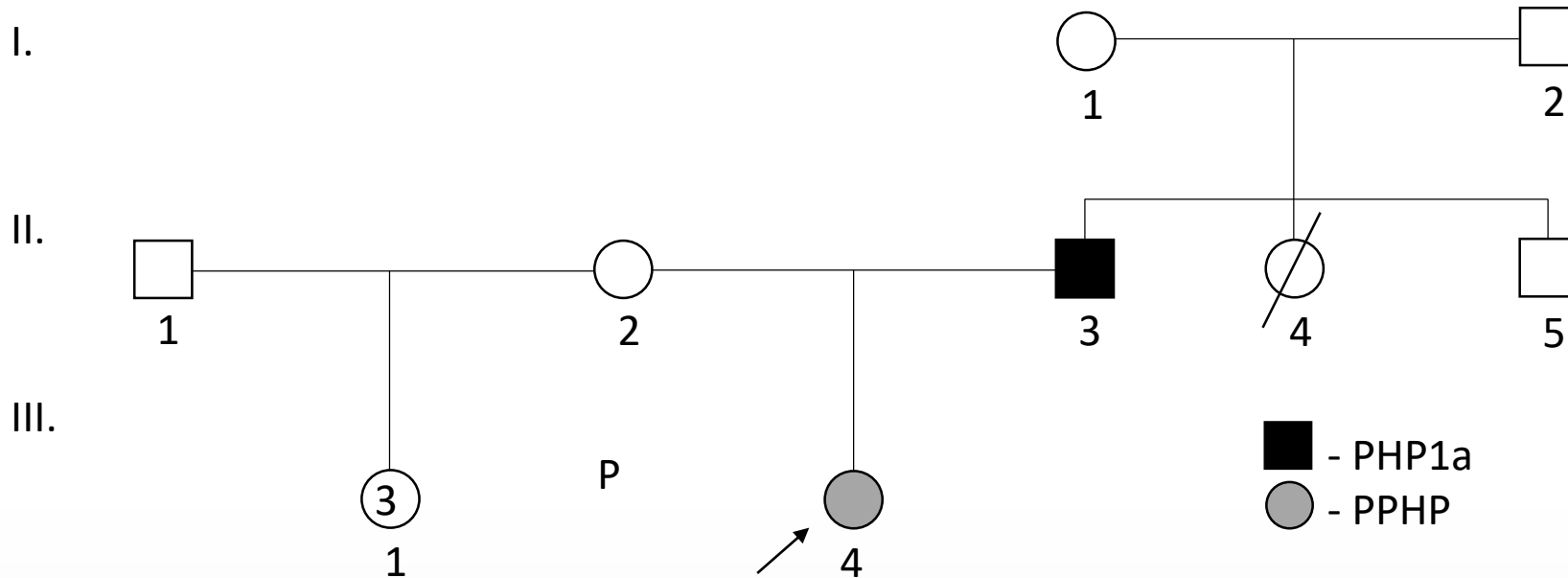
[§]The resistance to parathormone (PTH) is characterized by high serum PTH, hypocalcemia and hyperphosphatemia and it can be associated with resistance to other hormones, such as thyroid stimulating hormone (TSH).

[#]Described Albright's Hereditary Osteodystrophy (AHO) features are obesity, round face, short stature, brachydactyly type E, subcutaneous ossification and mild mental retardation.

The crossed alleles correspond to the inactivated alleles of the *GNAS* gene.

Adapted from Turan S., Bastepe M. (2015) *Curr Osteoporos Rep*

Maternally inherited loss of function mutations lead to **PHP1A**, whereas paternally inherited loss of function mutations lead to **PPHP**. Therefore, the two phenotypes usually coexist in the same family, but never in the same sibship.



Clinical manifestation	III.4	II.3
AHO features	Yes	Yes
Intrauterine growth retardation	Yes	No
- Serum calcium	N	N*
- Serum phosphate	N	N
- Serum PTH	N	↑
- Thyroid dysfunction	No	Yes
Clinical diagnosis	PPHP	PHP1a

N – normal level

*At the low limit of the normal range

GNAS sequencing

GNAS exons 1- 13 were sequenced and interpreted following the routine protocol.

In silico splicing prediction algorithms (SPiP and Alamut) were used.

GNAS expression analysis

Quantification

qRT-PCR was used to assess the expression of *GNAS* in cDNA in the proband and in a healthy, sex and aged-paired control.

Primers spanned the coding region of exons 1 and 2.

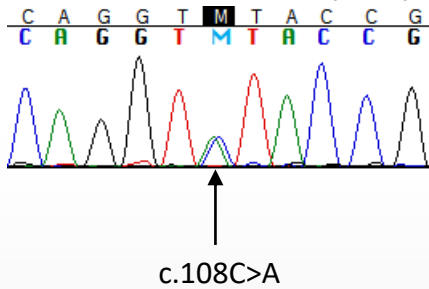
Transcript study and sequencing

RT-PCR using a gene-specific primer localized in the exon 5 was followed by a PCR using primers localized in exons 1 and 2.

A capillary electrophoresis analysis allowed to isolate and characterize the PCR products before sequencing.

GNAS sequencing

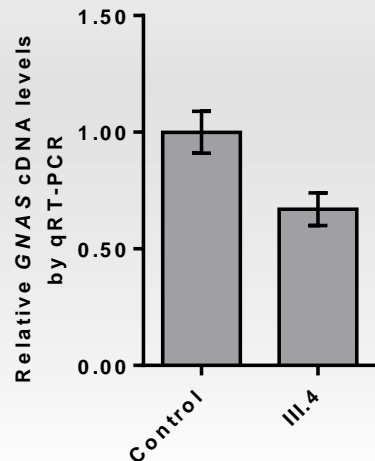
A heterozygous variation Chr20(GRCh37):g.57466889C>A, NM_001077488.2:c.108C>A p.(Val36=) localized in the exon 1 of *GNAS* gene, absent from the local and international databases was identified in III.4., inherited from her father (II.3):



Chromatogram from DNA sequencing of a nucleotide substitution in the proband (III.4)

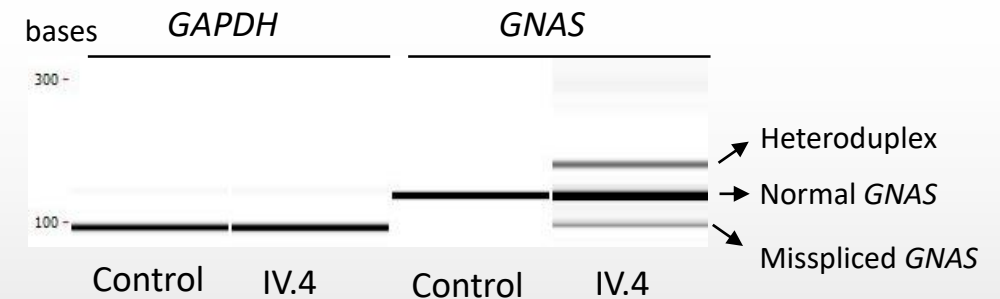
GNAS expression analysis

The expression quantification showed a reduction of *GNAS* expression by 35% in comparison with a normal control:



*Relative *GNAS* expression level measured by qRT-PCR using oligo(dT)18 primer, normalized to *GAPDH* expression level in the proband (III.4) and a healthy control.*

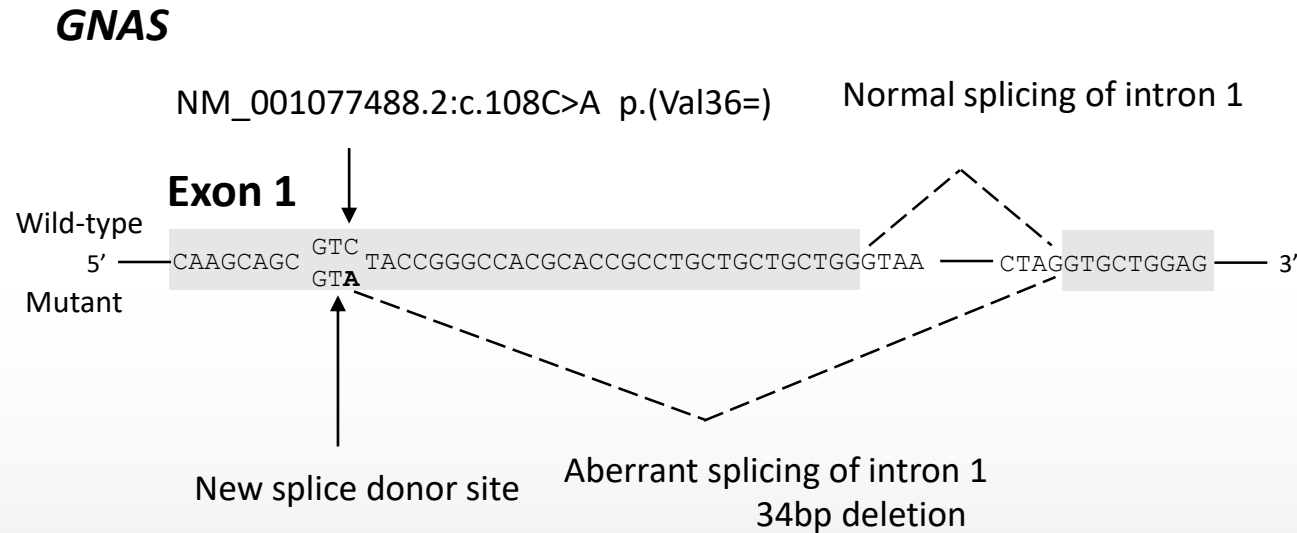
A second RT-PCR using a gene-specific primer allowed to amplify the expected PCR product and 2 additional products in smaller quantities, a shorter one and a longer one, respectively corresponding to the wild-type, the mutant allele and a heteroduplex (a mixture of wild-type and mutant allele) product:



*Capillary electrophoresis performed on the cDNA obtained by RT-PCR using *GNAS* gene specific primer.*

The sequencing of these PCR products showed a 34bp deletion in the mutant allele.

We describe the first family with PHP1a/PHP presenting a pathogenic synonymous variant in the *GNAS* gene, responsible for a splicing defect leading to the premature splicing of the first exon and to a probably unstable RNA or truncated protein:



This observation supports the increasing interest in the identification of splicing variants, particularly those predicted as synonymous, which are often systematically categorized as benign on this sole element.

Splice-prediction algorithms could be added to the bioinformatic pipelines for massive parallel sequencing data in routine analysis to improve the detection of such variations.