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RESEARCH LETTER

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# Inheritance of c.628-6G>A *GNB5* hypomorphic allele uncovers another challenge in the pathogenic prediction of genomic variants

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The molecular characterization of undiagnosed patients through genomic approaches is sheding light on disease-causing hypomorphic genetic variants, which exert subtle effects on gene function.[1] Here, we present the case of a compound heterozygous patient, who carried a variant inherited from the asymptomatic homozygous father. Written informed consent was obtained from the patient's parents, and the study received approval from the Ethics Committee.

The patient is an 18-year-old undiagnosed woman characterized by severe intellectual disability, absence of language, myopia magna, and two episodes of seizures. Using trio-whole exome sequencing, we identified two variants in the *GNB5* gene (MIM*604447): the unreported variant c.514delT; p.Ser172Leu*fs**5 in exon 7, classified as likely pathogenic following the ACMG/AMP guidelines, inherited from the asymptomatic heterozygous mother that causes a premature translation-termination codon (PTC); and the c.628-6G>A variant in intron 7, classified as a variant of uncertain significance, inherited from the asymptomatic homozygous father. c.628-6G>A has a frequency of 0.000012 and is not reported in homozygous form in the gnomAD. c.628-6G>A is located at the splice acceptor site (SAS) and is predicted to impact the splicing by creating a new SAS (Figure 1A,B).

*GNB5*-related neurodevelopmental disorder encompasses a spectrum of phenotypes,[2] including Lodder-Merla syndromes, type 1 [MIM#617173] and type 2 [MIM#617182]. Given that the patient's symptoms were consistent with *GNB5*-developmental disorder,[2] we considered *GNB5* as a candidate gene. The 24-h Holter electrocardiographic study, requested following the genetic results, revealed the presence of sinus arrhythmia alternating with nodal rhythm, with multiple sinus pauses of 3.5 s. Patient's clinical reassessment and phenotyping uncovered that she required thickened food until the age of 5 years and still experiences difficulties swallowing water, potentially attributable to gastroesophageal reflux problems. Furthermore, the patient showed distal motor axonal neuropathy and spastic paraparesis, which could be related to Gβ5 absence.[3]

Janet Hoenicka and Francesc Palau are joint senior authors.

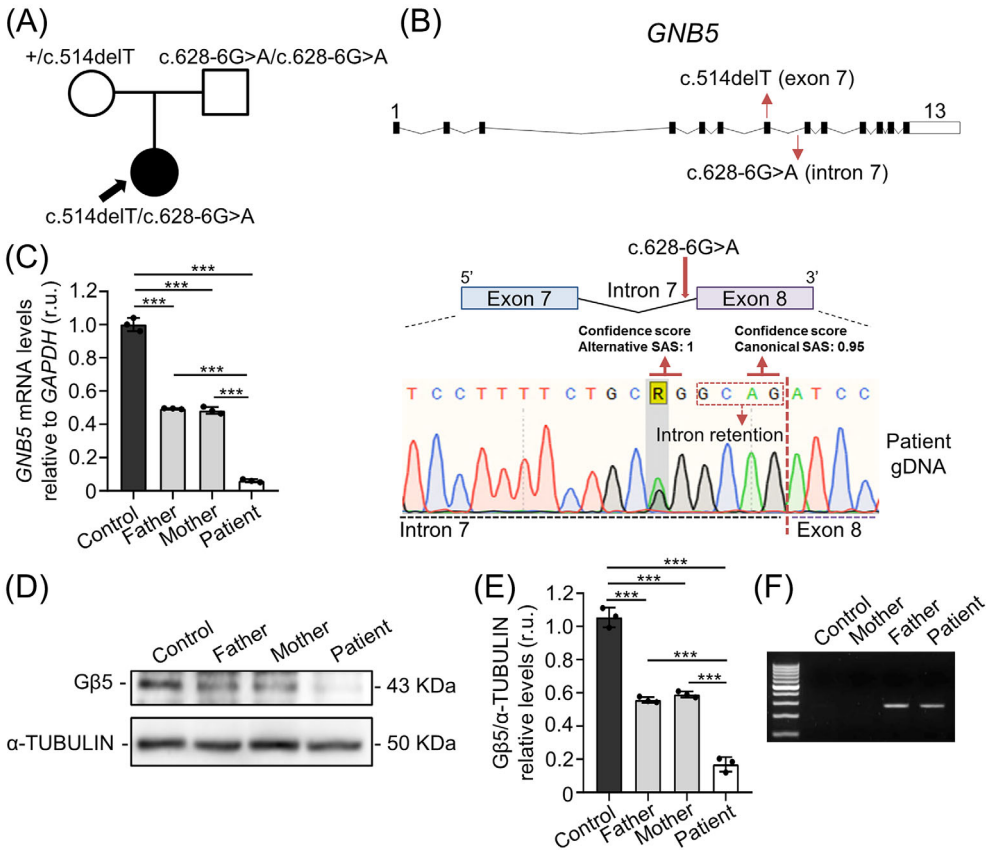


**FIGURE 1** Functional studies in *GNB5* family. (A) Proband's pedigree (arrow) and family. (B) Upper panel: c.514delT and c.628-6G>A variants' locations in the *GNB5* gene structure (NM_016194.4); black squares denote coding exons. Lower panel: Patient's gDNA electropherogram showing c.628-6G>A and the predicted retention of 4 intron nucleotides. (C) *GNB5* RT-qPCR in fibroblast total RNA samples (relative mean ± SD; $n = 3$ independent replicates). (D) Semi-quantitative Western blot of Gβ5 in fibroblast samples. (E) Gβ5 protein levels related to α-TUBULIN (relative mean ± SD; $n = 3$ independent replicates). (F) Specific RT-PCR analysis of the transcript generated by c.628-6G>A splicing variant in fibroblast samples. Oligonucleotides used: Fw: 5′-CAACTCTGACATGCAGGCAG-3′ and Rv: 5′-TGGGGTAGTACCGGACACTG-3′. Statistical analysis was performed using one-way ANOVA followed by the Tukey–Kramer post hoc test (***$p < 0.001$). r.u., relative units. [Colour figure can be viewed at wileyonlinelibrary.com]

*In-silico* and functional biological studies provided evidence that c.628-6G>A is pathogenic in the context of specific genotypes. We found that this variant creates a new SAS that could generate a new transcript, which includes an insertion of the last four nucleotides of intron 7, causing a PTC. The strength of the alternative SAS is almost equal to the canonical SAS (NetGene2: SAS wild-type, 0.95; SAS alternative, 1). The study of *GNB5* mRNA expression in fibroblasts by RT-qPCR showed nearly absence in the patient and a decrease of 50% in the parents probably related to a nonsense-mediated RNA decay mechanism (Figure 1C). These results were consistent with the protein levels in these samples (Figure 1D,E). By allele-specific RT-PCR, we corroborated that c.628-6G>A creates a new SAS in both the patient and the father (Figure 1F).

c.628-6G>A appears to show context-dependent pathogenicity. Our hypothesis is that the biallelic expression of c.628-6G>A in the father allows reaching 50% of the expression of *GNB5* while the patient shows a monoallelic expression of c.628-6G>A, because the maternal allele rapidly suffers from NMD. In the patient, the use of both SAS of the paternal variant might not be well balanced with a significant reduction to less than 25% of *GNB5* mRNA and protein.

Our findings underscore the significance of functional genomics as a second-tier diagnostic tool to establish the pathogenicity of variants in the diagnostic process.

## AUTHOR CONTRIBUTIONS

Francesc Palau, Janet Hoenicka, and Jordi Pijuan contributed to the conceptualization, data analyses and drafted the manuscript. Alba Vilanova-Adell contributed to data analyses. Dídac Casas-Alba and Jaume Campistol carried out the clinical evaluation. All authors approved the final version.

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## CONFLICT OF INTEREST STATEMENT

The authors have no competing interests.

## PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/cge.14454.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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