

GNAO1 Haploinsufficiency: The Milder End of the GNAO1 Phenotypic Spectrum

GNAO1 variants are typically associated with severe, early-onset movement disorders (MDs) with life-threatening and drug-resistant paroxysmal exacerbations, neurodevelopmental disorders, and epilepsy. Recently, the phenotypic spectrum has broadened to include milder phenotypes with late-onset dystonia, minor cognitive impairment, and other neurological signs, including parkinsonism and myoclonus. GNAO1 haploinsufficiency has been evoked as a putative mechanism underlying milder clinical presentations.^{1,2} To date, however, the functional consequences of this class of variants have not yet been evaluated.

We report on an 8-year-old boy with subtle neurological signs, including generalized tonic-clonic seizures during fever, mild language impairment, dystonic postures of lower limbs during walking, and occasional tongue dyskinetic movements (see Data S1 for more details and Video 1). A next generation sequencing-based epilepsy panel revealed a de novo NM_020988.3:c.163_164del variant in GNAO1. No additional candidate variants were identified. Reverse transcription polymerase chain reaction showed an approximately 50% decrease in the expression of the endogenous GNAO1 gene in cells from the affected child compared with cells from the unaffected father (Figs. 1A and S2), suggesting nonsense-mediated mRNA decay (NMD). If translated, the c.163_164delAT allele was predicted to generate a truncated protein (p.Ile55Hisfs*3). As expected, Western blotting performed in transiently transfected HEK293T cells revealed the lack of the truncated form of Gαo (Fig. 1B), which was not restored by MG132 or



VIDEO. 1. The patient at the age of 7 years while walking, running, standing, and jumping on one foot. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

Video content can be viewed at <https://onlinelibrary.wiley.com/doi/10.1002/mds.29585>

bafilomycin treatments, inhibitors of the ubiquitin/proteasome and autophagy pathways, respectively. These findings demonstrate that the c.163_164delAT transcript undergoes NMD, leading to GNAO1 haploinsufficiency.

Genotype/phenotype correlations in GNAO1 encephalopathy are still far from being elucidated. Recent studies suggest that pathogenic variants have a loss-of-function effect on Gαo-mediated signaling,³⁻⁷ but the consequences on Gβγ signaling that regulates cyclic adenosine monophosphate production remain unclear. Emerging data show that haploinsufficiency is associated with milder clinical features and later onset than missense changes underlying developmental and epileptic encephalopathy type 17 (Mendelian inheritance in man [MIM]#615473) or neurodevelopmental disorder with involuntary movements (MIM#617493). This finding has important implications. First, given the different phenotypic output, variants associated with the canonical form of GNAO1 encephalopathy cannot have a simple loss-of-function effect; rather, they behave as dominant-negative alleles or alter Gα/Gβγ association, as recently shown for a subset of changes.³⁻⁷ Second, the phenotype associated with GNAO1 haploinsufficiency is likely attributed to increased levels of free Gβγ in the brain, which, in turn, could lead to increased receptor-independent Gβγ signaling in neurons. Finally, the association of GNAO1 haploinsufficiency with a subtle but distinctive phenotype may help to design a proper gene therapy strategy. Allele-specific silencing by antisense oligonucleotides or short-interfering RNAs is unlikely to be a reasonable approach because 50% of the gene dosage is not neutral and single nucleotide substitutions hardly confer a complete discrimination for allele-specific targeting. In contrast, AAV-mediated gene supplementation coupled with silencing of the mutant allele is expected to effectively alleviate the disease phenotype.

Our findings also indicate that GNAO1 variants may be more frequent than previously estimated and encourage testing for this gene in patients with mild neurological signs

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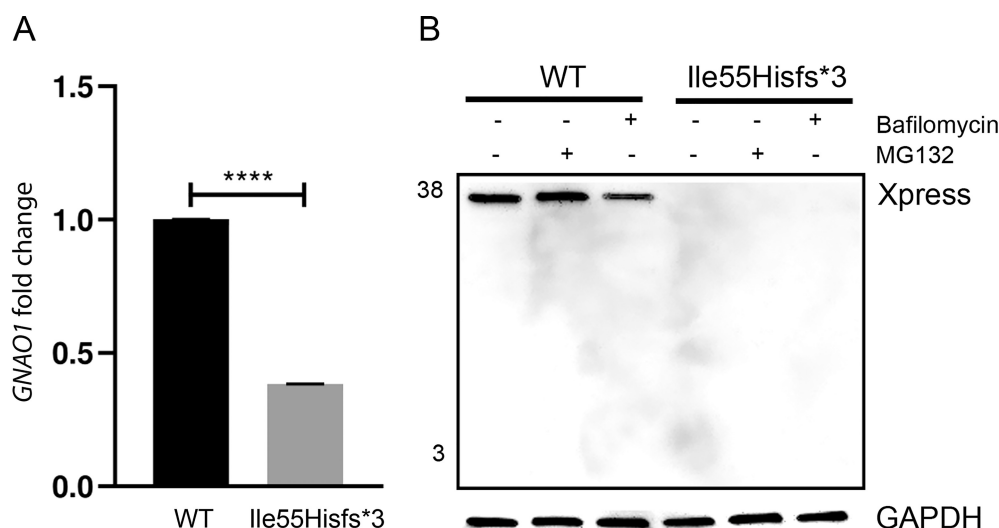


FIG. 1. The c.163_164delAT variant leads to *GNAO1* haploinsufficiency. (A) Reverse transcription polymerase chain reaction shows a significant decrease in endogenous *GNAO1* mRNA levels in cells from the affected child compared with cells from the unaffected father. Values are normalized to the expression of WT *GNAO1*. GAPDH was used as an internal control. Bars indicate mean values \pm standard deviation of three independent experiments, each performed in triplicate. The fold change was calculated using the $\Delta\Delta\text{CT}$ formula. Results were analyzed using a two-tailed unpaired *t* test with Bonferroni correction ($P < 0.0001$). (B) Western blot analysis shows the lack of the mutant protein, which is confirmed following treatment with MG132 (proteasome inhibitor) or bafilomycin (autophagy inhibitor), further supporting that the c.163_164delAT variant causes mRNA decay. Equal amounts of cell lysates were resolved by 4% to 20% polyacrylamide gradient gel electrophoresis. Membranes were probed with a mouse monoclonal anti-Xpress antibody and re-probed with a mouse monoclonal anti-GAPDH antibody for protein normalization. The blot is representative of three independent experiments. $\Delta\Delta\text{CT}$, delta delta CT; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; WT, wild type.

featuring epilepsy and/or MDs without a definite diagnosis. The progression into more severe phenotypes, and possible neurological deterioration induced by triggering events, typical for this condition, deserve a careful and prolonged clinical follow-up. ●

Ethical Compliance Statement

Written informed consent for offline and online video distribution of the video material was obtained from parents and is available upon request. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines. The authors confirm that the approval of an institutional review board was not required for this work. ●

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Data Availability Statement

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

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.