

FULL-LENGTH ORIGINAL RESEARCH

Spectrum of neurodevelopmental disease associated with the GNAO1 guanosine triphosphate-binding region

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Summary

Objective: To characterize the phenotypic spectrum associated with *GNAO1* variants and establish genotype-protein structure-phenotype relationships.

^aSee the Appendix for members of the Undiagnosed Diseases Network.

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Methods: We evaluated the phenotypes of 14 patients with *GNAO1* variants, analyzed their variants for potential pathogenicity, and mapped them, along with those in the literature, on a three-dimensional structural protein model.

Results: The 14 patients in our cohort, including one sibling pair, had 13 distinct, heterozygous *GNAO1* variants classified as pathogenic or likely pathogenic. We attributed the same variant in two siblings to parental mosaicism. Patients initially presented with seizures beginning in the first 3 months of life (8/14), developmental delay (4/14), hypotonia (1/14), or movement disorder (1/14). All patients had hypotonia and developmental delay ranging from mild to severe. Nine had epilepsy, and nine had movement disorders, including dystonia, ataxia, chorea, and dyskinesia. The 13 *GNAO1* variants in our patients are predicted to result in amino acid substitutions or deletions in the GNAO1 guanosine triphosphate (GTP)-binding region, analogous to those in previous publications. Patients with variants affecting amino acids 207-221 had only movement disorder and hypotonia. Patients with variants affecting the C-terminal region had the mildest phenotypes.

Significance: *GNAO1* encephalopathy most frequently presents with seizures beginning in the first 3 months of life. Concurrent movement disorders are also a prominent feature in the spectrum of *GNAO1* encephalopathy. All variants affected the GTP-binding domain of GNAO1, highlighting the importance of this region for G-protein signaling and neurodevelopment.

KEYWORDS

developmental and epileptic encephalopathy, *GNAO1*, mosaicism, movement disorders

1 | INTRODUCTION

GNAO1, or guanosine nucleotide-binding protein G(o) subunit α , is a gene that has been associated with neurodevelopmental disorders, including early onset developmental and epileptic encephalopathy (DEE),¹ developmental delay without epilepsy, and a range of movement disorders.²⁻¹⁶ *GNAO1* encodes a G-protein α subunit that, along with dimerized β and γ subunits, forms a heterotrimeric G-protein complex. G-protein α subunits contain guanosine triphosphate (GTP)-binding sites and dissociate from both the G-protein-coupled receptor and the β - γ dimer of the complex when activated. Alpha subunits are generally classified as stimulatory, inhibitory, or other (o). *GNAO1* encodes an "o" type α subunit; beyond GTP binding, it has a less well-established role in signaling.¹⁰ *GNAO1* is highly expressed in the central nervous system and is involved in neuronal excitability and neurotransmission.^{2,10}

Here, we report 14 previously unpublished patients with heterozygous, de novo *GNAO1* variants classified as pathogenic or likely pathogenic, including one variant shared by two siblings attributable to parental mosaicism. Two key components of our in silico variant analyses

Key Points

- Pathogenic variants in *GNAO1* result in early onset epilepsy and/or movement disorders, with seizures as the most common presenting feature
- Pathogenic variants in *GNAO1* lead to alterations in the GTP-binding region of the GNAO1 protein
- Genotype-phenotype correlations have emerged, with one region of the protein associated with a movement disorder and hypotonia phenotype without seizures
- Parental mosaicism can lead to siblings carrying the identical *GNAO1* variant that appeared to be de novo

were the comparison of variants in our cohort with those present in the general population and the variant location on the predicted GNAO1 protein structure. Both analyses demonstrate the importance of the GTP-binding region of the protein. We expand and add evidence to the previously reported phenotypic spectrum of *GNAO1*

encephalopathy with the addition of our 14 patients. In addition, we identify a region of the GNAO1 protein that correlates with a movement disorder–dominated phenotype without epilepsy.

2 | MATERIALS AND METHODS

Research was conducted with approval from the institutional review board at Boston Children's Hospital (BCH). Patient ascertainment included the Epilepsy Genetics Program, Genetics and Genomics Clinic, and Movement Disorders Clinic at BCH, the National Institutes of Health Undiagnosed Diseases Network, and the Australian Epilepsy Research Centre. Written informed consent was obtained from the parents or legal guardians of all patients included.

Deep phenotyping was performed, including review of medical records, electroencephalogram (EEG) reports, magnetic resonance imaging (MRI), and imaging reports when available, by our multidisciplinary team consisting of pediatric neurologists, including those with training in epilepsy and movement disorders, geneticists, a neuroradiologist, and a genetic counselor.

All 14 individuals had *GNAO1* variants identified through clinical testing, either by whole exome sequencing (WES; Patients 1–6, 8, 9, 13, and 14) or by targeted next generation sequencing epilepsy panels (Patients 7 and 10–12). Parental testing was completed for all but one patient. In the case of two siblings with the same variant, each reported as de novo, we reanalyzed parental WES data for evidence of parental mosaicism using the BCH Connect analysis platform. We conducted a thorough review of the literature (<https://www.ncbi.nlm.nih.gov/pubmed/?term=gnao1>, accessed October 2017) and ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/?term=GNAO1>, accessed March 2018) to identify additional cases for phenotypic comparison, to evaluate whether a given variant was previously reported in the literature or in ClinVar, and to evaluate for potential pathogenicity. Additionally, in silico predictions regarding pathogenicity were assessed using a combination of predictors, including the Alamut software suite (<http://www.interactive-biosoftware.com/alamut-visual/>) and PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>). We compared the presence and location of variants in patients, including those in the literature and ClinVar, to those in the population databases ExAC (<http://exac.broadinstitute.org/>) and gnomAD (<http://gnomad.broadinstitute.org/>).¹⁷ Variant classifications were determined based on the guidelines of the American College of Medical Genetics and Genomics (ACMG).¹⁸

Using the GNAO1 protein sequence (canonical transcript ENST00000262494, CCDS10757, NM_020988.2), we analyzed the position of the 13 variants in this report

along with 41 additional variants identified through our literature search (Table S1), totaling 54 disease-associated variants. For the following analyses, we excluded the intronic variant c.723+1G>A, because it cannot be annotated into the protein sequence.¹⁵ To assess variant pathogenicity, we evaluated the missense variant tolerance ratio (MTR) based on regional depletion in the general population.¹⁹ The MTR is a statistically normalized measure of how often a coding region “tolerates” missense variation based on the variant burden observed in gnomAD ($n = 138\,632$). We then evaluated the amino acid gene-family paralog conservation (Parazscore, <https://doi.org/10.1101/159780>) to assess for enrichment of patient-related variants compared to gnomAD variants in conserved regions. The Parazscore leverages amino acid conservation across gene-family members assuming that conserved sites are more likely to be important for protein function and thus more likely to be present in patients than controls. Statistical comparison between the variant counts of patients versus gnomAD was conducted using a two-sided Fisher's exact test with nominal two-sided P values < 0.05 considered significant.

We assessed genotype-phenotype correlations by comparing the locations of variants and the phenotypic features associated with each variant, with a focus on epilepsy and movement disorders. A model of the GNAO1 protein structure was generated on a template from the Protein Data Bank²⁰ identifier 3C7K,²¹ using SWISS-MODEL²² and mapped on complex structures using deconStruct.²³ Illustrations were generated using PyMol.²⁴

2.1 | Data availability

Anonymized data will be made available by request from any qualified investigator.

3 | RESULTS

3.1 | Genetic variants in GNAO1

We identified 13 unique heterozygous *GNAO1* variants in 14 patients (Table 1, Figure 1). Variants were reported as de novo in 13 patients after parental testing; one did not have parental testing performed. Parental mosaicism was suggested in one family by the same GNAO1 G40E variant present in two siblings, Patients 3 and 4.

Five variants previously reported in the literature, in other patients, are present in Patient 1 (p.G40R),^{4,15} Patient 6 (p.R209C),¹² Patient 7 (p.R209H),^{6,7,9,11} Patient 9 (p.Y231C),⁴ and Patient 11 (p.I279N).² Variants in our cohort affecting the previously implicated GNAO1 G40 amino acid site^{4,15} are present in Patient 2 (p.G40W) and Patients 3 and 4 (p.G40E). We identified six novel variants

TABLE 1 Pathogenicity predictions of de novo variants in *GNAO1*

Gender	Patient 1 Male	Patient 2 Female	Patient 3 Male	Patient 4 Female	Patient 5 Male	Patient 6 Female	Patient 7 Female	Patient 8 Female	Patient 9 Male	Patient 10 Female	Patient 11 Male	Patient 12 Female	Patient 13 Female	Patient 14 Female
Age, y	8	2	20	15	10	2	2	15	4	6	2	5	13	10
Variant	c.118G>C (p.G40R)	c.118G>T (p.G40W)	c.119G>A (p.G40E)	c.119G>A (p.G40E)	c.620C>A (p.S207Y)	c.625C>T (p.R209C)	c.626G>A (p.R209H)	c.662C>A (p.A221D)	c.692A>G (p.Y231C)	c.818A>T (D273V)	c.836T>A (p.I279N)	c.871T>A (p.Y291N)	c.1030_ 1032delATT (p.I344del)	c.1046_ 1055del10ms10 (p.R349_ G352delinsQGCA)
Conservation	High	High	High	High	High	High	High	High	High	High	High	High	N/A	N/A
Align- GVGD	C15	C15	C0	C0	C65	C65	C25	C65	C65	C15	C0	C0	N/A	N/A
SIFT	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious	N/A (in- frame deletion)	N/A
Mutation Taster	Disease causing	Disease causing	Disease causing	Disease causing	Disease causing	Disease causing	Disease causing	Disease causing	Disease causing	Disease causing	Disease causing	Disease causing	N/A	N/A
PolyPhen-2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.989	0.999	0.993	1.0	0.997	N/A	N/A
ClinVar (number of cases)	Pathogenic (1); uncertain significance (1)	Pathogenic (1)	Pathogenic (1)	Pathogenic (1)	Pathogenic (1)	Pathogenic/ likely pathogenic (3)	Pathogenic (2); uncertain significance (1)	N/A	Likely pathogenic (1)	N/A	Pathogenic (1)	Likely pathogenic (1)	N/A	N/A
ExAC AF	0	0	0	0	0	0	0	0	0	0	0	0	0	0
gnomAD AF	0	0	0	0	0	0	0	0	0	0	0	0	0	0

GNAO1 variants were annotated based on transcript NM_020988.2.
AF, allele frequency; N/A, not available.

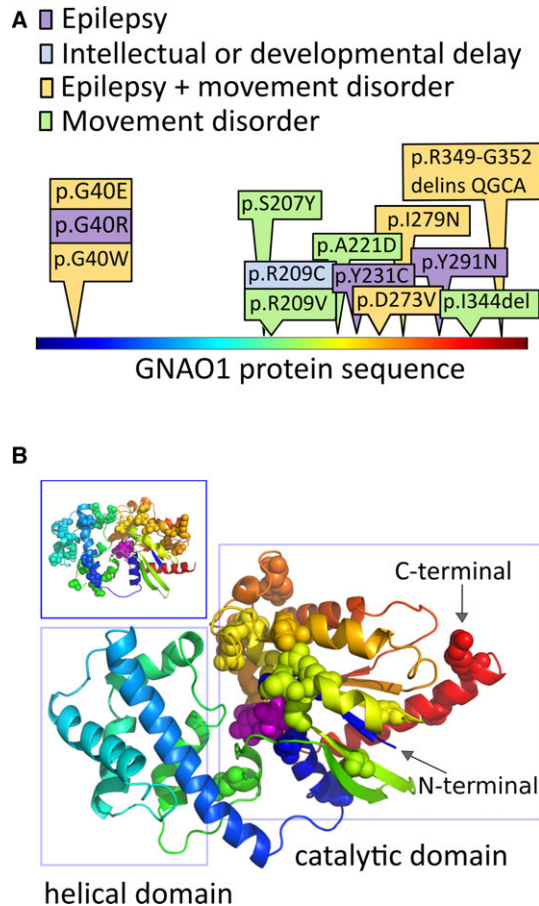


FIGURE 1 Patient variants in *GNAO1* affect the guanosine triphosphate-binding region. Disease-associated amino acid residues in the *GNAO1* protein localize to the catalytic domain. A, The protein is shown in linear representation, colored using a “rainbow” scheme, starting with blue at the N-terminal, and ending with red at the C-terminal. The amino acid substitutions altered by the *GNAO1* variants in our series are depicted here, with colors depicting the neurological features present in each case. B, The positions of disease-associated variants, from this series and the literature, are shown as spheres on the model of the *GNAO1* structure. The *GNAO1* substrate is also shown as spheres, colored magenta. The same coloring scheme is used in A and B. Patient variants cluster in the region of the catalytic domain (right rectangle), which starts at the very N-terminal aspect of the sequence, weaves into the helical domain, and returns back to complete the catalytic domain structure. The long helix on the N-terminal is not shown, because its position in the active conformation of $G\alpha$ is uncertain, and it carries no reported disease-associated amino acid positions. Inset (upper left-hand box): The distribution of missense variant amino acids in ExAC¹⁷ covers both structural domains of the protein and does not overlap with the disease-associated amino acid changes in our series

localized to previously unreported sites in the protein: c.620C>A (p.S207Y), c.662C>A (p.A221D), c.818A>T (p.D273V), c.871T>A (p.Y291N), c.1030_1032delATT (p.I344del), and c.1046_1055del10ins10 (p.R349_G352delinsQGCA). All *GNAO1* variants are annotated based on transcript NM_020988.2.

3.1.1 | Assessment of pathogenicity of *GNAO1* variants

Ten of the 13 unique variants have been reported in ClinVar, adding support for their pathogenicity (Table 1); five were classified as pathogenic, one with conflicting classification as pathogenic versus likely pathogenic, two as likely pathogenic, and two as pathogenic versus uncertain significance (Table 1). The two variants with conflicting classifications as pathogenic or uncertain significance (Patient 1 with p.G40R^{4,15} and Patient 7 with p.R209H^{6,7,9,11}) were de novo variants resulting in different amino acid substitutions at residues previously reported as pathogenic in multiple patients; thus, we classified them as pathogenic in accordance with ACMGG variant classification criteria.¹⁸ All variants were additionally evaluated for pathogenicity using Alamut. The three variants not listed in ClinVar were classified as pathogenic based on in silico predictions and in accordance with ACMGG criteria (Table 1).¹⁸

3.1.2 | Siblings with apparently de novo *GNAO1* variants due to parental mosaicism

Patients 3 and 4 were a 20-year-old brother and a 15-year-old sister. Within 2 hours of life, Patient 3 presented with episodes of apnea and lip smacking. Subsequently, at 2 months, he developed infantile spasms and hypsarrhythmia as well as focal motor seizures with left eye twitching, left head turning, and left arm extension. His sister also presented with seizures, initially described as bilateral tonic-clonic, on her first day of life. She eventually developed multiple seizure types including focal motor seizures, described as left eye twitching, tonic seizures, and infantile spasms. Additional phenotypic features are summarized in Table 2. Family history was notable for febrile seizures in the father, which we consider likely to be unrelated to the siblings' presentation given their *GNAO1* findings. Extensive genetic and metabolic testing for both siblings was initially nondiagnostic. Initially, a mitochondrial disorder was suspected based on muscle biopsy in Patient 4 suggesting a defect in complex IV of the respiratory chain that was later considered to be a secondary finding. Genetic evaluation included a normal karyotype, chromosomal microarray, and infantile epilepsy gene panel in 2015. WES performed for both siblings and their parents showed a pathogenic variant in *GNAO1* at c.119G>A (p.G40E) that appeared to be de novo in each sibling, leading to the presumption of either germline or gonadal parental mosaicism. Reevaluation of parental WES data revealed the variant allele (c.119G>A) in one parent in three of 150 reads (2%) consistent with germline mosaicism. This level of mosaicism is, however, below the current reporting threshold of Clinical Laboratory Improvement Amendments–certified laboratories.

TABLE 2 Genotype and phenotype of 14 patients with disease-associated variants in *GNAO1*

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14
Age, y/sex	8/M	2/F	20/M	15/F	10/M	2/F	2/F	15/F	4/M	6/F	2/M	5/F	13/F	10/F
Variant	c.118G>C (p.G40R)	c.118G>T (p.G40W)	c.119G>A (p.G40E)	c.119G>A (p.G40E)	c.620C>A (p.S207Y)	c.625C>T (p.R209C)	c.626 G>A (p.R209H)	c.662C>A (p.A221D)	c.692A>G (p.Y231C)	c.818A>T (D273V)	c.836T>A (p.I279N)	c.871T>A (p.Y291N)	c.1030_1032delATT (p.L344del)	c.1046_1055del10ins10 (p.R349_ G352delins QGCA)
Novel/ reported ^{14,15}	Reported	Novel	Novel	Novel	Novel	Reported ¹²	Reported ^{6,7,9,11}	Novel	Reported ⁵	Novel	Reported ²	Novel	Novel	Novel
Initial symptom (age)	Seizures (2.5 mo)	Seizures (5 wk)	Seizures (15 h)	Seizures (2 h)	Develop- mental delay (infancy)	Hypotonia (6 mo)	Develop- mental delay (6 mo)	Develop- mental delay (9 mo)	Seizure (5 d)	Seizures (2 d)	Seizures (1 h)	Seizures (2 mo)	Movement disorder (12 mo)	Developmental delay (6 mo)
Epilepsy type	DEE → LGS	DEE	DEE	DEE	N/A	N/A	N/A	N/A	DEE	DEE	DEE	DEE	N/A	Focal seizures
Onset	Spasms	Focal seizures	Spasms	GTC	N/A	N/A	N/A	N/A	Myoclonic seizures	GTC	Spasms	Focal seizures	N/A	Focal seizures
Other seizure types	Tonic, atonic, myoclonic seizures, GTC, spasms	N/A	Myoclonic, tonic seizures, GTC, spasms	Spasms	N/A	N/A	N/A	N/A	Focal, myoclonic seizures	N/A	Focal seizures	N/A	N/A	
Movement disorder	Anaxia	N/A	N/A	Left arm dystonia	Dystonia	N/A	Dystonia, ataxia	Dystonia	N/A	Dyskinesia	Chorea, akathisia	N/A	Dystonia, chorea	Facial dyskinesia, tremor
Cognition	ID	ID	ID	ID	Normal	ID	MID	MID	ID	ID	ID	ID	MID	ID
Motor	Nonambulatory	Delay	Nonambulatory	Nonambulatory	Delay	Delay	Delay	Delay	Delay	Nonambulatory	Delay	Nonambulatory	Delay	Oromotor apraxia
Speech	Nonverbal	Nonverbal	Nonverbal	Nonverbal	Delay	Delay	Nonverbal	Dysarthria	Delay	Nonverbal	Delay	Nonverbal	Delay	Delay, dysarthria
Tone	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia
EEG	3 y: slow spike and wave, multifocal spikes	9 mo: right temporal seizures, focal spikes, focal slowing	2 mo: hypersarhythmia; 14 y: generalized onset of tonic seizures and epileptic spasms, generalized slowing	9 d: multifocal spikes; 14 y: focal spikes and waves, absence of normal awake and sleep features	N/A	15 mo: slow posterior dominant rhythm	13 mo: normal	11 y: normal; 15 y: abnormal during sleep, frequent sharp waves	Neonatal: multifocal epileptiform sharp waves; 20 mo: frequent bioccipital spikes	Abnormal epileptiform activity	Neonatal: multifocal sharp waves with high-amplitude bursts (not burst suppression); 8 mo: modified hypersarhythmia	1 y: multifocal spikes, focal seizures; 3 y: intermittent posterior slowing	9 y: normal	7 y: sleep-activated posterior temporal and occipital spikes

(Continues)

TABLE 2 (Continued)

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14
MRI	4 mo: mildly prominent bifrontal subarachnoid spaces	8 mo: bilateral mesial temporal sclerosis, diffuse parenchymal atrophy, delayed myelination	2 y: status posttemporal lobectomy, left cerebral atrophy	15 mo: nonspecific signal increase in globi pallidi, normal myelination	9 y: normal	1 y: generalized thinning of corpus callosum, relative paucity of deep white matter	13 mo: frontal lobe volume loss	15 y: normal	2 y: prominent subarachnoid spaces	Normal	2.5 y: moderate to progressive atrophy with delayed myelination	2.5 y: normal	8 y: normal	9 y: normal
Other features	Swallowing dysfunction, chronic respiratory dysfunction	Gastroesophageal reflux	Swallowing dysfunction, cortical visual impairment, scoliosis	Astigmatism, myopia, alternating exotropia, scoliosis	N/A	N/A	N/A	Neonatal feeding difficulties	Spastic quadriplegia	Swallowing dysfunction	Intermittent exotropia	Hyperopia, esotropia	Single seizure at age 4 y	Swallowing dysfunction

All variants are confirmed de novo except Patient 10 (D273V), for whom parental testing has not been possible. DEE, developmental and epileptic encephalopathy; EEG, electroencephalogram; F, female; GTC, generalized tonic-clonic seizures; ID, intellectual disability; LGS, Lennox-Gastaut syndrome; M, male; MID, mild intellectual disability; MRI, magnetic resonance imaging; N/A, not available.

3.2 | Analysis of *GNAO1* variants in patients compared to controls

Based on the linear amino acid sequence of *GNAO1* (canonical transcript ENST00000262494, CCDS10757, NM_020988.2), we compared the position of the exonic variants in 54 patients with the variants identified in the gnomAD reference database.¹⁷ Collectively, our 14 patients plus 40 from the literature comprise 30 unique variants affecting 23 different amino acid positions (Figure 2), whereas gnomAD variants affect 95 different amino acids. To further assess variant pathogenicity, we implemented MTR scores and Parazscores for variant interpretation. As expected, both MTR scores ($P = 4.407\text{e-}04$) and Parazscores ($P = 1.756\text{e-}5$) from patient variants were significantly lower than those observed in gnomAD controls, indicating population and evolutionary constraint of patient variants, respectively (Figure 2). Furthermore, within the amino acid boundaries of the GTP-binding domain, we observed high intolerance for missense mutations (ie, lower MTR scores) and strong conservation across family members (ie, greater Parazscores). Taken together, these predictions strongly support the pathogenicity of *GNAO1* variants in our cohort, and for patient variants found within the GTP-binding domain.

3.3 | Phenotypes associated with *GNAO1* variants

Our 14 patients ranged in age from 2 to 20 years; five were male, and nine were female (Table 2). Nine of 14 (64%) had epilepsy, nine of 14 (64%) had movement abnormalities, and five of 14 (36%) had both epilepsy and movement abnormalities. A diagnosis of epilepsy was reported in nine patients. Eight patients had early onset DEE, with seizure onset at <3 months, epileptiform activity on EEG, and developmental stagnation or regression (Patients 1-4 and 9-12). The EEGs of all 9 patients with epilepsy showed epileptiform activity, including hypsarrhythmia in two 2 patients (Table 2). Patient 13 had a single afebrile seizure at 4 years of age and a normal EEG. Of the four patients without a history of epilepsy or seizure, three had an EEG; one was normal, one showed background slowing, and one was initially normal but later showed frequent focal spikes during sleep (Table 2).

Hypotonia was reported in all patients. Movement disorders were present in nine patients; five had severe dystonia (Patients 4, 5, 7, 8 and 13), two had ataxia (Patients 1 and 7), two had dyskinesia (Patients 10 and 14), two had chorea (Patients 11 and 13), one had akathisia (Patient 11), and one had tremors (Patient 14). Patient 14 had intermittent facial dyskinesia, swallowing dysfunction, and dysarthria, which has been reported in four patients with a severe phenotype.

Occipitofrontal circumference (OFC) data were available for 13 of 14 patients. One patient had microcephaly, Patient 3 with OFC 41 cm at 10 months (2.8%). Brain

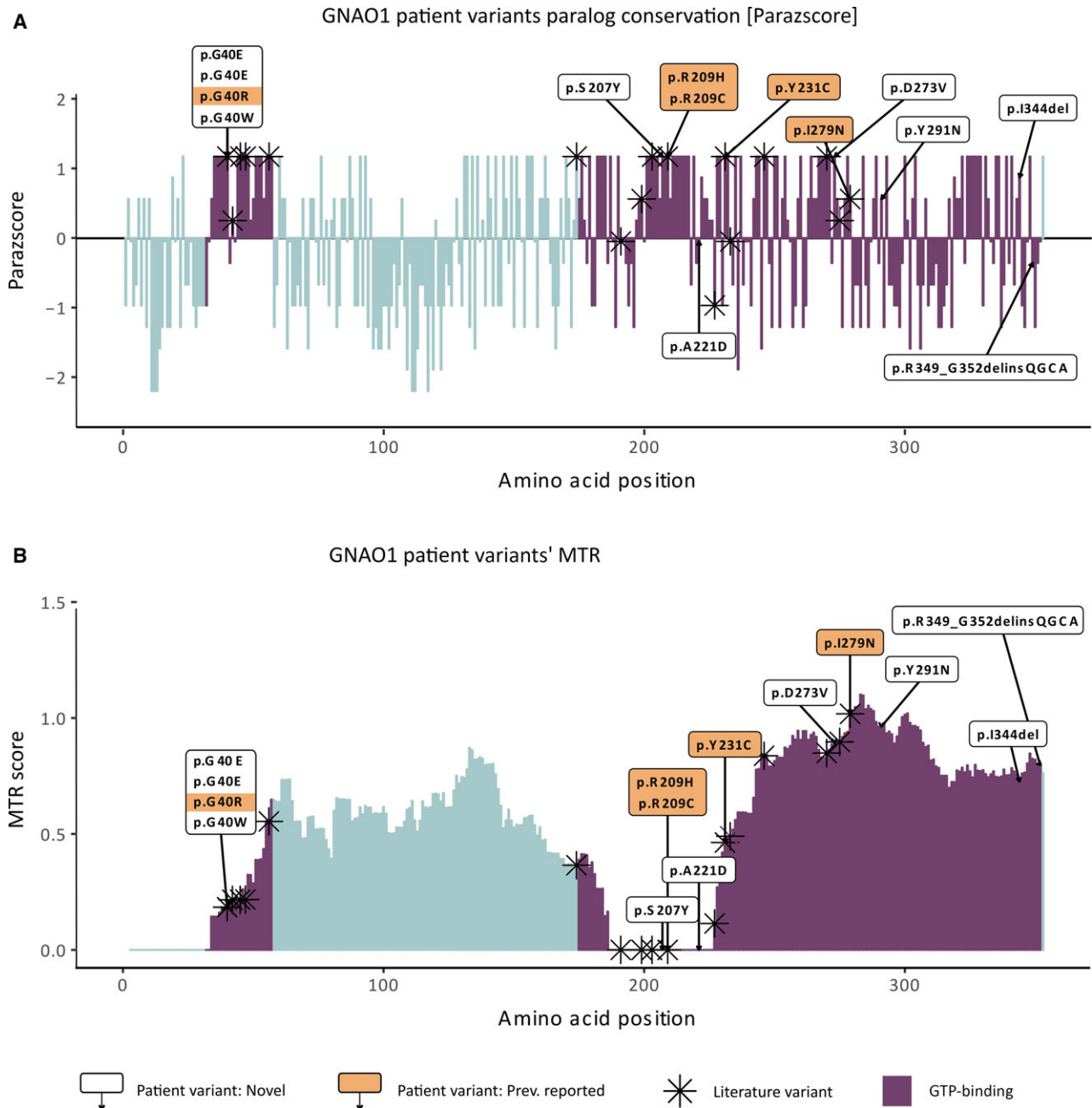


FIGURE 2 *GNAO1* patient variant evolutionary conservation and population constrained assessment. A, *GNAO1* patient variants' paralog conservation score (Parazscore) across the linear protein sequence. Comparing the amino acid sequence of the *GNAO1* protein to that of paralogous proteins in its gene family, the gene family-wide paralog conservation is shown for each amino acid of *GNAO1* protein sequence. Parazscore values range from negative values, representing less conservation at a given amino acid position, to positive values, representing high conservation, with the highest value indicating that identical amino acids are present in all related proteins. B, *GNAO1* patient variants' missense tolerance ratio (MTR).¹⁹ The score visualizes the tolerance to missense across the *GNAO1* protein sequence based on depletion of variants in population controls from the gnomAD database (ExAC v2).¹⁷ MTR values range from 0 (extremely intolerant to missense variant amino acids) to 1.5 (for positions tolerant to missense variant amino acids). For both graphs, patient variant-related amino acid substitutions not previously reported in the literature (white boxes, $n = 9$) and patient variant-related amino acid substitutions previously reported in the literature (shaded boxes, $n = 5$) are labeled alongside amino acids altered by *GNAO1* variants in the literature (asterisks, $n = 40$). Altogether, 23 amino acid positions are affected. Amino acids belonging to the guanosine triphosphate (GTP)-binding domain are marked in blue and represent the vast majority of amino acids affected in patients. Intronic variant c.723+1G>A was not included.¹⁵ All patient variants fall within the boundaries of the GTP-binding domain (blue bars)

magnetic resonance (MR) images were reviewed for 10 patients; MRI reports only were available for the remaining patients. The MR images were either normal or had

nonspecific abnormalities, including diffuse atrophy (six patients) and abnormal myelination (two patients). One MR image showed globus pallidus/dentate signal increase,

possibly related to vigabatrin use, and one MR image showed signs of mesial temporal sclerosis (Table 2).

All patients had developmental delay and showed a broad spectrum of severity. Five patients showed profound impairment and were nonverbal and nonambulatory. Patients 5, 13, and 14 had the mildest developmental abnormalities. Patient 5 presented with delayed speech and motor development but was reported to have met motor and language milestones and to have normal cognition at 10 years. Patient 13 presented with language delay, speaking single words at 2.5 years and full sentences at 4 years, and mild intellectual disability. Patient 14 presented with oromotor apraxia and moderate intellectual disability. At 11 years, she could recognize colors, some words and numbers, and understand early math concepts but is making very slow academic progress.

We compared the phenotypes of our 14 patients with those of 41 individuals with disease-associated *GNAO1* variants in the literature (Table S1).^{2–16,25} Striking phenotypic patterns emerged, despite incomplete phenotype information in some patients.

Epilepsy and movement disorders are common phenotypic features. Forty-two of 55 individuals (76%) had movement disorders, 35 of 55 (64%) had epilepsy, and 23 of 55 (42%) had both. The range of movement disorders includes chorea, dystonia, dyskinesia, stereotypies, and ataxia. The 35 individuals with epilepsy most often had DEE (24/35, 69%), including infantile spasms with hypsarrhythmia; seven individuals had focal seizures only, two had generalized seizures only, one had both generalized and focal seizures, one had infantile spasms only, and three reports did not contain enough information as to the type of epilepsy to allow further classification.

All patients had developmental delay in our cohort and the cases in the literature, with a wide range of severity, including our patients with mild delay. Hypotonia was commonly reported (76%), and microcephaly was reported in 20% of those cases for which data were available. MRI was reported to be normal in 20 of 55 individuals; 16 showed atrophy (both generalized and focal), 12 had corpus callosum abnormalities (most often a thin corpus callosum), seven showed delayed myelination, three had mild hypoplasia of the caudate nuclei, two had bilateral globus pallidus abnormalities (hypointense signal in a case with p.E246K⁶ and hyperintense signal in Patient 4 with p.G40E), and one had a left frontal astrocytoma thought to be unrelated to the *GNAO1* variant.¹⁵

3.4 | Relationship of phenotype with amino acid position and protein domain

Given the high sequence similarity of *GNAO1* to crystallized forms of $G\alpha$ proteins, its structure is predicted to

consist of two domains: one helical and one catalytic with a GTPase-binding pocket. Mapping the disease-associated variants reported to date onto this model structure demonstrates that disease-associated variants are located preferentially in the catalytic domain. This is even more intriguing when taking into account that the two globular domains of *GNAO1* are formed by nonconsecutive regions of the protein sequence (Figure 1). In contrast, variants present in the general, unaffected population can be found in both the helical and catalytic domains (Figure 1B, inset).

Evaluating the disease-associated variants on the predicted protein structure, the variants associated with movement disorders exclusively are found outside the catalytic pocket (Figure S2), between AA207 and AA221. Reviewing our patients and those in the literature, most patients with variants affecting AA207-AA246 present with movement disorder but not with epilepsy, with the exception of two patients with *GNAO1* p.Y231C (Patient 9, this report) and p.A227V.¹² Conversely, any variant in or near the catalytic pocket resulted in epilepsy, either alone or in association with movement disorders.

The most commonly affected amino acids R209 and G40 are both located in the catalytic domain. Examining the phenotypes associated with variants at R209 (p.R209C¹² in Patient 6 and p.R209H^{6,7,9,11} in Patient 7), only three of 10 individuals (30%) were reported to have seizures, one generalized tonic-clonic and two focal. By comparison, variants at G40 produce a far more severe phenotype (p.G40R^{4,15} in Patient 1, p.G40W in Patient 2, and p.G40E in Patients 3 and 4). The G40 site has been implicated in seven patients with epilepsy, including six with DEE. Furthermore, three of these seven individuals are reported to have both a movement disorder and epilepsy classified as DEE.^{4,15,25} Six of these seven also had developmental delay and/or intellectual disability, hypotonia, and abnormal findings on MRI.

4 | DISCUSSION

GNAO1 encephalopathy has a broad phenotypic spectrum, most commonly presenting with seizures and less frequently with movement disorders. Developmental delay and hypotonia occur in nearly all cases, although they led less frequently to medical attention. The severe phenotype, occurring in 44% of cases, comprises an early onset profound DEE, with seizure onset soon after birth in 24 of 55 cases.^{2–5,8,10,12,14–16,26}

MRI studies have not shown major structural abnormalities in patients with *GNAO1* variants. Progressive diffuse atrophy and delayed myelination are noted in some cases with thinning of the corpus callosum.^{2,5,6,8,10,12,14–16} For patients with *GNAO1*-related movement disorders, treatments such as deep brain stimulation have been undertaken^{9,13,15} and require neuroimaging. Therefore, longitudinal assessment of the MRI

features of patients with movement disorders will likely be available in the future.

Previous reports mention a slight female predominance in patients with *GNAO1*-related neurodevelopmental disorders¹⁰ and an apparent excess of sibling pairs identified with apparently de novo *GNAO1* pathogenic variants.^{9,11} In our cohort, nine of 14 (64%) patients are female, and of the 40 cases in the literature identified as male or female, 24 (60%) were female; as more cases continue to accrue, the sex ratio can be more accurately determined. Together with our series, there are now three affected sibling pairs as well as an individual whose brother had previously passed away with the same phenotype.^{9,11} These variants appeared to occur de novo based on “negative” parental testing. However, their recurrence in siblings leads to the hypothesis of parental mosaicism, either in the germline with low allele frequency undetectable by standard clinical sequencing or restricted to the gametes. In our case, reanalysis of the clinical sequencing data identified parental mosaicism in 2% (3/150) WES reads. The phenomenon of parental mosaicism has been observed in association with other disease-associated genes, including those associated with neurodevelopmental disorders.²⁷ At present, we do not have evidence to conclude that *GNAO1* is more likely to be associated with parental mosaicism than other genes.²⁸ However, the possibility of parental mosaicism needs to be taken into account when providing genetic counseling to families that have one child with a de novo variant in *GNAO1*. A subsequent affected child must be considered, and if further children are planned, testing with high depth coverage in the parents offers an opportunity to assess this risk.

GNAO1 encodes a G-protein α subunit highly expressed in the brain. Heterozygous de novo variants are thought to cause a gain of function, as demonstrated in the *GNAO1*+/*G184S* mutant mouse model.²⁹ However, recent in vitro studies suggest that the functional consequences of *GNAO1* variants depend on their location within the gene.³⁰ The G-protein α subunit consists of two sections of a P-loop structure containing a GTP-binding domain. The amino acids between these domains create a helical insertion domain that isolates and stabilizes the guanine nucleotide upon GTP binding and must be displaced for GTP/guanosine diphosphate dissociation to occur. It is in this critical region that the majority of patient variants are located, both in our patients and in the literature.²

We observed interesting correlations between phenotype and genotype across all reported cases according to amino acid position and protein structure. Not surprisingly, the picture was dominated by pathogenic or likely pathogenic variants affecting the GTP-binding regions of the protein in all but one of the published variants, with the only variant identified in the helical insertion domain located at AA174,² a site adjacent to the start of the GTP-binding protein domain. The localization of patient variants in the GTP-binding domain is consistent

with our current understanding of the $G\alpha$ function; both the catalytic pocket and interactions with its protein partners are affected through this domain (Figure S1). It is possible that the role of the helical domain is simply to function as the “lid” in the active conformation of the protein, requiring a conserved amino acid sequence. A dysfunctional *GNAO1* catalytic pocket may lead to extended GTP hydrolysis time and consequently the failure of dissociation between $G\alpha$ and the $G\beta\gamma$ dimer. This in turn, through the failure of ion channel regulation exerted by the $G\beta\gamma$ dimer, may lead to neuronal hyperexcitability and an epilepsy phenotype; this hypothesis will require further functional testing, as pioneered by Feng et al.³⁰

Many of the shared features identified in the current *GNAO1* patient population are not specific to any single variant or amino acid position. Epilepsy was seen across variants affecting amino acids throughout the protein, and different types of epilepsy seem to be equally distributed across the gene. Movement disorders were associated with amino acid positions across the gene as well, particularly associated with the AA207-246 region, which may act as a “hotspot” for movement disorder phenotypes typically, although not always, without epilepsy. In addition, it is worth noting that patients with variants affecting *GNAO1* AA40 had particularly severe phenotypic presentations including all features.

As the case number for *GNAO1*-associated neurodevelopmental disorders grows, expansion of the clinical phenotypic spectrum continues to emerge. When *GNAO1* variants are identified in individuals, it is most often by WES rather than by targeted testing. This likely reflects the lag time between gene discovery and inclusion of a given new gene on targeted multigene panels as well as a lag in clinician awareness for the gene. Although the number of individuals undergoing WES is increasing, we would support clinical testing laboratories adding *GNAO1* to their panels for early onset epilepsy, DEE, and movement disorders. Earlier genetic identification of a variant associated with DEE might change management, in that epilepsies arising from variants in signal transduction genes are generally not treated by focal brain resection (as had been pursued in one of our patients). Similarly, recognition of a *GNAO1* variant in a patient with a mixed movement disorder should prompt evaluation for therapy with deep brain stimulation, as it has been shown to be effective in a number of cases.^{9,13} Patients with *GNAO1* variants have often been mistakenly diagnosed with secondary movement disorders, which derive much lower benefit from deep brain stimulation,³¹ in contrast to a *GNAO1*-related disorder.

5 | CONCLUSIONS

The phenotypic spectrum of *GNAO1*-related neurodevelopmental disease includes epilepsy and a range of movement disorders, often with epilepsy as the presenting feature. A

range of movement disorders are seen in the majority of patients, even if not as the presenting symptoms. Hypotonia and developmental delay were present in all patients in our series with a wide range of severity.

All patients had variants in the GTP-binding region of GNAO1, highlighting the importance of this region for normal neurodevelopment. We identified a small cluster of variants apparently associated only with movement disorders but not epilepsy. The presence of two siblings with the same pathogenic variant, with parents who initially appear to lack that variant, highlights the importance of considering parental mosaicism when counseling families.

In addition to expanding and refining the phenotypic spectrum of *GNAO1*-related neurodevelopmental disease in a cohort rigorously analyzed for variant pathogenicity and phenotypic features, we highlight more generally the importance of considering protein structure-phenotype correlations, which may help determine disease prognosis in children with early onset neurodevelopmental diagnoses. Our findings highlight the importance of pursuing a genetic etiology in patients with a wide range of presenting symptoms, including patients who were previously diagnosed with acquired etiologies for their multisymptom neurodevelopmental conditions. Particularly when the course of treatment may be altered by the presence of a pathogenic variant in *GNAO1*—currently deep brain stimulation for movement disorders, but perhaps one day a more targeted treatment addressing the epilepsy and other features of the disorder—early genetic diagnosis should be pursued.

Epilepsia is a member of COPE (Committee on Publication Ethics), and we adhere to its principles (<http://publicationethics.org/>).

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DISCLOSURE

The authors have no conflicts of interest to report. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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APPENDIX

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