EXPANDING THE PHENOTYPE RELATED TO GNAO1 MUTATIONS

Highlighting the Dystonic Phenotype Related to GNAO1

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ABSTRACT: Background: Most reported patients carrying GNAO1 mutations showed a severe phenotype characterized by early-onset epileptic encephalopathy and/or chorea.

Objective: The aim was to characterize the clinical and genetic features of patients with mild GNAO1-related phenotype with prominent movement disorders.

Methods: We included patients diagnosed with GNAO1-related movement disorders of delayed onset (>2 years). Patients experiencing either severe or profound intellectual disability or early-onset epileptic encephalopathy were excluded.

Results: Twenty-four patients and 1 asymptomatic subject were included. All patients showed dystonia as prominent movement disorder. Dystonia was focal in 1, segmental in 6, multifocal in 4, and generalized in 13. Six patients showed adolescence or adulthood-onset dystonia. Seven patients presented with parkinsonism and 3 with myoclonus. Dysarthria was observed in 19 patients. Mild and moderate ID were present in 10 and 2 patients, respectively.
**Conclusion:** We highlighted a mild GNAO1-related phenotype, including adolescent-onset dystonia, broadening the clinical spectrum of this condition. © 2022 The Authors. Movement Disorders published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society

**Key Words:** dystonia; GNAO1; phenotypes; mutation

GNAO1 mutations have been associated with two phenotypes: a severe, early-infantile epileptic encephalopathy with burst-suppression (EIEE17, OMIM 615473) and a neurodevelopmental disorder with involuntary movements (NEDIM, OMIM 617493), with or without seizures. GNAO1 encodes the α-subunit of a heterotrimeric guanine nucleotide-binding protein (Gαo), which is widely expressed in the central nervous system, playing an important role in signal transduction through AMPc metabolism in the striatum. As the number of reports increased, it became evident that GNAO1-related encephalopathies encompass a continuous spectrum of neurological syndromes featuring variable association of movement disorders, psychomotor delay, intellectual disability (ID), and different types of epilepsy. GNAO1-related movement disorder usually starts in infancy. Choreoathetosis is usually described with spontaneous or trigger-induced exacerbations, potentially leading to status dystonicus, as a hallmark of the disease. Most patients reported so far showed a severe phenotype, with recurrent exacerbations and significant disability. However, in a few atypical, milder cases, with movement disorder onset in late childhood or adolescence, no acute exacerbation and less-severe disability have been identified using next-generation sequencing techniques. In this study, we characterized the clinical and genetic features of a cohort of patients with mild GNAO1-related phenotype characterized by prominent movement disorders, further expanding the spectrum of this condition.

**Patients and Methods**

**Patients**

Patients carrying causative heterozygous variants in GNAO1 and exhibiting mild phenotypes were included from 18 neurology and neuropsychiatric movement disorders reference centers from the United States, France, Israel, Switzerland, the United Kingdom, and Italy. Mild phenotype was defined by (1) lack of severe or profound ID, (2) lack of early-onset epileptic encephalopathy, (3) late-onset (ie, after age 2 years) appearance of movement disorders, and (4) acquisition of walk. Patients were recruited through an international collaboration mediated by the online platform Genematcher. All patients were assessed by neurologists or neuro-pediatricians with an expertise in movement disorders. Patients’ phenotypes from family 6 and family 4, which were previously reported elsewhere, were added in the cohort as further clinical data were obtained.

**Genetic Analysis**

CGH-array, gene panel, exome, and genome sequencing were performed as previously reported. Detailed procedures of the sequencing, including library preparation and bioinformatic analysis, are available in Supplementary Data. Variants were considered as causative if they fulfilled the following criteria: (1) known disease mutation reported in ClinVar; (2) loss-of-function variant, including protein truncating variants, frameshift indel, large deletion, and splice site changes predicted to cause aberrant splicing; or (3) missense variant with a CADD score >20, absent in GnomAD and predicted to be deleterious by at least two additional algorithms (Polyphen-2, SIFT and Mutation taster). In addition, variant class of pathogenicity was reported according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

**Ethics**

All patients and relatives provided written informed consent before genetic analysis. Strasbourg University Hospital review board gave approval for the exome sequencing of families 4 and 6 that was performed in a research framework. Genetic analysis for other families was performed for diagnostic purposes.

**Results**

We included 24 patients (15 women) and 1 asymptomatic carrier from 20 different families. Patients’ clinical characteristics are provided in Table 1. Mean age at inclusion was 23.8 years (range: 5–66), mean age at disease onset was 6.6 years (range: 0.25–47), and mean age of dystonia onset was 10.1 years (range: 2–47). Initial manifestations included dystonia in 10 (41%), myoclonus or seizure in 1, developmental delay in 13, language delay in 4, motor delay in 9, and hypotonia in 4 patients. Seven patients were from three unrelated families showing autosomal dominant inheritance, while all others were sporadic cases due to de novo mutations. Pedigrees of these three families and videos of patients are available in Supplementary Data.

Dystonia was the main movement disorder in all patients, prominently affecting multiple segments of the upper body part in 21 patients or being limited to the cervical segment in 1 patient. Dystonia was isolated, namely not associated to any other symptom, in
### TABLE 1  Clinical and genetic features of GNAO1 mutation carriers

| Patient ID | Ancestry  | Gender | Age at last assessment | Age at first symptoms | First symptom | Dyskinesia | Topography | Progression | Acute exacerbations | Paroxysms | Myoclonus | Chorea | Hypertonia | Intellectual disability | Seizures | Speech | Other | Treatment response | GNAO1 variant |
|------------|-----------|--------|------------------------|-----------------------|---------------|------------|------------|-------------|-------------------|-----------|-----------|-------|-----------|-----------------------|---------|--------|-------|-----------------------|---------------|
| Family 1   | North African | Female | 28 y                   | 4 y                   | Dystonia      | 12 y       | Segmental; face, neck, upper limbs | No           | No               | No           | No | No       | Mild | Yes       | Normal               | None    | No     | No     | Mild response to levodopa, moderate improvement with trihexyphenidyl | [NM_020988.3]: c.68 T > C; p.L23P, hts |
| Case A     |           |       |                        |                       |              |            |            |             |                   |           |           |       |           |                      |         |        |       |                      |               |
| Family 2   | North African | Female | 5 y                     | 1 y                   | Developmental delay (motor delay) | 2 y       | Generalized, oromandibular, trunk, dystonia | Yes         | No               | No           | No | No       | Yes  | No      | No                   | None    | NA     | No     | No response to levodopa and trihexyphenidyl, minimal improvement with gabapentin and trihexyphenidyl | [NM_020988.3]: c.137A > G; p.K46R, hts |
| Case A     |           |       |                        |                       |              |            |            |             |                   |           |           |       |           |                      |         |        |       |                      |               |
| Family 3   | European | Male   | 19 y                   | 3 y                   | Developmental delay (motor delay, dysarthria) | 12 y       | Generalized, left upper limbs, cervical and axial dystonia | No           | No               | No           | No | No       | Yes  | No      | Yes                  | None    | NA     | No     | No response to levodopa and trihexyphenidyl, mild worsening by Gpi-DBS | [NM_020988.3]: c.535A > G; p.R179G, hts |
| Case A     |           |       |                        |                       |              |            |            |             |                   |           |           |       |           |                      |         |        |       |                      |               |
| Family 4   | European | Male   | 24 y                   | 15 y                  | Dystonia     | 15 y       | Segmental, oromandibular and cervical dystonia | No           | No               | No           | No | No       | No   | No      | Yes                  | None    | NA     | No     | No response to levodopa and trihexyphenidyl | [NM_020988.3]: c.617G > A; p.R206Q, hts |
| Case A     |           |       |                        |                       |              |            |            |             |                   |           |           |       |           |                      |         |        |       |                      |               |
| Family 4   | European | Female | 53 y                   | 47 y                  | Dystonia     | 47 y       | Brachial, cervical | No           | No               | No           | No | No       | No   | No      | Normal              | None    | NA     | No     | No response to levodopa | [NM_020988.3]: c.644G > A; p.C215Y, hts |
| Case B     |           |       |                        |                       |              |            |            |             |                   |           |           |       |           |                      |         |        |       |                      |               |
| Family 4   | European | Female | 57 y                   | 30 y                  | Dystonia     | 30 y       | Multifocal, upper and lower limbs, oromandibular dystonia with dysarthria | No           | No               | No           | No | No       | No   | No      | No                   | None    | NA     | No     | No response to levodopa | [NM_020988.3]: c.644G > A; p.C215Y, hts |
| Case C     |           |       |                        |                       |              |            |            |             |                   |           |           |       |           |                      |         |        |       |                      |               |
| Family 5   | European | Female | 5 y 11 mo              | 3 mo                  | Developmental delay (motor delay, dysarthria) | 5 y        | Multifocal, lower limbs | No           | No               | No           | No | No       | Yes  | No      | No                   | None    | NA     | No     | No response to levodopa and trihexyphenidyl, mild response to levodopa | [NM_020988.3]: c.622G > C; p.E208N, hts |
| Case A     |           |       |                        |                       |              |            |            |             |                   |           |           |       |           |                      |         |        |       |                      |               |
| Family 6   | European | Male   | 81 y                   | 3 y                   | Dystonia     | 3 y        | Generalized, ataxia, oromandibular dystonia | Yes          | No               | No           | No | No       | Yes  | No      | No                   | None    | NA     | No     | No response to levodopa, no effect of trihexyphenidyl | [NM_020988.3]: c.644G > A; p.C215Y, hts |
| Case A     |           |       |                        |                       |              |            |            |             |                   |           |           |       |           |                      |         |        |       |                      |               |
| Family 6   | European | Female | 66 y                   | 5 y                   | Dystonia     | 5 y        | Generalized, oromandibular, distal, dystonia, facial dystonia, left foot | Yes          | No               | No           | Yes | No       | No   | No      | Mild     | None    | NA     | No     | Subjective response to levodopa | [NM_020988.3]: c.644G > A; p.C215Y, hts |
| Case B     |           |       |                        |                       |              |            |            |             |                   |           |           |       |           |                      |         |        |       |                      |               |
| Family 7   | European | Male   | 48 y                   | 6 y                   | Dystonia     | 6 y        | Generalized, oromandibular, cervical, trunk, arm, neck | Yes          | No               | No           | No | No       | Mild | No      | No                   | None    | NA     | No     | Subjective response to levodopa, no effect of trihexyphenidyl | [NM_020988.3]: c.644G > A; p.C215Y, hts |
| Case A     |           |       |                        |                       |              |            |            |             |                   |           |           |       |           |                      |         |        |       |                      |               |
| Family 8   | European | Female | 16 y                   | 6 y                   | Dystonia     | 6 y        | Segmental, oromandibular, neck and upper limbs | Yes          | No               | No           | No | No       | Mild | No      | No                   | None    | NA     | No     | Mild improvement with gabapentin, no benefit with trihexyphenidyl | [NM_020988.3]: c.644G > A; p.C215Y, hts |
| Case A     |           |       |                        |                       |              |            |            |             |                   |           |           |       |           |                      |         |        |       |                      |               |
| Patient ID | Ancestry | Genotype | Gender | Age at last assessment | Age at first symptom | First symptom | Topography | Prognosis | Acute exacerbations | Parkinsonism | Myoclonus | Chorea | Hypotonia | Intellectual disability | Seizures | Speech | Other | Treatment response | GNAO1 variant |
|------------|----------|----------|--------|-----------------------|---------------------|---------------|-------------|-----------|-------------------|-------------|-----------|-------|-----------|----------------------|---------|--------|-------|----------------------|----------------|
| Family 9   | African American | Male | 13 y 7 mo | Developmental delay (language delay) | 5 y | Generalized cervical, neck, and upper limb dystonia with axial, trunk, and oromandibular involvement | Yes | No | No | No | No | MILD | No | Dysesthesia | None | No response to levodopa, trihexyphenidyl, and baclofen; rash with clonazepam; minimal improvement with baclofen |
| Family 10  | Moroccan | Female | 15 y 1 y | Developmental delay (motor delay) | 5 y | Generalized dystonia | No | No | No | No | No | MILD | No | Dysesthesia | None | Tactile hyperreflex | No response to levodopa, trihexyphenidyl, and baclofen |
| Family 11  | Caucasian | Female | 18 y 6 mo | Developmental delay (motor delay) | 7 y | Generalized dystonia | Yes | No | No | No | No | Yes | MILD | No | Anarthria | None | No response to levodopa, trihexyphenidyl, and baclofen; good response to bilateral Gpi-DBS |
| Family 12  | Caucasian | Male | 20 y 2 mo | Developmental delay (motor delay, language delay) | 11 y | Generalized bilateral upper limb, axial, trunk, cervical, and oromandibular dystonia | Yes | Yes | No | No | No | Yes | MILD | No | ADHD | None | No response to amantadine and levodopa; moderate and transient response to trihexyphenidyl and tetrabenazine, good response to Gpi-DBS |
| Family 13  | Caucasian | Male | 13 y 3 y | Developmental delay (language delay) | 11 y | Segmental bilateral upper limb dystonia | No | No | No | Yes (upper limbs) | No | Yes | MILD | No | Normal | ADHD | No response to anticholinergic and amantadine; improvement in dystonia with trihexyphenidyl |
| Family 14  | Chinese | Male | 13 y 3 y | Developmental delay (motor delay) | 2 y | Generalized upper and lower limb dystonia | Yes | No | No | Yes (upper limbs) | Yes (left sided) | Yes | No | No | Dysesthesia | ADHD | Moderate response to tetrabenazine on dystonia, good response to trihexyphenidyl and tetrabenazine, and levodopa |
| Family 15  | Caucasian | Female | 11 y 8 mo | Developmental delay | 2 y | Generalized upper and lower limb dystonia | Yes | No | No | Yes (upper limbs) | No | No | No | No | Dysesthesia | ADHD | None | Good response to levodopa |
| Family 16  | Caucasian | Female | 11 y 8 mo | Developmental delay | 2 y | Generalized upper and lower limb dystonia | No | No | No | Yes (left sided) | Yes | No | No | No | Dysesthesia | None | None | Response to antipsychotic and levodopa |
| Family 17  | Caucasian | Male | 18 y 2 y | Developmental delay (language delay) | 2 y | Segmental dystonia, posturing of fingers and hand | No | No | No | No | No | No | No | No | Dysesthesia | None | No response to haloperidol, tetrabenazine, and trihexyphenidyl |
| Family 18  | Caucasian | Female | 20 y 4 mo | Dystonia | 6 y | Generalized dystonia, | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No | Anarthria | None | No response to haloperidol, tetrabenazine, and trihexyphenidyl |

(Continues)
| Patient ID | Ancestry | Gender | Age at last assessment | First symptom | Total symptom | Dysostasia | Topography | Progression | Acute exacerbations | Parkinsonism | Myoclonus | Chorea | Hypertonia | Intellectual disability | Seizures | Speech | Other | Treatment response | GNAO1 variant |
|------------|----------|--------|------------------------|---------------|---------------|------------|------------|-------------|-------------------|-------------|-----------|-------|----------|----------------------|---------|--------|-------|----------------------|----------------|
| Family 19  | European | Female | 13 y                   | 6 y           | Dystonia      | 6 y        | Multifocal | Bilateral   | Upper limb       | Cervical    | No        | No    | No      | No                   | No      | No     | No    | No                   | c.765dupT; p.N256*, hts |
| Family 19  | European | Female | 39 y                   | 16 y          | Dystonia      | 16 y       | Multifocal | Bilateral   | Upper limb       | Cervical    | No        | No    | No      | No                   | No      | No     | No    | No                   | Heterozygous deletion in 16q12.2 (273–375kb) encompassing GNAO1 |
| Family 20  | Caucasian| Male   | 9 y                    | By 1 y        | Developmental delay (motor delay, hypotonia) | 9 y | Generalized | Upper limb | Multifocal       | #1 | Yes       | No      | No      | No      | Yes      | No       | No       | No       | No       | No       | Normal  | No       | No       | No       | No       | No       | No       | Heterozygous deletion in 16q12.2 (273–375kb) encompassing GNAO1 |

**Summary**

- Gender: Male, Female
- Mean age at last assessment: 23.6 y
- Mean age at disease onset: 6.6 y
- Developmental delay: 13
- Motor delay: 9
- Language delay: 4
- Dysostasia: 10
- Hypertonia: 4
- Seizures: 1
- Myoclonus: 1
- Parkinsonism: 7
- Myoclonus: 3
- Chorea: 2
- Hypertonia: 11
- Intellectual disability: 12
- Seizures: 3
- Dysostasia: 19
- Pyramidal: 1
  - Myoclonus: 1
  - MDD: 4
  - OHD: 2
  - ADHD: 1
  - Exaggerated startle reflex: 1

**Abbreviations:** hts: heterozygous, NA, not available; BFMDRS: Burke Fahn Marsden Dystonia Rating Scale, dystonia score; GPi-DBS: globus pallidus internal deep brain stimulation; MDD, major depressive disorder; ADHD, attention deficit with hyperactivity disorder; ASD, autism spectrum disorder; ID, intellectual disability.
7 patients (29%). Dystonia was the only movement disorder in 14 patients and was combined with other movement disorders in 10, namely myoclonus in 3, chorea in 2, and parkinsonism in 7 (with 2 patients combining three movement disorders). Only 3 patients presented an acute exacerbation of dystonia. Dystonia course was non-progressive for 11 patients. Dystonia topography was generalized in 13 patients (54%), multifocal in 4 patients, segmental in 6 patients, and focal in 1 patient. Dystonia was associated with dysarthria/anarthria in 19 patients. Early-onset hypotonia preceded dystonia in 11 patients. Dystonia was associated with ID in 12 patients (mild for 10 and moderate for 2). Seizures occurred in 3 patients between age 4 and 19 years. Magnetic resonance imaging was unremarkable for all patients.

Movement disorders response to medication, including anticholinergic drugs, levodopa, tetrabenazine, amantadine, clonazepam, or methylphenidate, was variable. Six patients received pallidal deep brain stimulation (DBS), with significant improvement for 5 of them. Detailed treatment outcomes are available in Supplementary Data.

Mutations carried by the patients are presented in Table 1. Details regarding pathogenicity assessment are available in Supplementary Data. Apart from the p.R206Q, all variants were classified as pathogenic (class V) according to the ACMG criteria. In family 4, we identified 3 patients with the R206Q variant, which was classified as a variant of uncertain significance due to the presence of an unaffected carrier, 4-D, son of 4-C, despite meeting other criteria of pathogenicity (absent from GnomAD, unanimously predicted damaging by in silico tools, affecting a highly conserved residue located in a hot spot without benign variation) (criteria PM1, PM2, PP2, and PP3). A recurring splicing variant (c.724-8G > A), previously reported in ClinVar, was identified in 8 patients (33%) showing late-onset and/or segmental dystonia. Previous report showed this variant to damage the natural splice acceptor site and create a stronger cryptic splice acceptor site in intron 6, resulting in the insertion of two amino acids leading to protein mislocation. Two patients were carrying a nonsense variant (p.N256*), and one was carrying a

![FIG. 1.](http://wileyonlinelibrary.com)
large deletion encompassing GNAO1. Other mutations (p.L23P; p.K46R; p.R179G; p.R206Q; p.E208N; p.C215Y; and p.N242T and p.E246V) were all missense variants absent from GnomAD. The previously reported pathogenic p.C215Y variant was found in three unrelated families. All variants were close to known mutational hot spots (Fig. 1).

Discussion

Here, we report a large cohort of patients with mild GNAO1-related phenotypes, experiencing prominent movement disorders without severe chronic encephalopathy. The typical phenotype was a nonprogressive generalized or focal/segmental upper-body dystonia appearing beyond infancy, associated with dysarthria. Acute exacerbation occurred only in 3 patients, and 29% of patients showed isolated dystonia without additional neurological manifestation. Our inclusion criteria were able to identify these phenotypes that were in contrast with most of the previously reported patients with GNAO1-related movement disorders, who showed severe hyperkinetic encephalopathy with recurrent dystonic exacerbations, and profound developmental delay with or without epilepsy in the first year of life. Dystonia distribution was segmental or focal in 7 patients, and clinical course was nonprogressive in 11 patients, while most of the previously reported patients with GNAO1-related movement disorders had generalized and rapidly progressive dystonia. Dystonia topography revealed prominent upper-body distribution in most of our patients, reminiscent of the clinical pictures associated with other dystonia-related genes, such as GNAL or ANO3. Seven patients also exhibited mild parkinsonism, which is consistent with the role of $G_{\alpha_0}$ in the signal transduction within the striatal projection neurons downstream of the dopamine receptors.

We identified 3 autosomal dominant families where multiple symptomatic relatives carried heterozygous variants, which was in contrast with all the previously reported patients who showed de novo mutations. One p.R206Q carrier did not present any clinical sign evocative of GNAO1-related disorders. The similarities between this family’s phenotype and the other cases—all showing upper-body distribution—argue for the implication of the variant, while no other class IV to V variant in a dystonia-related gene was identified. In addition, a family member carrying this variant had disease onset in his 40s, meaning the 30-year-old asymptomatic carrier could be potentially symptomatic. Future identification of autosomal dominant family with GNAO1-related dystonia and follow-up of this patient might confirm whether incomplete penetrance is possible in GNAO1-related disorders.

Response to medication was variable in our cohort. No significant response to levodopa was identified in our cohort, but 3 patients had partial response to anticholinergic drugs, which was in accordance with previous findings from the literature. Conversely, the outcome was good in 5 of 6 patients who received DBS, further confirming its efficacy in GNAO1-related dystonia.

Most of the variants identified in the present work were not reported among previously published cases showing severe phenotype, and two variants recurred in multiple families, suggesting that mild phenotypes could be related to specific mutations. However, the variants we identified were close to previously reported hot spots (Fig. 1), leading to amino-acid substitution in the same functional domains. Further studies are needed to elucidate if these different variants have a milder impact on protein function. In addition, we identified two putative loss-of-function variants (a nonsense variant and a whole-gene deletion), presumably affecting protein expression and possibly causing GNAO1 haploinsufficiency. All 3 carriers were presenting late-childhood/adolescence onset dystonia without ID. Thus far, no report described the phenotype of patients harboring GNAO1-nonsense variants. A few patients with chromosome 16q deletions encompassing GNAO1 have been described, all harboring significantly larger deletions compared to our case and showing variable associations of dysmorphisms, microcephaly, seizures, and developmental delay. Although previous research suggested that loss-of-function variants were mainly responsible for epileptic encephalopathy while gain-of-function mutations were mostly associated with a movement disorders prominent phenotype, recent evidence suggests that pathogenic variants exert their effect through a combination of dominant-negative and loss-of-function mechanisms, and each mutation likely produces circuit-selective effects through a peculiar mechanism of signaling disruption. The expanding spectrum of associated phenotypes and disease-causing variants provides further evidence that genotype-phenotype correlations are nuanced, and GNAO1-related disorders shape a continuous spectrum of overlapping phenotypes rather than distinct entities. Our study carries some limitations, including the retrospective design and the lack of formal assessment in several cases. Here, we highlighted the milder GNAO1-related phenotypes, broadening this condition-clinical spectrum. GNAO1 mutations should be considered as a cause of adolescent or adult-onset nonprogressive dystonia, particularly in the presence of a speech involvement even in the absence of seizures or ID.

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Data Availability Statement
Anonymized data pertaining to the research presented will be made available upon reasonable request from external investigators.

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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.