Both subthalamic and pallidal deep brain stimulation are effective for GNAO1-associated dystonia: three case reports and a literature review

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Abstract

Background: Mutations in the G-protein subunit alpha o1 (GNAO1) gene have recently been shown to be involved in the pathogenesis of early infantile epileptic encephalopathy and movement disorders. The clinical manifestations of GNAO1-associated movement disorders are highly heterogeneous. However, the genotype-phenotype correlations in this disease remain unclear, and the treatments for GNAO1-associated movement disorders are still limited.

Objective: The objective of this study was to explore diagnostic and therapeutic strategies for GNAO1-associated movement disorders.

Methods: This study describes the cases of three Chinese patients who had shown severe and progressive dystonia in the absence of epilepsy since early childhood. We performed genetic analyses in these patients. Patients 1 and 2 underwent globus pallidus internus (GPI) deep brain stimulation (DBS) implantation, and Patient 3 underwent subthalamic nucleus (STN) DBS implantation. In addition, on the basis of a literature review, we summarized and discussed the clinical characteristics and outcomes after DBS surgery for all reported patients with GNAO1-associated movement disorders.

Results: Whole-exome sequencing (WES) analysis revealed de novo variants in the GNAO1 gene for all three patients, including a splice-site variant (c.724–8G>A) in Patients 1 and 3 and a novel heterozygous missense variant (c.124G>A; p. Gly42Arg) in Patient 2. Both GPI and STN DBS were effective in improving the dystonia symptoms of all three patients.

Conclusion: DBS is effective in ameliorating motor symptoms in patients with GNAO1-associated movement disorders, and both STN DBS and GPI DBS should be considered promptly for patients with sustained refractory GNAO1-associated dystonia.

Keywords: de novo variant, deep brain stimulation, dystonia, GNAO1-associated movement disorders, whole-exome sequencing analysis

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Highlights

- Expanding the phenotypic and genotypic spectrum of GNAO1-associated disease.
- Providing a new basis for the STN as an effective target for DBS to treat GNAO1-associated movement disorders.

Introduction

Movement disorders are generally classified as hyperkinetic and hypokinetic.1,2 Many inherited diseases can cause movement disorders in children. GNAO1 has been recently identified to be involved in the pathogenesis of early-onset genetic...
movement disorders.\textsuperscript{3–5} Mutations in the \textit{GNAO1} gene have been associated with a complex spectrum of neurological disorders, including early infantile epileptic encephalopathy 17 (EIEE17, OMIM: 615473) and neurodevelopmental disorder with involuntary movements (NEDIM, OMIM: 617493; also known as GNAO1-associated movement disorders).\textsuperscript{3,5,6} Patients with \textit{GNAO1}-associated movement disorders are predominantly characterized by various combinations of dystonia, chorea, myoclonus, hypotonia, tremor, and orofacial dyskinesia with or without epileptic seizures.\textsuperscript{4} To date, approximately 35 variants in the \textit{GNAO1} gene have been reported.\textsuperscript{4,6–12} Treatments with levodopa, topiramate, and tetrabenazine are considered somewhat effective in improving movement dysfunction;\textsuperscript{13,14} however, most \textit{GNAO1}-associated movement disorders are refractory to medications.\textsuperscript{5,15,16} Deep brain stimulation (DBS) has been established as a safe and effective treatment in patients with inherited dystonia, and patients with \textit{GNAO1} mutations suffering from progressive hyperkinetic movement disorders have shown a beneficial response to globus pallidus internus (GPI) DBS.\textsuperscript{7–9,17,18} However, the genotype–phenotype correlations of \textit{GNAO1}-associated disease remain unclear, and the application of DBS in this field is still limited. In this study, we report the cases of three Chinese patients with \textit{de novo} \textit{GNAO1} variants who manifested similar symptoms of severe dystonia and developmental delay in the absence of epilepsy since early childhood. All of them were responsive to DBS. Our study expands the phenotypic spectrum of \textit{GNAO1} variants and suggests that DBS is a safe and effective option for the symptoms of patients with \textit{GNAO1}-associated movement disorders.

\textbf{Materials and methods}

\textit{Clinical study}

Three patients from different Chinese families were recruited from the Department of Peking University First Hospital and the Department of Neurology of the First Hospital of China Medical University. All of them presented with childhood-onset movement disorders and developmental delay. Clinical data were collected, and detailed neurological examinations, mental status examinations, laboratory examinations, electroencephalography, and brain magnetic resonance imaging (MRI) were performed in these patients.

\textit{Genetic analysis}

Whole-exome sequencing (WES) was performed in all three patients. Blood samples were obtained from these three patients and their parents with informed consent. Genomic DNA was extracted from peripheral leukocytes using standard methods. Paired-end 150-bp sequencing runs were performed on a HiSeq ×10 instrument (Illumina, San Diego, CA, USA) to cover the mean read depth of the potential sites by 100×. Sequencing data were aligned to the human reference genome (UCSC hg19) by the Burrows–Wheeler Aligner. Variants were called using the Genome Analysis Tool Kit and annotated with the ANNOVAR tool (annovar.openbioinformatics.org/en/latest/). All variants were further filtered using the 1000 Genomes Project (http://phase3browser.1000genomes.org/index.html) and the Exome Sequencing Project (http://evs.gs.washington.edu/EVS). The potential impact of single-nucleotide variants was predicted by the Mutation Taster, SIFT, \textit{GERP++}, and \textit{PolyPhen-2} programmes. The Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/) was used to confirm whether the detected variants were known or novel. Finally, Sanger sequencing (BigDye® Terminator v3.1, Applied Biosystems, Foster City, CA, USA) was performed to determine whether the variants were inherited or \textit{de novo}.

\textit{DBS}

All patients in our cohort were evaluated by the interdisciplinary movement disorders group, which comprised neurologists, neurosurgeons, and neuropsychologists. After pre-operative evaluation, the three patients were treated with DBS. The target nucleus for DBS was the bilateral GPI in Patients 1 and 2 and the bilateral subthalamic nucleus (STN) in Patient 3. For the GPI and STN targets in the three patients, direct targeting was performed using pre-operative MRI (3-T MRI, General Electric) and an intra-operative microelectrode recording (MER) technique. The Medtronic 3387 electrode (IPG, Activa-PC, Medtronic) was used on Patient 1, with contact 0 placed at the bottom of the GPI target. PINS (PINS Medical Co., Ltd, Beijing, China) L301 DBS leads were used on Patients 2 and 3. Two different types of lead kits were used in the three patients. In general, the PINS and Medtronic intracranial electrode systems were essentially the
same, and neither of the electrodes used was directional. The stimulators have the following subtle differences: the stimulator of the PINS can be set at different frequencies on each side and has a frequency conversion mode, while the stimulator of the Medtronic leads has an interactive electrical pulse mode. Detailed stimulator parameters are listed in Table 1. Post-implantation imaging for all three patients was performed to reconfirm the correct electrode placement (Figure 1). In addition, we assessed these patients with the Burke–Fahn–Marsden Dystonia Rating Scale (BFMDRS) both pre-operatively and 14–24 months after surgery.

Written informed consent was obtained from Patient 1 (a 17-year-old male) and the parents of Patient 2 (a 4-year-old girl) and Patient 3 (a 5-year-old boy) before genetic testing and DBS surgery in this study. We also obtained written informed consent from the patients or their parents for the publication of clinical data and images.

### Results

**Case presentation**

Patient 1 was a 17-year-old male with non-consanguineous parents. His perinatal course was uneventful. According to his parents’ description, however, he showed some abnormalities at 2 years old, such as walking on his tiptoes and mild slurred speech. He was diagnosed with cerebral palsy and received only symptomatic treatment. At the age of 15, he was referred to the hospital due to the slow progressive aggravation of generalized dystonia with mild intellectual disability as assessed by Raven’s Progressive Matrices. He was given various oral drugs, including clonazepam, levodopa, and diazepam. However, these drugs were not effective for him, even the maximum doses of levodopa (0.25 g three times per day). When he was admitted to the hospital at the age of 17, his movement symptoms had worsened, as indicated by the development of torsional trunk movement to the left, which was accompanied by a twisting of the left arm, as well as left torticollis (Figure 2(a)). His severe dystonia symptoms interfered with his activities of daily living, posture, and gait. However, neither episodes of epilepsy nor acute dystonic exacerbations were seen throughout the course of his illness. Neurological examinations showed considerable dystonia in the extremities and trunk torsion with spontaneous head tremor. Notably, no choreic or athetoid movement was present. In addition, he had remarkable scoliosis, with a Cobb angle of 25° as seen on a radiograph of the spine (Figure 2(b)), which was due to long-term severe trunk dystonia. The laboratory biochemical examination results of creatine kinase, liver enzymes, ceruloplasmin, and lactic acid were normal. The brain MRI and electroencephalography showed no abnormalities.

Patient 2 was a 4-year-old girl. Her family history was noncontributory. From the age of 6 months, some signs progressively appeared that were unusual for her developmental age. Increased muscle tone of the extremities, decreased muscle strength,
developmental delay, and intellectual disability were the most representative symptoms. When she came to the clinic at the age of four, she was unable to walk or grasp objects independently because of dystonic postures in the extremities, with severe contractures. Neurological examinations showed a twisting and repetitive flexion of the metacarpophalangeal and interphalangeal joints because of the increased muscle tone of the hand flexor muscles (Figure 2(c)). She underwent multiple medication trials, such as levodopa (60 mg two times per day) and levocarnitine, but these were discontinued because no effects were observed. Moreover, she could not speak complete words; she could only utter simple sounds. Extensive workup found nothing abnormal until WES was performed.

Patient 3 was a 5-year-old boy whose birth history was normal. Abnormal movements were noted at approximately 6 months of age. Over time, his dystonia in the limbs and trunk progressed, and he developed slurred speech and dysphagia. When he was hospitalized at the age of 5, he could stand without external support, but he had difficulty walking unaided (Figure 2(d)). Neurological examinations indicated severe dystonia involving his trunk, upper and lower limbs, and bulbar muscles, with increased muscle tone in the extremities and trunk. In addition, skeletal deformities, such as remarkable knee flexion and mild scoliosis, were observed. Furthermore, although the brisk Achilles tendon reflex was also observed, the Babinski sign was absent. Laboratory biochemical examinations and brain...
Figure 2. Dystonic postures and skeletal deformities in affected individuals with GNAO1 variants: (a) picture of Patient 1 before surgery showing remarkable dystonia in his left limbs and trunk torsion. (b) The spine radiograph before surgery demonstrating remarkable scoliosis with a Cobb angle of 25° in Patient 1. (c) Picture of Patient 2 before surgery showing dystonic postures in her hands, with severe contractures. (d) Picture of Patient 3 before surgery showing dystonia in the upper and lower limbs with knee flexion and scoliosis. (e) Patient 1 exhibited a significant improvement in limb and trunk dystonia when evaluated 14 months after GPi DBS. (f) Patient 2 showed an obvious improvement in upper limb dystonia at a follow-up 24 months after surgery. (g) Dystonic postural improvement is shown in Patient 3 15 months after STN DBS surgery.
MRI showed no significant findings. Many drugs, including levodopa, benzhexol, oxcarbazepine, and levocarnitine, were given without any substantial benefits. Notably, the response to levodopa was not assessable because the patient took the drug only once at the starting dose of 25 mg; he then stopped taking the drug because of the occurrence of hyperkinesia in the extremities, especially in the lower limbs.

Before surgery, none of the patients suffered from any episodes of acute dystonic exacerbations. However, their dystonia and abnormal postures progressively worsened. Patients 1 and 3 had persistent dystonia and abnormal postures except during sleep and anaesthesia, whereas the dystonia and abnormal postures of Patient 2 persisted even in sleep and anaesthesia but were less severe than when awake. The detailed clinical presentations of the patients are summarized in Table 2.

### Table 2. Summary of key clinical features of patients with GNAO1 mutations in our cohort.

| Patient number | 1 | 2 | 3 |
|----------------|---|---|---|
| **Gender**     | M | F | M |
| **Age at onset (years/months)** | 2 year | 6 m | 6 m |
| **Inheritance** | De novo | De novo | De novo |
| **GNAO1 mutation** | c.724-8G > A | c.124G > A | c.724-8G > A |
| **Clinical symptoms** | Dystonia in extremities | + | + | + |
| Trunk torsion | + | + | + |
| Chorea or athetosis | – | – | – |
| Seizures | – | – | – |
| **Skeletal deformities** | Scoliosis | Metacarpophalangeal and interphalangeal joint contractures | Remarkable knee flexion and mild scoliosis |
| **Other symptoms** | + | + | Dysphagia and dysarthria |
| Raven’s progressive matrices | Mild intellectual disability | NA | NA |
| Gesell developmental schedule | NA | Moderate developmental delay | Mild developmental delay |
| Brain MRI | N | N | N |
| EEG | N | N | N |

EEG, electroencephalogram; F, female; GNAO1, G-protein subunit alpha o1; M, male; MRI, magnetic resonance imaging; N, normal value; NA, not available; +, present; –, absent.

**Genetic analysis**

Heterozygous variants in the GNAO1 gene were identified in all three patients. The heterozygous splicing variant c.724–8G > A, which is located in intron 6, was identified Patients 1 and 3, who were unrelated (Figure 3(a) and (c)). This variant was a de novo variant (PS2) and was absent from controls (1000 Genomes Project and ExAC databases) (PM2). This variant has been detected in at least five patients, and its frequency was significantly higher in the affected population than controls (PS4).19,20 According to HGMD and ClinVar database information, this variant is predicted to disrupt the natural splice acceptor site.
and create a stronger cryptic splice acceptor site in intron 6 (https://useast.ensembl.org/info/docs/tools/vep/index.html). Thus, variant c.724–8G > A is classified as a pathogenic variant according to the American College of Medical Genetics (ACMG) standard.21

In Patient 2, WES identified a novel heterozygous missense variant in exon 2 of the GNAO1 gene (c.124G > A), resulting in a substitution of glycine to arginine at position 42 (p. Gly42Arg) (Figure 3(b)). This variant was not reported as a pathogenic variant in the HGMD or 1000 Genomes Project databases. Theoretical prediction based on in silico analysis by the MutationTaster, SIFT, GERP++, and PolyPhen-2 programmes suggested that this variant might have a deleterious effect on protein function. Subsequently, Sanger sequencing confirmed that this substitution was not found in the patient’s parents or in 50 healthy Chinese controls, indicating that the c.124G > A in the GNAO1 gene is a pathogenic de novo variant.

No other pathogenic variants were identified in the present cohort.

**Brain stimulation**

After presurgical evaluation and obtaining parental permission, Patient 1 underwent bilateral GPi DBS implantation on October 20, 2020, at the Department of Neurosurgery of the First Hospital of China Medical University. Patient 2 underwent bilateral GPi DBS implantation on December 30, 2019, and Patient 3 underwent bilateral STN DBS implantation on September 18, 2020, at the Department of Paediatric Surgery of Peking University First Hospital. No post-operative complications or morbidities occurred. At the first follow-up (approximately 1 month after surgery), the extremity and trunk dystonia symptoms of Patients 1 and 3 had improved significantly. However, the dystonia symptoms of Patient 2, especially the hand dystonia that bothered the patient the most, did not improve significantly in the early post-operative period. After several attempts to modulate the DBS parameters, the patient’s hand dystonia improved at the 4-month post-operative follow-up, as evidenced by her ability to grasp some objects, such as a pen, and extend the metacarpophalangeal and interphalangeal joints. Over a follow-up period of 14–24...
months, improvement based on the BFMDRS score ranged from 18.18% to 52.30% in all three patients, as summarized in Table 3. Thus, as of the time of submission, DBS showed beneficial effects on the movement functions of all three patients (Figure 2(e)–(g)).

**Discussion**

In this study, we report the cases of three patients with de novo heterozygous variants in GNAO1 who benefitted from DBS treatment.

A review of the literature showed that GNAO1 variants were recently discovered as rare causes of epileptic encephalopathies and early-onset hereditary movement disorders. To date, studies of GNAO1 pathological mutations at the molecular level have yielded conflicting results. An earlier study based on the effects of their variants on GNAO1-mediated cAMP signalling in non-neuronal cells, classified GNAO1 pathological mutations into three groups: loss-of-function, gain-of-function, and normal-function mutations.6 Subsequent studies challenged this hypothesis, showing that GNAO1 has a complex biology and pathogenic variants may alter Gαo function in a neuron-type-specific fashion via a combination of dominant-negative and loss-of-function mechanisms.22 In brief, the functional effects and mechanisms of GNAO1 pathological mutations remain unresolved. Furthermore, approximately 90% of the known GNAO1 variants clustered in exons 6 and 7, including c.607G>A (p.G203R) and c.736G>A (p.E246 K), which together account for approximately 50% of cases, indicating that GNAO1-related diseases may have hotspot variants.

Our study identified two de novo variants in the GNAO1 gene in three patients who shared the characteristics of severe and progressive movement disorders and developmental delay. Among the clinical, imaging, and genetic findings of the three patients in our cohort, there were some similarities, including no definite family history, de novo variants, increased muscle tone, developmental delay, and normal brain MRI results. None of the patients had seizures during the course of their disease. Patient 1 was misdiagnosed with cerebral palsy in early childhood and experienced torsion spasm after an exacerbation of dystonia. Patients 2 and 3 were also misdiagnosed with cerebral palsy before a confirmed diagnosis was made on the basis of WES results. Patients diagnosed with cerebral palsy in full-term births without specific MRI findings may have a genetic disease disguised as cerebral palsy;13,23 therefore, the diagnosis of GNAO1-associated movement disorders is often made after a considerable delay. Clinicians’ discernment capability and the decision to perform genetic analysis as soon as possible play an important role in the precise diagnosis and treatment of GNAO1-associated disorders.

In this study, we detected the novel c.124G>A (p. Gly42Arg) missense variant in Patient 2. Notably, a different variant in the same amino acid position, c.124G>C, leading to the same amino acid change (p. Gly42Arg), has previously been described in a patient with predominant choreoathetosis, developmental delay, and seizure.24 We found some similarities when comparing the clinical data from Patient 2 with the data from the patient with the c.124G>C variant, such as early-onset involuntary movements and developmental delay. However, the differences were considerable and remarkable. Hypotonia and epilepsy were significant in the patient with

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**Table 3.** Summary of the management and outcomes of our patients with GNAO1 mutations.

| Patient number | Age at surgery (years) | BFMDRS score at baseline | BFMDRS score after DBS | Improvement rate (%) |
|----------------|------------------------|--------------------------|------------------------|---------------------|
| 1              | 17                     | 86                       | 41 after 4 months      | 52.30 after 4 months |
|                |                        |                          | 41 after 14 months     | 52.30 after 14 months |
| 2              | 4                      | 77                       | 71 after 14 months     | 7.79 after 14 months |
|                |                        |                          | 63 after 24 months     | 18.18 after 24 months |
| 3              | 5                      | 62                       | 42 after 5 months      | 32.26 after 5 months |
|                |                        |                          | 35 after 15 months     | 43.55 after 15 months |

BFMDRS, Burke–Fahn–Marsden Dystonia Rating Scale; DBS, deep brain stimulation; GNAO1, G-protein subunit alpha o1.
the c.124G > C variant and other previously reported patients with GNAO1 variants but were not detected in Patient 2 in this study, reinforcing the notion that the clinical features are heterogeneous in GNAO1-associated diseases.

It is noteworthy that Patients 1 and 3 were confirmed to harbour the same de novo splice-site variant, c.724–8G > A, in intron 6 of GNAO1. According to previous studies, this variant has been observed in two individuals with abnormalities of the nervous system. However, there is no specific description of the neurological symptoms caused by this variant in previous reports. To the best of our knowledge, our study is the first to describe two patients with this pathogenic variant who shared extremely similar clinical symptoms of generalized dystonia, developmental delay, and skeletal changes in the absence of seizures. Considering all the patients reported to date, variants affecting the arginine 209, glycine 203, and glutamate 246 residues seem to be variant hotspots significantly associated with a prominent movement disorder phenotype. To date, at least four patients, including ours, have been reported to harbour the c.724–8G > A variant; therefore, we hypothesize that this splice-site variant might be an emerging hotspot in GNAO1-related diseases that primarily feature a series of clinical signs of motor symptoms and developmental delay without seizures. However, Patients 1 and 3 showed slightly different characteristics. For example, it is noteworthy that Patient 3 had dysphagia and dysarthria since early childhood, but Patient 1 did not, which reinforces the notion that the clinical features are heterogeneous in GNAO1-associated disorders. In addition, we suspect that the presence of some modifying genetic factors for this phenotype might explain this observation, similar to other hereditary neurological diseases. Further studies with larger patient numbers should help to clarify the genotype–phenotype correlation.

It has remained challenging to find suitable treatments for GNAO1-associated movement disorders. According to the documented literature, risperidone was effective for both a mouse model and patients with GNAO1-related movement disorders. However, tetrabenazine, a representative vesicular monoamine transporter-2 (VMAT-2) inhibitor, was the most successful medication for controlling chorea symptoms among patients with GNAO1 variants. In addition, antiepileptics and other agents, such as levodopa, are the standard pharmacological treatments, but they show only partial effectiveness. Furthermore, previous studies have indicated that movement disorder exacerbations may be refractory to treatment and result in severe complications. Similar to previously described patients, all three patients in our cohort were placed on a variety of medications, such as some neuroleptics and levodopa, a few years before surgery, but none seemed to have any effect, and the dystonia symptoms progressively worsened, suggesting refractoriness to drugs.

Encouragingly, DBS has been recently recognized as a safe and effective symptomatic treatment in patients with GNAO1-associated movement disorders, and GPi DBS has been reported to generally reduce the frequency and severity of movement disorder exacerbations, but without complete remission. We summarized the clinical features and outcomes after DBS surgery of all reported patients with GNAO1-associated movement disorders in Table 4. The movement disorder phenomenology of these patients mainly incorporates hypotonia, chorea, dystonia, ballismus, and orofaciolingual dyskinesia in various combinations. Our statistical analysis showed that 68.42% (13 of 19) of patients underwent GPi DBS implantation for the treatment of exacerbation episodes of hyperkinetic movements such as choreoathetosis, and 31.58% (6 of 19) of patients underwent DBS implantation for slowly progressive dystonia (including ours). Overall, all patients had good outcomes. DBS was effective in preventing acute exacerbations for 13 patients and in improving motor functions with incomplete remission for six patients. These results better established the efficacy of GPi DBS in the treatment of GNAO1-associated movement disorders, despite the limited number of reports.

In our study, although different DBS targets were selected by different neurological surgeons, we found that the severity of GNAO1-associated movement disorders and the associated disability improved to varying degrees both in terms of movement symptoms and clinical examination results after DBS in all three patients, despite their dystonia relief being incomplete. In addition, BFMDRS scores showed that DBS was significantly effective for Patients 1 and 3, but the effect for Patient 2 was not as obvious at
| Case (ref.) | Gender | Age at onset (years/months) | Age at surgery (years) | GNAO1 mutation | DBS target | DBS settings after surgery (months) | MD phenomenology | Episodic status dystonicus | Seizures | Drugs tried | Outcome | Post-surgical complications |
|------------|--------|-----------------------------|------------------------|----------------|------------|------------------------------------|----------------|-------------------------|----------|-------------|---------|-----------------------------|
| 1 (Benato et al.19) | F | 13 months | 5 years | c.736G > A, p. Glu246Lys | Bilateral GPi | Settings: L 4 V, 120 μs, 210 Hz; R 6 V, 120 μs, 210 Hz | Dystonia, chorea, ballismus involving the limbs | Yes | No | Trihexyphenidyl, nitrazepam, clonazepam, tetraethylzine, levodopa, levetiracetam, and phenobarbital | Return to pre-SD baseline; two SD recurrences involving right hemibody | Skin erosion above the left electrode (no infection), successfully managed with cutaneous flap; left electrode displacement (MRI-documented) as a result |
| 2 (Benato et al.19) | F | 4 years | 13 years | c.736G > A, p. Glu246Lys | Bilateral GPi | Settings: L 4.6 V, 90 μs, 210 Hz; R 4.6 V, 90 μs, 210 Hz | Recurrent generalized hyperkinetic spells | Yes | No | Flunitrazepam, baclofen, trihexyphenidyl, tetraethylzine, and pimozide | Return to pre-SD baseline with elimination of hyperkinetic exacerbations | None |
| 3 (Waak et al.18) | M | 3 months | 11 years | c.709G > A, p. Glu237Lys | Bilateral GPi | Settings: L 6.6 mA, 280 μs, 130 Hz; R 6.6 mA, 280 μs, 130 Hz, 26 months | Chorea, dystonia, ballism Oro-facio-lingual dyskinesia | Yes | No | Levodopa, benzhexol, diazepam, clonazepam, clonidine, predinisolone, and oral baclofen, botulinum toxin (local), tetraethylzine, clonazepam, phenobarbital, sodium valproate, and oxcarbazepine | Functional improvement (able to sit in wheelchair and drive electrical wheelchair, improved feeding and communication, discharge from hospital, and weaning of baseline medications) | Stimulator site infection |
| 4 (Waak et al.18) | F | 3 months | 6 years | c.736G > A, p. Glu246Lys | Bilateral GPi | Settings: L 4.4 mA, 100 μs, 130 Hz; R 4.4 mA, 100 μs, 130 Hz, 28 months | Chorea, dystonia, ballism Oro-facio-lingual dyskinesia | Yes | No | Levodopa, benzhexol, carbamazepine, Nitrazepam, (carazepam, clonazepam, acetacolamide, clonidine, biotin, Botulinum toxin (local)) | Functional improvement (able to tolerate sitting in wheelchair, improved feeding, and communication, discharge from hospital, and weaning of baseline medications) | Lead displacement requiring reinsertion |
| 5 (Waak et al.18) | M | 6 months | 13 years | c.625C > T, p. Arg209Cys | Bilateral GPi | Settings: L 0.5 mA, 450 μs, 130 Hz; R 0.5 mA, 450 μs, 130 Hz, 16 months | Chorea, dystonia, ballism Oro-facio-lingual dyskinesia | Yes | Generalized tonic-clonic seizures from age 10 | Clonidine, benzhexol | Functional improvement - improved mobility - GMFRS: 5 to 2, improved communication and feeding, weaning of all baseline medication) | NA |
| 6 (Key et al.11) | F | 3 years | 9 years | c.723 + 1G > T | Bilateral GPi | NA | NA | Yes | No | NA | No more exacerbation, improved motor function, BFMDRS-M 76,5 dropped to 66,5, BFMDRS-D 29 dropped to 18 | NA |

(Continued)
| Case (Ref.) | Gender | Age at onset (Years/ months) | Mutation | DBS setting | DBS After surgery (months) | Drugs tried | Outcome | Post-surgical complications |
|------------|--------|-----------------------------|----------|-------------|---------------------------|-------------|---------|------------------------------|
| 7 (Koy et al.) | F      | 14 years                     | c.625 > T, p. Arg209Cys | Bilateral GPi | NA | Metopimazine and trihexyphenidyl | Yes | Yes | Post-surgical complications | Improvement in dystonia and chorea at 6 years, exacerbation of dyskinesia at 14 years |
| 8 (Koy et al.) | M      | 15 years                     | c.625 > T, p. Arg209Cys | Bilateral GPi | NA | Tetrabenazine | Yes | Yes | BFMDRS-M 114 dropped to 84,5 | BFMDRS-D 30 to 27 |
| 9 (Koy et al.) | M      | Neonate                      | c.709G > A, g.56370758G > A, p. Glu237Lys | Bilateral GPi | NA | Carbidopa, levodopa, bromocriptine | Yes | No | Carbidopa, levodopa, bromocriptine | Complete remission of hyperkinesia and dystonia at rest, improvement of nonverbal communication, hand function, and mobility |
| 10 (Koy et al.) | M      | 8 years                      | c.709G > A, p. Glu237Lys | Bilateral GPi | NA | Carbidopa, levodopa, bromocriptine | Yes | No | Carbidopa, levodopa, bromocriptine | Complete remission of hyperkinesia and dystonia at rest, improvement of nonverbal communication, hand function, and mobility |
| 11 (Honey et al.) | M      | 18 months                   | The chr16: 56,370,675 G > T variant | Bilateral GPi | NA | Carbidopa, levodopa, bromocriptine | Yes | No | Carbidopa, levodopa, bromocriptine | Complete remission of hyperkinesia and dystonia at rest, improvement of nonverbal communication, hand function, and mobility |
| 12 (Yilmaz et al.) | M      | 13 months                   | c.698A > C, p. (Q233P) | Bilateral GPi | NA | Clonazepam, haloperidol, carbamazepine, acetazolamide, and diazepam | Yes | No | Clonazepam, haloperidol, carbamazepine, acetazolamide, and diazepam | Improvement in dystonia and chorea at 6 years, exacerbation of dyskinesia at 14 years, severe deterioration of his clinical condition, several lead replacements due to hardware infection |
| Case (ref.) | Gender | Age at onset (years/months) | Age at surgery (years) | GNAO1 mutation | DBS target | DBS settings after surgery (months) | MD phenomenology | Episodic status dystonicus | Seizures | Drugs tried | Outcome | Post-surgical complications |
|------------|--------|-----------------------------|------------------------|----------------|------------|-----------------------------------|----------------|--------------------------|-----------|-------------|---------|---------------------------|
| 13 (Kulkarni et al.17) | M | 18 months | 5 years | c.626G > A, p. Arg209His | Bilateral GPi | NA | 18 month/34 month/ hypotonia; 34 month/ choreoathetoid movements of arms and legs, head jerks | No | No | Clonazepam, valproic acid | Motor function has improved, and he is described as less encephalopathic | NA |
| 14 (Kulkarni et al.17) | M | 2 years | 7 years | c.626G > A, p. Arg209His | Bilateral GPi | NA | 2 years/abnormal movements began with his mouth and face and spread to the remainder of his body, irregular writhing movements of all extremities | No | No | Clonidine, clonazepam | Severe motor delay: Fahn–Marsden Dystonia Rating Scale score dropped from 65.5 pre-operatively to 34.0 after 10 months of DBS | NA |
| 15 (Danti et al.17) | M | 6 months | 7 years | c.737A > G, p. Glu246Gly | Bilateral GPi | NA | 6 month/2 year/mild central hypotonia; 7 year/mild-severe generalized and orobucal dystonia, lower limb spasticity; 7 year/severe exacerbation of hyperkinetic movements | Yes | Generalized tonic-clonic seizures and focal dyscognitive seizures | Tetraabenazine was effective in baseline management of the severe involuntary movements; anaesthetic agents | Almost complete remission of hyperkinesia with persistent residual dystonia | NA |
| 16 (Yamashita et al.28) | F | 6 months | 17 years | c.620 C > T (p.S207F) | Bilateral GPi | Settings: L 0.9 mA, 50 μs, 130 Hz R 0.9 mA, 50 μs, 130 Hz | Choreoathetosis, dystonia, hypotonia, and bradykinesia | No | No | Tetraabenazine, trihexyphenidyl, clonazepam, butotatum toxin, pram-pimozol, and levodopa | The Gross Motor Function Measure improved by 65% (from 15.6% to 60.6%). | None |
| Present 1st | M | 2 years | 17 years | c.724-80 > A | Bilateral GPi | Settings: L 3.0 V, 70 μs, 140 Hz R 2.40 V, 60 μs, 130 Hz | Exacerbation of dystonia in left limbs, torsion spasm, and spontaneous head tremor | No | No | Clonazepam, levodopa, and diazepam | BFMDRS score from 86 dropped to 41 after 14 months | None |
| Present 2nd | F | 6 months | 4 years | c.124G > A, p. Gly42Arg | Bilateral GPi | Settings: L 3.3 V, 90 μs, 140 Hz R 3.3 V, 80 μs, 140 Hz | Exacerbation of dystonia in left limbs, torsion spasm, and spontaneous head tremor | No | No | Medopar and levcarnitine | BFMDRS score from 77 dropped to 63 after 24 months, useful grasping | None |
| Present 3rd | M | 6 months | 5 years | c.724-80 > A | Bilateral STN | Settings: L 3.5 V, 90 μs, 140 Hz R 3.3 V, 80 μs, 140 Hz | Hypokinetic movements involved both of his upper extremities, and gonoacampsis | No | No | Medopar, benzhexol, oxcarbazepine, and levocarnitine | BFMDRS score from 62 dropped to 35 after 15 months, walk independently | None |

BFMDRS, Burke–Fahn–Marsden Dystonia Rating Scale; BFMDRS-D, Burke–Fahn–Marsden Dystonia Rating Scale disability; BFMDRS-M, Burke–Fahn–Marsden Dystonia Rating Scale motor; DBS, deep brain stimulation; GMFSC, gross motor function classification system; GNAO1, G-protein subunit alpha o1; GPi, globus pallidus internus; MD, movement disorder; MRI, magnetic resonance imaging; NA, not available; SD, status dystonicus; STN, subthalamic nucleus.

*Current study.
follow-up. However, the slight improvement in dystonia in patient 2 was associated with some functional improvement, such as useful grasping. According to previous studies, patients with a shorter duration of dystonic symptoms had better outcomes. When patients suffer from contractures or fixed skeletal deformities, DBS is likely to have a limited effect. Thus, we speculate that the surgical outcome of Patient 2 was not as good as that of the other two patients because she had exhibited much more severe distal limb contractures since early childhood. Since our finding was consistent with other case reports demonstrating dystonia relief, DBS therapy should be considered in medication-refractory individuals with GNAO1 variants, especially those with minimal contractures, although the exact mechanism of dystonia relief after DBS remains unclear. In addition, the course of disease of Patient 2 is generally consistent with that of Patient 3, but the outcome of Patient 2 was less satisfactory than that of Patient 3. We speculated that genetic factors, such as different pathogenic variants and changes in protein function, may account for differences in the severity of this disease and the response to DBS therapy.

Importantly, Patients 1 and 3, who harboured the same de novo splice-site variant in GNAO1 and had similar clinical symptoms, obviously benefited from DBS surgery, although different surgical targets were selected. According to the case reports of refractory patients with GNAO1 variants, most of them underwent GPi DBS implantation, and no patients have been treated with STN DBS thus far. After careful consideration, we chose the STN as the DBS target in Patient 3 for the following reasons: first, recent studies have found that the STN can be an alternative target for DBS in patients with isolated, focal, or generalized dystonia. In China, there have been several successful clinical studies about the long-term efficacy of STN DBS in primary dystonia, refractory tardive dystonia, and Meige syndrome. In addition, several strengths of STN DBS for dystonia compared with GPi DBS were also observed. STN DBS has a longer battery life and provides rapid improvement compared with GPi DBS. In addition, the neurosurgical team had greater experience with STN than with GPi DBS; therefore, we expected similarly favourable results for STN in GNAO1-associated dystonia. Second, as depicted in our study, GPi DBS improved some symptoms in Patient 2; however, the results were less than satisfactory. Moreover, from a surgical perspective, more accurate positioning may be more easily achieved in the sensorimotor area of the STN than in the GPi target. Another potential reason for concern for the choice of the STN target was that most side effects associated with STN DBS can be mitigated by adjusting the parameters, although dyskinesia was the most common adverse effect of STN DBS. Therefore, we chose the STN as the target for DBS for Patient 3 and achieved relatively satisfactory results. We speculate that patients with GNAO1-associated movement disorders without dyskinesia and acute hyperkinetic exacerbations can be considered for STN DBS.

Our findings suggest that the STN may be a candidate DBS target for patients with GNAO1-associated dystonia, and further investigation is necessary to provide a better understanding of whether the STN can be chosen as a suitable DBS target for GNAO1-associated dystonia in the future. In addition, our research provides a new basis for the STN as a target for DBS in the treatment of hereditary dystonia. More studies are needed to compare the long-term tolerability and sustained effectiveness of STN DBS and GPi DBS for the treatment of GNAO1-associated dystonia. Nevertheless, we also observed that the rate of improvement in Patient 1, who was treated with GPi DBS, was slightly higher than that in Patient 3, who was treated with STN DBS (52.30% vs 43.55%). This finding highlighted the notion that the STN can be considered an effective DBS target. An understanding of the differences between STN DBS and GPi DBS in terms of the long-term efficacy for dystonia depends on the verification of these findings in larger samples in the future.

In conclusion, GNAO1 is one of the causative genes in early-onset encephalopathy and movement disorders. As most known variants in the GNAO1 gene are de novo, many patients lack a family history of the disorder. Thus, when early-onset dyskinesia of unknown aetiology, whether accompanied by seizures or not, is encountered, early GNAO1 gene variant screening is necessary even if there is no family history. In addition, evidence from the relevant literature and our own data suggest that DBS is effective in ameliorating motor symptoms in patients with GNAO1-associated movement disorders, and both STN DBS and GPi DBS should be considered...
promptly in patients with sustained refractory GNAO1-associated movement disorders. The role of DBS and the genotype–phenotype correlation in GNAO1-associated movement disorders should be investigated in future studies.

**Ethics statement and patient consent**

Our study did not require an ethics board approval because drug refractory hereditary dystonia is one of the indications for deep brain stimulation (DBS) surgery according to the consensus of Chinese experts on dystonia. Written informed consents were obtained from Patient 1 (the 17-year-old young male) and the parents of Patient 2 (the 4-year-old girl) and Patient 3 (the 5-year-old boy) before genetic testing and DBS surgery. Furthermore, all patients or their parents provided written informed consent for the publication of their clinical data and images.

**Author contribution(s)**

**Ye Liu:** Writing – original draft; Writing – review & editing.

**Qingping Zhang:** Visualization.

**Jun Wang:** Methodology; Resources; Supervision; Writing – review & editing.

**Jiyuan Liu:** Data curation; Validation.

**Wuyang Yang:** Validation; Visualization.

**Xuejing Yan:** Resources; Software.

**Yi Ouyang:** Conceptualization; Formal analysis; Funding acquisition; Project administration; Resources.

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**Conflict of interest statement**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**References**

1. Stoessl AJ and McKeown MJ. Movement disorders. *Handb Clin Neurol* 2016; 136: 957–969.

2. Wilson RB and Keener AM. Movement disorders in children. *Adv Pediatr* 2018; 65: 229–240.

3. Schirinzi T, Garone G, Travaglini L, et al. Phenomenology and clinical course of movement disorder in GNAO1 variants: results from an analytical review. *Parkinsonism Relat Disord* 2019; 61: 19–25.

4. Kelly M, Park M, Mihalek I, et al. Spectrum of neurodevelopmental disease associated with the GNAO1 guanosine triphosphate-binding region. *Epilepsia* 2019; 60: 406–418.

5. Feng H, Khalil S, Neubig RR, et al. A mechanistic review on GNAO1-associated movement disorder. *Neurobiol Dis* 2018; 116: 131–141.

6. Feng H, Sjögren B, Karaj B, et al. Movement disorder in GNAO1 encephalopathy associated with gain-of-function mutations. *Neurology* 2017; 89: 762–770.

7. Danti FR, Galosi S, Romani M, et al. GNAO1 encephalopathy: broadening the phenotype and evaluating treatment and outcome. *Neurol Genet* 2017; 3: e143.

8. Koy A, Cirak S, Gonzalez V, et al. Deep brain stimulation is effective in pediatric patients with GNAO1 associated severe hyperkinesia. *J Neurol Sci* 2018; 391: 31–39.

9. Yilmaz S, Turhan T, Ceylaner S, et al. Excellent response to deep brain stimulation in a young girl with GNAO1-related progressive choreathetosis. *Childs Nerv Syst* 2016; 32: 1567–1568.

10. Okumura A, Maruyama K, Shibata M, et al. A patient with a GNAO1 mutation with decreased spontaneous movements, hypotonia, and dystonic features. *Brain Dev* 2018; 40: 926–930.
11. Arya R, Spaeth C, Gilbert DL, et al. GNAO1-associated epileptic encephalopathy and movement disorders: c.607G>A variant represents a probable mutation hotspot with a distinct phenotype. *Epileptic Disord* 2017; 19: 67–75.

12. Wirth T, Tranchant C, Drouot N, et al. Increased diagnostic yield in complex dystonia through exome sequencing. *Parkinsonism Relat Disord* 2020; 74: 50–56.

13. Malaquias MJ, Fineza I, Loureiro L, et al. GNAO1 mutation presenting as dyskinetic cerebral palsy. *Neurrol Sci* 2019; 40: 2213–2216.

14. Sakamoto S, Monden Y, Fukai R, et al. A case of severe movement disorder with GNAO1 mutation responsive to topiramate. *Brain Dev* 2017; 39: 439–443.

15. Ananth AL, Robichaux-Viehoffer A, Kim YM, et al. Clinical course of six children with GNAO1 mutations causing a severe and distinctive movement disorder. *Pediatr Neurol* 2016; 59: 81–84.

16. Dhamija R, Mink JW, Shah BB, et al. GNAO1-associated movement disorder. *Mov Disord Clin Pract* 2016; 3: 615–617.

17. Kulicarni N, Tang S, Bhardwaj R, et al. Progressive movement disorder in brothers carrying a GNAO1 mutation responsive to deep brain stimulation. *J Child Neurol* 2016; 31: 211–214.

18. Waak M, Mohammad SS, Coman D, et al. GNAO1-related movement disorder with life-threatening exacerbations: movement phenomenology and response to DBS. *J Neurol Neurosurg Psychiatry* 2018; 89: 221–222.

19. Monies D, Abouelhoda M, Assoum M, et al. Lessons learned from large-scale, first-tier clinical exome sequencing in a highly consanguineous population. *Am J Hum Genet* 2019; 104: 1182–1201.

20. Retterer K, Juusola J, Cho MT, et al. Clinical application of whole-exome sequencing across clinical indications. *Genet Med* 2016; 18: 696–704.

21. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; 17: 405–424.

22. Muntean BS, Masuho I, Dao M, et al. Gao is a major determinant of cAMP signaling in the pathophysiology of movement disorders. *Cell Rep* 2021; 34: 108718.

23. Takezawa Y, Kikuchi A, Higinoya K, et al. Genomic analysis identifies masqueraders of full-term cerebral palsy. *Am Clin Transl Neurol* 2018; 5: 538–551.

24. Zhu X, Petrovski S, Xie P, et al. Whole-exome sequencing in undiagnosed genetic diseases: interpreting 119 trios. *Genet Med* 2015; 17: 774–781.

25. Larrivee CL, Feng H, Quinn JA, et al. Mice with GNAO1 R209H movement disorder variant display hyperlocomotion alleviated by risperidone. *J Pharmacol Exp Ther* 2020; 373: 24–33.

26. Benato A, Careccchio M, Burlina A, et al. Long-term effect of subthalamic and pallidal deep brain stimulation for status dystonicus in children with methylmalonic acidemia and GNAO1 mutation. *J Neural Transm* 2019; 126: 739–757.

27. Honey CM, Malhotra AK, Tarailo-Graovac M, et al. GNAO1 mutation-induced pediatric dystonic storm rescue with pallidal deep brain stimulation. *J Child Neurol* 2018; 33: 413–416.

28. Yamashita Y, Ogawa T, Ogaki K, et al. Neuroimaging evaluation and successful treatment by using directional deep brain stimulation and levodopa in a patient with GNAO1-associated movement disorder: a case report. *J Neurol Sci* 2020; 411: 116710.

29. Isaias IU, Alterman RL and Tagliati M. Outcome predictors of pallidal stimulation in patients with primary dystonia: the role of disease duration. *Brain* 2008; 131: 1895–1902.

30. Ostrem JL, San Luciano M, Dodenhoff KA, et al. Subthalamic nucleus deep brain stimulation in isolated dystonia: a 3-year follow-up study. *Neurology* 2017; 88: 25–35.

31. Schjerling L, Hjermind LE, Jespersen B, et al. A randomized double-blind crossover trial comparing subthalamic and pallidal deep brain stimulation for dystonia. *J Neurosurg* 2013; 119: 1537–1545.

32. Cao C, Pan Y, Li D, et al. Subthalamicus deep brain stimulation for primary dystonia patients: a long-term follow-up study. *Mov Disord* 2013; 28: 1877–1882.

33. Deng ZD, Li DY, Zhang CC, et al. Long-term follow-up of bilateral subthalamic deep brain stimulation for refractory tardive dystonia. *Parkinsonism Relat Disord* 2017; 41: 58–65.

34. Yao C, Horn S, Li N, et al. Post-operative electrode location and clinical efficacy of subthalamic nucleus deep brain stimulation in Meige syndrome. *Parkinsonism Relat Disord* 2019; 58: 40–45.

35. Liu Y, Zhu G, Jiang Y, et al. Comparison of short-term stimulation of the globus pallidus interna and subthalamic nucleus for treatment of primary dystonia. *World Neurosurg* 2019; 123: e211–e217.