THERAPEUTIC ADVANCES in
Neurological Disorders

Original Research

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

Ther Adv Neurol Disord

2022, Vol. 15: 1–15

DOI: 10.1177/17562864221093507

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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

Received: 29 October 2021; revised manuscript accepted: 24 March 2022.

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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.[3–5] Mutations in the *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).[3,5,6] Patients with *GNAO1*-associated movement disorders are predominantly characterized by various combinations of dystonia, chorea, myoclonus, hypotonia, tremor, and orofacial dyskinesia with or without epileptic seizures.[4] To date, approximately 35 variants in the *GNAO1* gene have been reported.[4,6–12] Treatments with levodopa, topiramate, and tetrabenazine are considered somewhat effective in improving movement dysfunction;[13,14] however, most *GNAO1*-associated movement disorders are refractory to medications.[5,15,16] Deep brain stimulation (DBS) has been established as a safe and effective treatment in patients with inherited dystonia, and patients with *GNAO1* mutations suffering from progressive hyperkinetic movement disorders have shown a beneficial response to globus pallidus internus (GPi) DBS.[7–9,17,18] However, the genotype–phenotype correlations of *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 *de novo 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 *GNAO1* variants and suggests that DBS is a safe and effective option for the symptoms of patients with *GNAO1*-associated movement disorders.

Materials and methods

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.

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, GERP++, and 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 *de novo*.

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

Table 1. Detailed DBS targets, implants, and stimulation parameters.

Case	1		2		3	
DBS targets	Bilateral GPi		Bilateral GPi		Bilateral STN	
Lead kit	Medtronic 3387		PINS		PINS	
IPG models	Activa-PC		G102RZ		G102RZ	
Side	Left	Right	Left	Right	Left	Right
Electrode configuration	C+8–9–	C+1–2–	C+2–	C+6–	C+3–	C+7–
Amplitude (V)	3.70	2.40	3.00	3.00	3.50	3.30
Pulse width (us)	60	60	90	90	90	80
Frequency (Hz)	130	130	140	140	140	140

DBS, deep brain stimulation; GPi, globus pallidus internus; STN, subthalamic nucleus.

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,

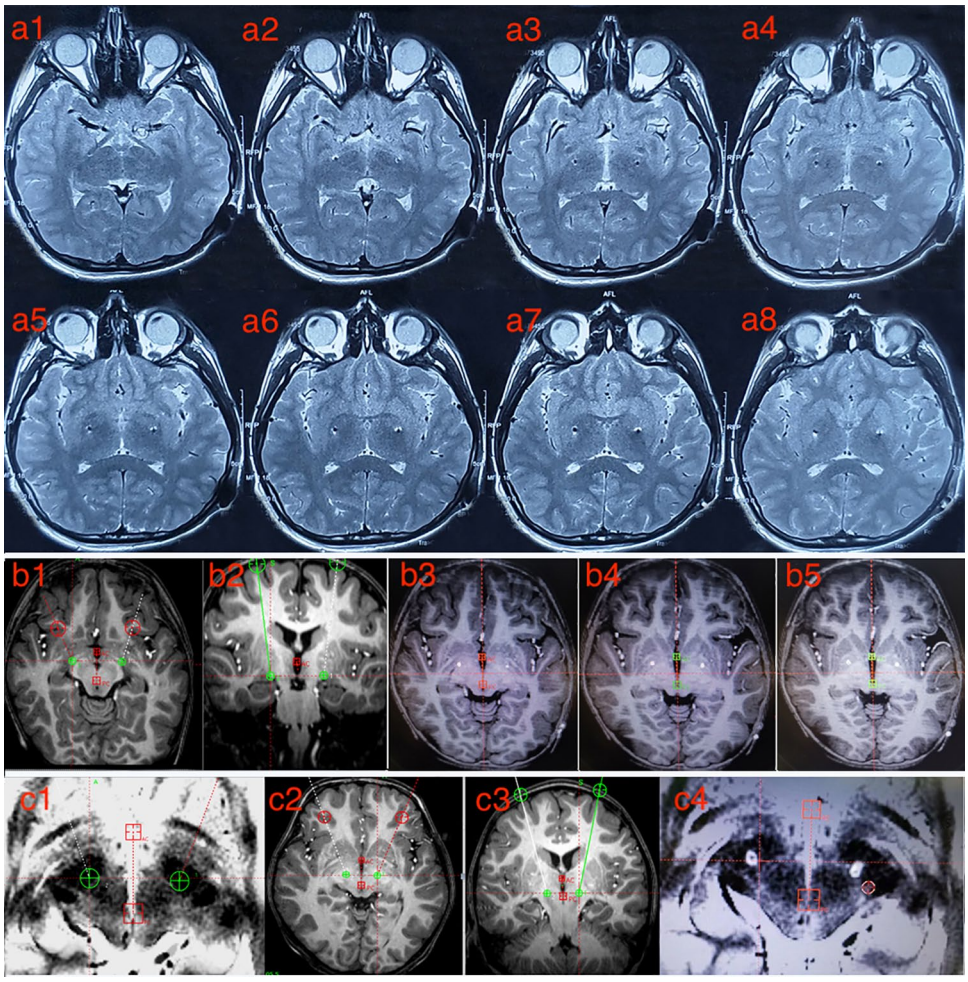

Figure 1. Pre- and post-operative images to confirm the electrode locations: (a1–a8) post-operative MRI (2-mm-layer thickness) for Patient 1 demonstrating the location of electrodes in the GPi targets. (b1 and b2) Presurgical planning of GPi targets for patient 2. (b3–b5) Post-operative computed tomography (CT) fused with pre-operative MRI showing the location of electrodes in the GPi targets. (c1–c3) Presurgical planning of STN targets for Patient 3. (c4) Post-operative CT fused with pre-operative MRI showing the location of electrodes in the STN targets.

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.

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.

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.

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

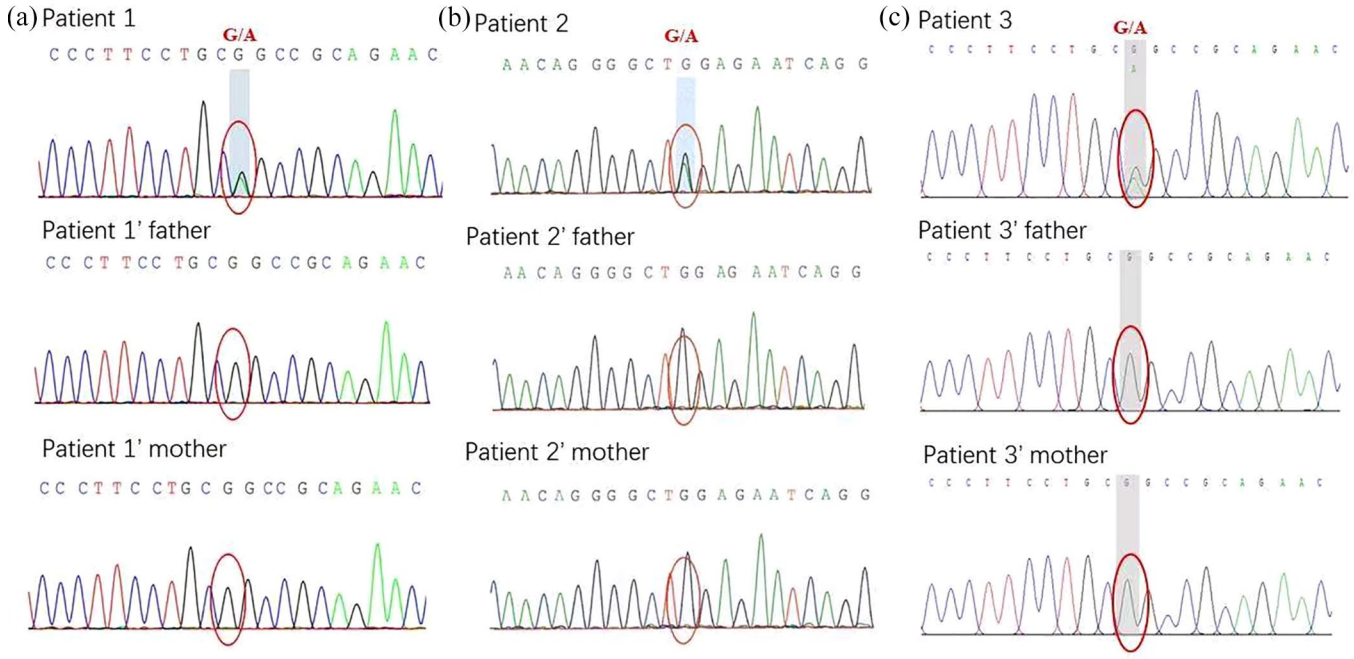

Figure 3. Sequence chromatograms of portions of intron 6 and exon 2 of the *GNAO1* gene from the three affected individuals and their parents in our series: (a) and (c) nucleotide sequence of intron 6 in the *GNAO1* gene. The G-to-A changes in Patients 1 and 3 with a heterozygous state and the wild-type sequence in their healthy parents are shown. (b) Nucleotide sequence of exon 2 in the *GNAO1* gene. A heterozygous G-to-A transversion at nucleotide 124 in Patient 2 is shown, but this heterozygous variant was not detected in her parents.

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

Table 3. Summary of the managements 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	41 after 14 months	52.30 after 4 months	52.30 after 14 months
2	4	77	71 after 14 months	63 after 24 months	7.79 after 14 months	18.18 after 24 months
3	5	62	42 after 5 months	35 after 15 months	32.26 after 5 months	43.55 after 15 months

BFMDRS, Burke–Fahn–Marsden Dystonia Rating Scale; DBS, deep brain stimulation; *GNAO1*, G-protein subunit alpha o1.

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

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.[20] However, there is no specific description of the neurological symptoms caused by this variant in previous reports.[20] 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.[5] 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.[14,25] 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.[5,15,16] In addition, antiepileptics and other agents, such as levodopa, are the standard pharmacological treatments, but they show only partial effectiveness.[5] 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.[7–9,17,18,26–28] The movement disorder phenomenology of these patients mainly incorporates hypotonia, chorea, dystonia, ballismus, and orofaciolingual dyskinesia in various combinations.[6] 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

Table 4. Summary of clinical features and outcomes after DBS surgery of all reported patients with *GNA01*-associated movement disorders, including our Patients 1–3.

Case (ref.)	Gender	Age at onset (years/ months)	Age at surgery (years)	*GNA01* mutation	DBS target	DBS settings after surgery (months)	MD phenomenology	Episodes of status dystonicus	Seizures	Drugs tried	Outcome	Post-surgical complications
1 (Benato *et al.*[26])	F	13 months	5 years	c.736G>A, p. Glu246Lys	Bilateral GPi	Settings: L 4 V, 120 µs, 210 Hz; R 4 V, 120 µs, 210 Hz	Dystonia, chorea, ballismus involving the limbs	Yes	No	Trihexyphenidyl, nitrazepam, clonazepam, tetrabenazine, baclofen, 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.*[26])	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, tetrabenazine, 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, clobazam, clonidine, prednisolone, and oral baclofen, botulinum toxin (local), tetrabenazine, clonazepam, phenobarbitone, 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, lorazepam, clobazam, 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, benxhexol	Functional improvement –improved mobility – GMFSC 5 to 2, improved communication and feeding, weaning of all baseline medications)	NA
6 (Koy *et al.*[8])	F	3 years	9 years	c.723 + 1G>T	Bilateral GPi	NA	3 year/ dyskinesia, during exacerbations: chorea, ballism, dystonia, oro-facio-lingual dyskinesia	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)

Table 4. (Continued)

Case (ref.)	Gender	Age at onset (years/ months)	Age at surgery (years)	*GNA01* mutation	DBS target	DBS settings after surgery (months)	MD phenomenology	Episodes of status dystonicus	Seizures	Drugs tried	Outcome	Post-surgical complications
7 (Koy *et al.*[8])	F	Infant	14 years	c.625 > T, p. Arg209Cys	Bilateral GPi	NA	Infant/generalized progressive dystonia and chorea, exacerbation of dyskinesia at 6 year, status hyperkineticus at 14 year	Yes	Yes	Metopimazine and trihexyphenidyl	BFMDRS-M 114 dropped to 84,5, BFMDRS-D 30 to 27	NA
8 (Koy *et al.*[8])	M	Infant	15 years	c.625 C > T, p. Arg209Cys	Bilateral GPi	NA	Infant/ central hypotonia; 11 year/dystonia and choreoathetosis of the arms, spasticity of all four limbs; 13 year/worsening of hyperkinesia; 15 year/two exacerbations: dystonia, chorea and ballism	Yes	Yes (10 y/ tonic clonic seizures, nocturnal partial complex frontal seizures)	Tetrabenazine	BFMDRS-M 101 dropped to 54 BFMDRS-D 30 to 24	NA
9 (Koy *et al.*[8])	M	Neonate	6 years	c.709G > A, g.56370758G > A p. Glu237Lys	Bilateral GPi	NA	Neonate/axial hypotonia; infant/ permanent dystonia and hyperkinesia; 4 year/ exacerbation: hyperkinetic state with rhabdomyolysis and dehydration	Yes	No	NA	Almost complete remission of hyperkinesia and dystonia at rest, improvement of nonverbal communication, hand function, and mobility	6.5 year reimplantation due to hardware infection
10 (Koy *et al.*[8])	M	8 years	10.5 years	c.709G > A, p. Glu237Lys	Bilateral GPi	NA	8 year/dystonia and dyskinesia; 10 year/exacerbation with hyperkinetic state with rhabdomyolisus	Yes	No	NA	14.8 year the child died due to refractory worsening of the hyperkinesia	Several subsequent lead replacements due to severe deterioration of his clinical condition
11 (Honey *et al.*[27])	M	18 months	10 years	The chr16: 56,370,675 G > T variant p. Arg209Leu, NP_620073	Bilateral GPi	Settings: L 3.0 V, 90 μs, 130 Hz; R 2.8 V, 90 μs, 130 Hz;	Constant severe dyskinetic movements of all four limbs and his mouth (chorea, ballismus, orofacial dyskinesia, and dystonia).	Yes	No	Carbidopa, levodopa, clonazepam, and bromocriptine	The paediatric Barry-Albright dystonia scale decreased from 27/32 to 1/32; no recurrences	NA
12 (Yilmaz *et al.*[9])	M	13 months	5 years	c.698A > C, p. (Q233P)	Bilateral GPi	NA	13 month/ marked hypotonia, insignificant dyskinesia; 2 year/sohnicificant choreoathetosis; 3 year/progressive involuntary movements	Yes	No	Clonazepam, haloperidol, carbamazepine, acetazolamide, and diazepam without any success; intravenous midazolam and fentanyl infusions along with oral pimozide could partially reduce involuntary movements	Fahn-Marsden Dystonia Rating Scale dropped 89.0 to 9.0 at the second month of operation; head control, sitting by support; awareness improved, stared to smile and cry	NA

(Continued)

Table 4. (Continued)

Case (ref.)	Gender	Age at onset (years/ months)	Age at surgery (years)	*GNAO1* mutation	DBS target	DBS settings after surgery (months)	MD phenomenology	Episodes of 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/ hypotonia; 34 month/ choreathetoid 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 year/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.*[7])	M	6 months	7 years	c.737A>G, p. Glu246Gly	Bilateral GPi	NA	6 month/moderate central hypotonia; 2 year/ moderate-severe generalized and orobuccal dystonia, lower limb spasticity; 7 year/ severe exacerbation of hyperkinetic movements	Yes	Generalized tonic-clonic seizures and focal dyscognitive seizures	Tetrabenazine was effecitve 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	Tetrabenazine, trihexyphenidyl, clonazepam, botulinum toxin, pramipexol, and levodopa	The Gross Motor Function Measure improved by 45% (from 15.6% to 60.6%).	None
Present 1[a]	M	2 years	17 years	c.724-8G>A	Bilateral GPi	Settings: L 3.70 V, 60 µs, 130 Hz R 2.40 V, 60 µs, 130 Hz 14 months	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 2[a]	F	6 months	4 years	c.124G>A, p. Gly42Arg	Bilateral GPi	Settings: L 3.0 V, 90 µs, 140 Hz R 3.0 V, 90 µs, 140 Hz 24 months	Exacerbation of dystonia in left limbs, torsion spasm, and spontaneous head tremor	No	No	Medopar and levocarnitine	BFMDRS score from 77 dropped to 63 after 24 months, useful grasping	None
Present 3[a]	M	6 months	5 years	c.724-8G>A	Bilateral STN	Settings: L 3.5 V, 90 µs, 140 Hz R 3.3 V, 80 µs, 140 Hz 15 months	Hypokinetic movements involved both of his upper extremities, and gonocampsis	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.
[a]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.[29] 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 benefitted 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.[30–32] In China, there have been several successful clinical studies about the long-term efficacy of STN DBS in primary dystonia,[32] refractory tardive dystonia,[33] and Meige syndrome.[34] 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.[32,35] 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.[34,35] 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.[35] 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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Acknowledgements

The authors thank the families that participated in this study. They thank Catherine Perfect, MA (Cantab), from Liwen Bianji (Edanz) (www.liwenbianji.cn/), for editing the English text of a draft of this manuscript.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by a grant from the National Natural Science Foundation of China (grant no. 81100879) and a grant from Liaoning Province Natural Science Foundation of China (grant no. 201602847).

Conflict of interest statement

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

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