GNAO1 encephalopathy
Broadening the phenotype and evaluating treatment and outcome

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ABSTRACT

Objective: To describe better the motor phenotype, molecular genetic features, and clinical course of GNAO1-related disease.

Methods: We reviewed clinical information, video recordings, and neuroimaging of a newly identified cohort of 7 patients with de novo missense and splice site GNAO1 mutations, detected by next-generation sequencing techniques.

Results: Patients first presented in early childhood (median age of presentation 10 months, range 0–48 months), with a wide range of clinical symptoms ranging from severe motor and cognitive impairment with marked choreoathetosis, self-injurious behavior, and epileptic encephalopathy to a milder phenotype, featuring moderate developmental delay associated with complex stereotypies, mainly facial dyskinesia and mild epilepsy. Hyperkinetic movements were often exacerbated by specific triggers, such as voluntary movement, intercurrent illnesses, emotion, and high ambient temperature, leading to hospital admissions. Most patients were resistant to drug intervention, although tetrabenazine was effective in partially controlling dyskinesia for 2/7 patients. Emergency deep brain stimulation (DBS) was life saving in 1 patient, resulting in immediate clinical benefit with complete cessation of violent hyperkinetic movements. Five patients had well-controlled epilepsy and 1 had drug-resistant seizures. Structural brain abnormalities, including mild cerebral atrophy and corpus callosum dysgenesis, were evident in 5 patients. One patient had a diffuse astrocytoma (WHO grade II), surgically removed at age 16.

Conclusions: Our findings support the causative role of GNAO1 mutations in an expanded spectrum of early-onset epilepsy and movement disorders, frequently exacerbated by specific triggers and at times associated with self-injurious behavior. Tetrabenazine and DBS were the most useful treatments for dyskinesia. *Neurol Genet* **2017;3:e143; doi: 10.1212/NXG.0000000000000143**

GLOSSARY

CK = creatine kinase; **DBS** = deep brain stimulation; **EOEE** = early-onset epileptic encephalopathy; **EVS** = Exome Variant Server; **ExAC** = Exome Aggregation Consortium; **GATK** = Genome Analysis Toolkit; **GPCR** = G protein–coupled receptor; **WES** = whole-exome sequencing; **WGS** = whole-genome sequencing.

De novo GNAO1 mutations (MIM 139311) were initially identified in children with early-onset epileptic encephalopathy (EOEE) and severe developmental delay,[1–3] with later development of dyskinetic movement disorders[1,4–6] and in children exhibiting developmental delay and severe dyskinesia without seizures.[7,8]

GNAO1 encodes a subclass (Gαo) of the Gα subunit of heterotrimeric guanine nucleotide-binding proteins and is highly expressed in the brain and involved in the regulation of neuronal excitability and neurotransmission. Mutations in *GNAO1* and other G-protein subunits (*GNAL*), adenylyl cyclase (*ADCY5*), cyclic nucleotide phosphodiesterase (*PDE10A*), and G protein–coupled receptor (GPCR) (*GPR88*)[9] in early-onset movement disorders, support the notion that disruption of the G-protein-cAMP pathway axis is a key contributor to the pathophysiology of dystonia and chorea. Although the pathophysiologic mechanisms underlying such genetic movement disorders remain yet to be elucidated, impaired modulation or transduction of transmembrane signaling, presynaptic autoinhibitory effects, and altered neuronal excitability are all putative disease mechanisms that might explain the co-occurrence of multiple neurologic manifestations such as epilepsy and hyperkinesia.

Here, we report 7 newly identified patients with *GNAO1* mutations, with the aim of characterizing in detail the clinical phenotype, response to treatment and outcome, as well as associated brain MRI features.

METHODS Molecular genetic testing. Patients 1–3, 6, and 7 were studied using whole-exome/genome (WES/WGS) sequencing. For patient 1, WES was performed on a SOLiD 5500XL sequencing platform (Life Technologies, Foster City, CA). Short reads were mapped against the GRCh37/hg19 human assembly by means of LifeScope software (Life Technologies). Variants were detected using the Haplotype Caller software package of the Genome Analysis Toolkit (GATK) suite and filtered to include only variants covered by at least 10 reads with mapping quality values exceeding 30. For patients 2, 3, and 6, WGS was performed with Illumina TruSeq DNA PCR-Free Sample Preparation kit on an Illumina Hiseq 2500 sequencing platform (Illumina Inc., San Diego, CA). For patient 3, WGS was performed as part of the National Institute for Health Research Bioresource Rare Disease Project. A minimum coverage of 15X (95% of the genome) was obtained, with an average coverage of ~30X. Reads were aligned using Isaac aligner (version 01.14) (Illumina Inc., Great Chesterford, UK) and mapped against the GRCh37 hg19 human assembly. Single nucleotide variants and insertions/deletions were identified using Isaac variant caller. Patients 4 and 5 were studied using a Haloplex panel (Agilent Technologies, Santa Clara, CA) of 95 epilepsy genes. Variants were called and annotated using the GATK[10] toolkit and the ANNOVAR tool.[11] Patient 7 was analyzed by WES using Nextera Rapid Capture Exome, Illumina NextSeq platform (Illumina Inc.) with an average coverage depth of 100–130X. An end-to-end in-house bioinformatics pipeline including base calling, primary filtering of low-quality reads and probable artefacts, and annotation of variants was applied.

For all patients, variants reported as validated polymorphisms with a minor allele frequency >0.01 (1%) in publicly available human variation resources (including dbSNP144, NHLBI GO Exome Sequencing Project, UK10, 1000 Genomes, Exome Variant Server [EVS], and Exome Aggregation Consortium [ExAC] browser) and variants present in in-house control individuals were filtered out. In silico prediction of the mutation pathogenicity was performed using ANNOVAR and the dbNSFP database (v3.0a) (sites.google.com/site/jpopgen/dbNSFP). Putative causative variants were validated by Sanger sequencing and investigated in the probands' parents to determine whether the mutations were inherited or had occurred de novo.

Standard protocol approvals, registrations, and patient consents. Written informed consent to disclose clinical information, neuroimaging, and video footage was obtained from all parents/guardians of the 7 participants. Informed consent for genetic testing was obtained according to the local institutional policy.

RESULTS Genetic analysis. We identified 7 patients carrying 6 different *GNAO1* (GenBank accession number NM_020988) mutations; all the variants occurred de novo and 4 are previously unreported (table, figure 1).

In patient 1, we detected the novel c.139A>G [p.(Ser47Gly)] missense variant, located in the N-terminal domain of the protein. In patients 2 and 4, we identified the c.625C>T [p.(Arg209Cys)] missense variant. This variant has previously been reported in 1 patient with developmental delay, intellectual disability, and severe chorea, associated with the later onset of complex partial seizures.[5] In patient 3, we observed the novel c.723+1G>A intronic variant, and in patient 5, the novel c.167T>C [p.(Ile56Thr)] missense variant, located in the N-terminal domain of the protein. In patient 6, we detected the c.118G>C [p.(Gly40Arg)] missense mutation, located in the N-terminal domain of the protein. A different mutation leading to the same amino acid change (c.118G>A [p.(Gly40Arg)]) has previously been described in a patient with infantile-onset epilepsy.[3] In patient 7, we identified the novel c.737A>G [p.(Glu246Gly)] missense variant. A different nucleotide change in the same amino acid position, c.736G>A [p.(Glu246Lys)], had been reported as disease causing in patients exhibiting developmental delay, intellectual disability, and a paroxysmal movement disorder.[5,8]
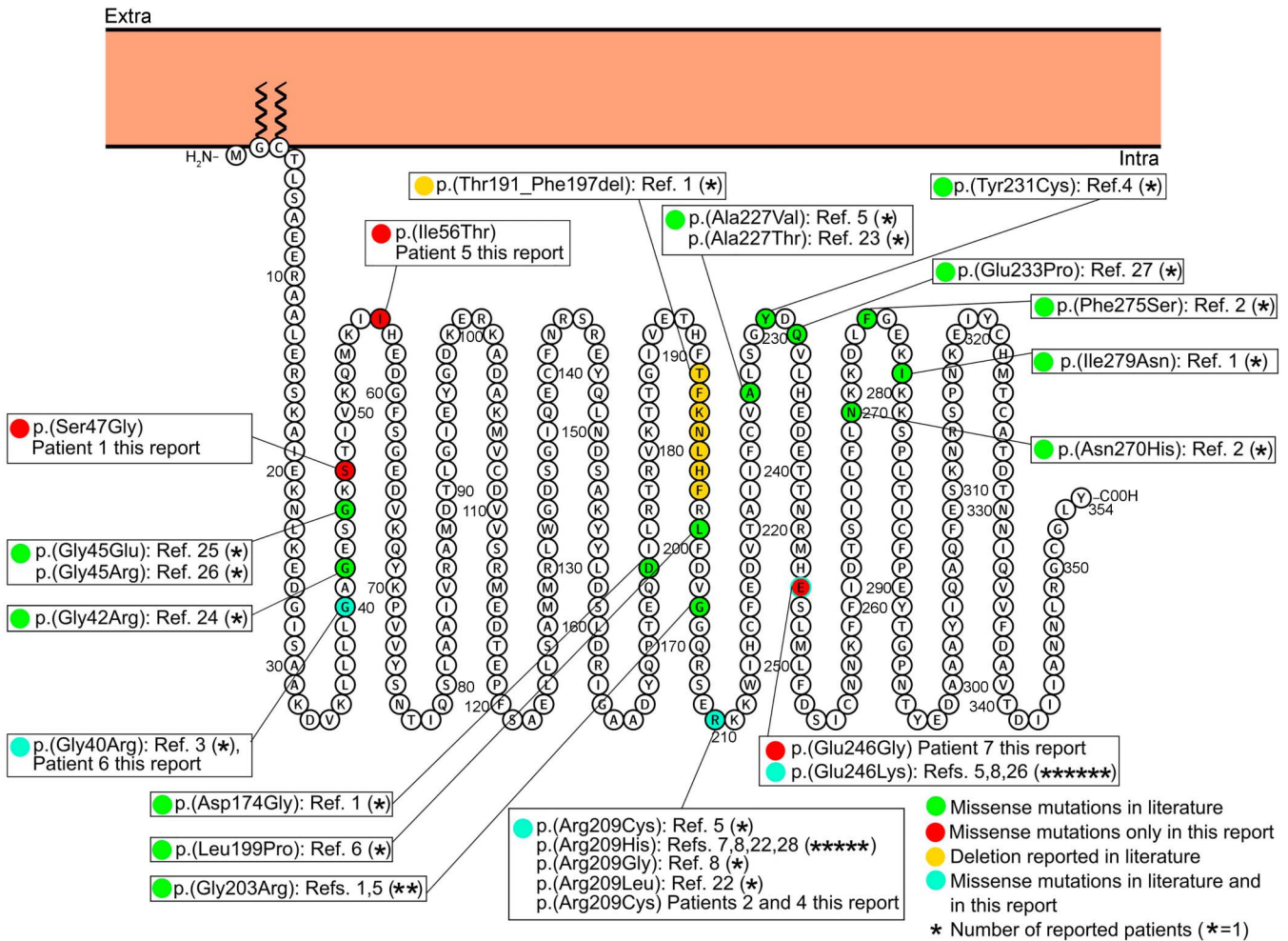
The 5 missense variants are predicted to be deleterious using the dbNSFP database and are not reported in the 1000 Genomes, ExAC, and EVS databases. The splicing mutation c.723+1G>A was predicted to cause the loss of a splice donor site

Table — Clinical features and genetic laboratory findings

Patient/sex	1/M	2/M	3/F	4/M	5/F	6/F	7/M
Age at first medical evaluation/follow-up	5 mo/5 y 5 mo	Birth/10 y 8 mo	4 mo/deceased at 4 y	5 mo/5 y 6 mo	4 y/16 y	2 mo/5 y 2 mo	6 mo/15 y
GNAO1 mutation (de novo)	c.139A>G [p.(Ser47Gly)]	c.625C>T [p.(Arg209Cys)]	c.723+1G>A	c.625C>T [p.(Arg209Cys)]	c.167T>C [p.(Ile56Thr)]	c.118G>C [p.(Gly40Arg)]	c.737A>G [p.(Glu246Gly)]
Novel/reported	Novel	Ref. 5	Novel	Ref. 5	Novel	Ref. 3	Novel
Clinical findings at follow-up	Severe ID/DD, hypotonia, movement disorder, seizures	Severe ID/DD, hypotonia, movement disorder, seizures, MC	Severe ID/DD, hypotonia, movement disorder, MC	Severe ID, focal epilepsy, movement disorder, MC	Mild ID, focal epilepsy, movement disorder	Severe ID, focal epilepsy, movement disorder, MC	Moderate-severe appendicular/orobuccal dystonia; moderate lower limb spasticity
Onset/type of abnormal movements	4 mo/severe hypotonia, mild dystonia; 2 y 3 mo/seHypM, dystonia; stereotypic hand movements; SIB (lips biting, hair pulling)	Birth/severe hypotonia; 9 y/seHypM; SIB (lips biting)	8 mo/continuous generalized choreoathetosis; orolingual dyskinesia	7 mo/complex motor stereotypies of hands/arms; dyskinetic motor pattern	5 y/subcontinuous dyskinetic tongue movements; mild upper limb dyskinesia	3 y/hand-to-mouth complex stereotypies, rocking, pelvic thrusting	6 mo/moderate hypotonia; 2 y/moderate-severe generalized and orobuccal dystonia, lower limb spasticity; 7 y/seHypM
Dysphagia	Yes	Yes	Yes	No	No	Yes, gastrostomy	Yes (progressive), gastrostomy
Head control	1 y 6 mo then regression	Yes	Yes	Yes	Yes	Yes	Yes
Sitting	No	9 mo then regression	No	Yes	Yes	No	Yes, with support
Language	No	No	Limited	No	Yes	No	Yes, with dysarthria
Onset/epilepsy/severity	3 y/FS with secondary generalization/well controlled on AED	6 y/GTCS/well controlled on AED	No	2 y/FS/well controlled on AED	4 y/FS/well controlled on AED	2 mo/EOEE (OS-FS soon followed by spasms, then FS and GTCS)/multiple AEDs, ketogenic diet, VNS	10 y/rare FS and GTCS/no AEDs
Interictal EEG	Right frontotemporal spikes	Slow background activity	NA	Bilateral centrotemporal spikes	Left frontotemporal spikes	Burst suppression at onset; slow background, multifocal spike waves	Normal
MRI	VE; thin and dysmorphic corpus callosum; mild HyCN	VE; thin corpus callosum; mild HyCN, hypoplasia of inferior vermis	VE and dilated subarachnoid spaces; moderate cortical atrophy; dysmorphic corpus callosum; mild HyCN	Normal	Left frontal lesion (diffuse astrocytoma WHO grade 2); low-lying cerebellar tonsils	Mild VE; thin corpus callosum	Mild loss of volume (atrophy) in generalized distribution
Neurotransmitter analysis in CSF	Methyltetrahydrofolate 34 nmol/L; 63–111	Methyltetrahydrofolate 66 nmol/L; 72–172	Normal	Normal	Normal	Normal	Normal

Abbreviations: AED = antiepileptic drugs; DD = developmental delay; EOEE = early-onset epileptic encephalopathy; FS = focal seizures; GTCS = generalized tonic-clonic seizures; HyCN = hypoplasia of caudate nuclei; ID = intellectual disability; MC = microcephaly; NA = not available; nv = normal values; OS = Ohtahara syndrome; seHypM = severe exacerbation of hyperkinetic movements; SIB = self-injurious behavior; VE = ventricular enlargement; VNS = vagus nerve stimulation.

Figure 1   Membrane topology modeling of *GNAO1*

Membrane topology was predicted using the Protter online tool[29] (P09471, GNAO_HUMAN). Known *GNAO1* missense mutations are indicated in green, the deletion is in yellow. Mutations found in this report and already described in the literature are indicated in light blue, novel mutations in red.

using Mutation Taster and Berkeley Drosophila Genome Project.
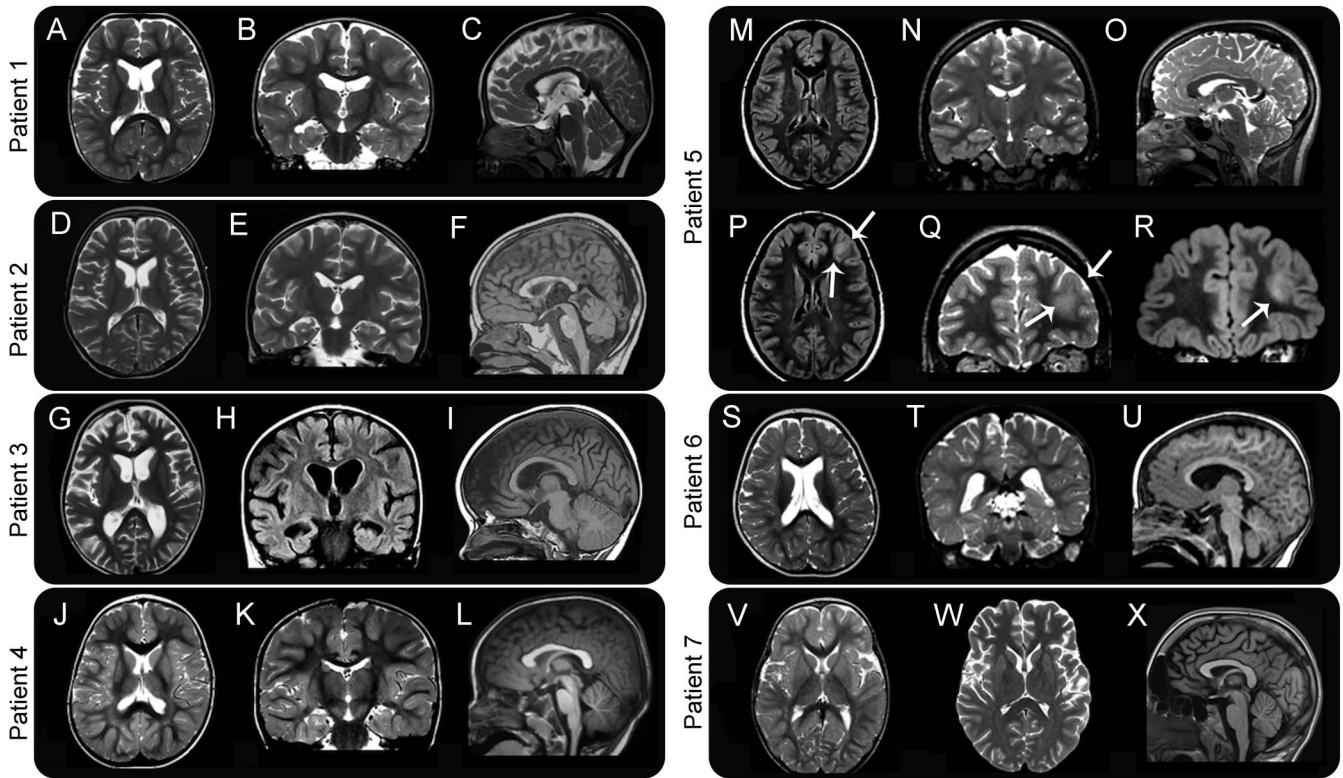
Clinical features and neurologic investigations. Clinical features, as obtained by history taking, direct clinical examination, and evaluation of video footage, neuroimaging, and genetic findings are detailed in the table, in figure 2 and in videos 1–5 at Neurology.org/ng. Within the cohort, we identified 4 boys and 3 girls. Six of the patients are alive, currently aged 5–16 years.

The onset of abnormal hyperkinetic movements ranged from 4 months to 9 years of age (median 2 years). Within the cohort, there was considerable clinical heterogeneity with regard to motor semiology and severity. Profound hypotonia preceded the onset of abnormal movements in patients 1, 2, and 7, who later developed dystonia, followed by severe episodes of paroxysmal choreoathetosis. Gradual development of facial dyskinesia was also reported in these patients

(videos 1, 2, and 5). Patient 3 experienced continuous generalized choreoathetosis and facial dyskinesia. At the age of 4, because of repeated and prolonged infective episodes, status dystonicus developed, requiring intensive care, with sedation, ventilation, tracheostomy, gastrostomy, and metabolic support. MRI showed progressive cerebral atrophy. Status dystonicus persisted, with rise in creatine kinase (CK) and renal failure, leading to multisystemic deterioration and death. Patient 4 exhibited complex motor stereotypies and a dyskinetic motor pattern (video 3). Patient 5 manifested almost continuous orolingual dyskinesia. She also had mild dyskinetic movements of the upper limbs (video 4). Patient 6 exhibited complex rocking, staring, back arching, and pelvic thrusting movements, associated with hand-to-mouth complex stereotypies.

Several triggers were reported to elicit or exacerbate abnormal movements, including emotion (all patients), intercurrent illnesses (patients 1, 2, and 7),

Figure 2 MRI characteristics of 7 patients with *GNAO1* mutations

For patients 1–6, a set of 3 images including an axial (A, D, G, J, M, and S), coronal (B, E, H, K, N, and T), and sagittal midline (C, F, I, L, O, and U) sequences is shown. For patient 5, in addition, 1 axial (P) and 2 coronal (Q and R) images are shown to illustrate better the neoplastic lesion in the left frontal lobe (arrows). For patient 7, axial images of 2 different investigations (V and W) and a sagittal image are shown to illustrate better the progression of atrophic changes. Images are at 1.5–3T. Images A–E, G, J, K, N, O, Q, S, T, and W are T2 weighted. Images F, I, L, U, and X are T1 weighted. Images H, M, P, and R are acquired as fluid attenuation inversion recovery (FLAIR) sequences. Patient 1: axial and coronal images show mild ventricular enlargement in the frontal horns, with concomitant hypoplasia of the caudate nuclei. The sagittal section shows a thin corpus callosum. Patient 2: axial and coronal images show mild ventricular enlargement in the frontal horns, with mild hypoplasia of the caudate nuclei, likewise seen in patient 1. The sagittal section shows a thin corpus callosum and a hypoplastic inferior vermis. Patient 3: axial and coronal images show moderate cortical atrophy, with ventricular enlargement and small caudate nuclei. The sagittal image shows a dysmorphic corpus callosum. Patient 4: no structural abnormality is visible on MRI. Patient 5: MRI shows an isolated abnormality (arrows) involving the anterolateral aspect of the left frontal lobe, including the cortex and underlying white matter, with increased signal intensity on both T2 and FLAIR images. Histopathology, after surgical removal of the lesion, showed characteristics consistent with a diffuse astrocytoma (WHO grade 2). Patient 6: axial and coronal images show dilated lateral ventricles; the sagittal cut shows a thin corpus callosum. Patient 7: the first axial MRI at age 2 (V) is normal, but a follow-up study at age 16 (W) shows loss of brain volume in generalized distribution. No abnormalities in the brainstem and cerebellum are visible in the sagittal image at age 16 (X).

high temperature (patient 1), intention, and purposeful movements (patients 1 and 7). Attacks frequently presented in clusters, lasting minutes (patient 1), hours or weeks (patient 2), or months (patient 7) (videos 1, 2, and 5). Such exacerbations were pharmacoresistant to standard therapies. Patients 1, 2, and 7 also exhibited associated autonomic instability, sweating, dehydration, and rise in CK, frequently requiring hospitalization and intensive care and, often, administration of anesthetic agents (patients 2 and 7). Worsening of baseline dystonia with hyperkinetic movements and loss of fine motor skills were reported in patient 7 after each episode. One such exacerbation determined a 3-month admission to the intensive care unit and anesthetic agents. The emergency placement of a deep brain stimulator (DBS) device bilaterally into the globus pallidus interna was transformative and resulted in almost complete remission of the pronounced hyperkinesia, although residual generalized dystonia persisted.

Six patients (1, 2, 4–7) exhibited epilepsy, with age of seizure onset ranging from 2 months to 10 years. While seizures were well controlled by medication in 4 patients, patient 7 presented rare generalized tonic-clonic seizures and focal dyscognitive seizures and patient 6 had a history of EOEE followed by drug-resistant focal epilepsy with dyscognitive seizures. Patient 3 never had seizures and died at age 4 years.

Additional clinical features were also reported in some patients. Patients 1 and 2 exhibited mild self-injurious behavior (lip biting, hair pulling). Patient 7 has a history of significant anxiety, requiring treatment with fluoxetine, sertraline, and risperidone.

In our cohort, diagnostic metabolic investigations were unremarkable, including CSF neurotransmitters, except for the reduction of CSF 5-methyltetrahydrofolate levels in patients 1 and 2. In both patients, calcium folinate administration was started but did not lead to discernible clinical improvement. Brain MRI (figure 2) was unremarkable in patients 4 and 7, while inconsistent changes were detected in the remaining patients (microcephaly, macrocephaly, progressive cerebral atrophy with dilated ventricles and subarachnoid spaces, caudate volume loss, and dysmorphic corpus callosum). In 1 patient (patient 5), a focal lesion involving the gray and white matter of the left frontal lobe was identified and confirmed as a diffuse astrocytoma (WHO grade 2) after surgical removal.

DISCUSSION Through its activation by GPCRs, Gαo is involved as a modulator or transducer in several transmembrane signaling pathways.[10,12,13] Gαo also mediates the widespread presynaptic auto-inhibitory effect of many neurotransmitters (via α2 adrenoreceptors, M2/M4 muscarinic, μ/δ opioid, GABAB, adenosine A1, or endocannabinoid CB1 receptors) through a reduction of sensitivity to membrane depolarization and a direct inhibitory effect on the vesicle fusion process.[14] Furthermore, Gαo indirectly activates G protein–coupled inwardly rectifying K+ channels that mediate neuronal excitability through a slower self-inhibitory postsynaptic potential.[15]

Gαo-deficient mice (Gnao1−/−) have occasional seizures, severe impairment of motor-control, hyperalgesia, and behavioral abnormalities with early postnatal lethality.[16,17] Although heterozygous Gnao1 knockout mice do not manifest seizures, a gain-of-function knock-in mutant murine model (Gnao1 Gly184Ser/+) has severe seizures with markedly increased frequency of interictal epileptiform discharges and sudden premature death. Animal model data thus suggest that pathogenic monoallelic *GNAO1* mutations in humans may also result in a gain-of-function effect. Moreover, impaired protein localization and decreased *GNAO1*-mediated inhibition of calcium currents by norepinephrine compared to the wild type have been showed in in vitro functional expression systems.[18] *GNAO1* has also been implicated in the etiology of brain tumors, such as ependymoma and glioblastoma multiforme.[19,20]

De novo *GNAO1* mutations were originally first reported in Ohtahara syndrome and EOEE in 4 children, associated with abnormal movements in 2.[1] Since this initial description, 30 additional patients have been reported with an emerging phenotype characterized by neurodevelopmental delay with an early onset of a hyperkinetic movement disorder, inconsistently associated with epilepsy. The epilepsy phenotype of the 7 patients we studied ranged from EOEE to mild drug-sensitive epilepsy, whose onset could precede or follow that of the movement disorder, which was certainly a prominent feature. The occurrence of stereotypies (previously reported in 2 patients with EOEE)[4,5] and characteristic paroxysmal exacerbations, associated often with clear triggers, may both be considered as 2 further important discerning clinical features of *GNAO1* encephalopathy.

As previously reported, standard investigations appear to be unyielding in patients with *GNAO1*-related disease. Two of our patients had low levels of CSF 5-methyltetrahydrofolate, although this was not consistently seen in the cohort. Brain MRI (figure 2) showed a combination of minor features in most, including a thin corpus callosum with dilated ventricles in 4, as previously reported[8] as well as hypoplastic caudate nuclei in 3 and a diffuse astrocytoma (WHO grade II) in the oldest patient (patient 5). The latter finding raises concerns about the possible role of the Gly40Arg mutation in tumorigenesis, in view of the reported role of *GNAO1* in promoting oncogenic transformation.[19,21] A somatic *GNAO1* mutation (p.Arg243Hys) has been identified in breast carcinomas where it promotes oncogenic transformation by rendering the Gα subunit constitutively activated and enhancing signaling pathways responsible for neoplastic transformation.[21] Although *GNAO1* was identified as part of the human plasma proteome, its high abundance was suggested to promote cancer cell viability via proapoptotic protein interference.[20] Larger series and longer follow-up data will be fundamental to determine whether patients carrying specific *GNAO1* mutations are at higher risk of developing tumors.

Twenty *GNAO1* mutations, 19 missense and 1 deletion, have been previously reported (figure 1).[1–8,22–28] The only reported deletion was described in a patient with a severe phenotype, including Ohtahara syndrome, developmental delay, and severe intellectual disability, who died secondary to respiratory-tract complications.[1] The 7 patients we are reporting harbored 6 different mutations, one of which was evident in 2 patients (figure 1). Two of the 6 mutations have been reported previously [p.(Arg209Cys) and p.(Gly40Arg)],[3,5] while 4 are novel [p.(Ser47Gly), c.723+1G>A, p.(Ile56Thr), and p.(Glu246Gly)]. The c.723+1G>A splicing mutation, likely resulting in abnormal mRNA splicing, was found in patient 3, who died at 4 years of age and exhibited the most severe phenotype. The p.(Arg209Cys) mutation falls within the switch II domain, which is important for guanidine nucleotide-dependent regulation of downstream effectors and is highly

conserved across vertebrate species.[5] The Arg209 amino acidic residue represents a mutation hotspot since, including our series, 10 patients carrying mutations affecting this residue have been reported. All these patients presented developmental delay and chorea or dystonia and 3 had seizures. The p.(Glu246Gly) mutation, although novel, affects an amino acid residue that represents a second mutation hotspot since 7 patients with mutations affecting this residue have been reported (including our own), all presenting with developmental delay and chorea, with only patient 7 developing late childhood–onset seizures. The Arg209 and Glu246 residues form a salt bridge that is important for the stabilization of the Gα-containing complexes, mainly in GTP-bound active state.[5] Hence, the variants involving these residues should disrupt their interaction, resulting in destabilization of the Gα-containing complexes.[5] In our study, the identification of 2 additional patients harboring mutations involving the Arg209 amino acid residue and 1 additional patient harboring a mutation involving the Glu246 amino acid residue confirm that these residues are *GNAO1* mutation hotspots.

Tetrabenazine was the most effective drug in the baseline management of the severe involuntary movements in patients 1 and 7; its positive effect was previously described in 6 patients[8] treated in association with neuroleptics (risperidone and haloperidol). In addition, a recently reported patient harboring the c.626G>A (p.Arg209His) exhibited an initial significant improvement with tetrabenazine and subsequent response to trihexyphenidyl.[28] Emergency DBS, during a severe prolonged exacerbation, was life saving in patient 7. Three patients with *GNAO1* mutations had previously been reported whose hyperkinetic exacerbations, usually preceded by illness, clearly improved after DBS insertion into the globus pallidus.[7,27] Our report provides further evidence that DBS should be promptly considered in all the patients with sustained intractable movement disorder due to *GNAO1* mutations.

Long-term outcome of early-onset *GNAO1*-related disease remains yet to be determined. We would suggest that prognosis of these patients should remain guarded, as 3 previously reported patients[1,8] and 1 in this series have died in childhood. Furthermore, 2 reported patients have experienced definite motor regression in the early stages of disease.[7,8] Identification of more patients will certainly aid delineating this newly identified disorder. Given the ever-growing disease spectrum, *GNAO1* mutations should be considered in the differential diagnosis for patients with unexplained paroxysmal/nonparoxysmal early-onset hyperkinetic movement disorders, especially in the context of neurodevelopmental delay with or without epilepsy.

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