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GNAO1-associated epileptic encephalopathy and movement disorders: c.607G>A variant represents a probable mutation hotspot with a distinct phenotype

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ABSTRACT – We describe a case of GNAO1-associated epilepsy and chorea in a patient with a *de novo* pathogenic mutation. This patient is unique in being the first reported male with this phenotype, and we propose that this genetic variant may represent a mutation hotspot that characterizes a unique phenotype. This 5.2-years-old boy presented with seizures, chorea, and severe global developmental delay. Brain imaging showed progressive diffuse cerebral atrophy. EEG monitoring revealed multifocal and diffuse discharges, along with generalized-onset seizures. Genetic testing found a *de novo* pathogenic variant in the *GNAO1* gene (c.607G>A; p.Gly203Arg). A review of the literature showed two other patients with similar phenotype and the same genetic variant. In contrast, other patients with neurological involvement had private mutations in the *GNAO1* gene. The neurological phenotypes associated with *GNAO1* mutations appear to lie on a spectrum, and it is possible that the c.607G>A (p.Gly203Arg) variant characterizes a phenotype with both severe epilepsy and chorea. [*Published with video sequence on www.epilepticdisorders.com*]

Key words: GNAO1, epileptic encephalopathy, epilepsy genetics, drug-resistant epilepsy, chorea

VIDEO ONLINE

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Massively parallel gene sequencing for comprehensive disease-specific panels and whole-exome sequencing are increasingly contributing to the diagnosis and improved understanding of the biology of epilepsies (Ottman *et al.*, 2010). In 2013, Nakamura *et al.* tested 367 individuals and demonstrated pathological consequences of variants in the *GNAO1* gene in four of them (Nakamura *et al.*, 2013). The *GNAO1* gene, which encodes the $G\alpha_o$ subunit of guanine-binding proteins (G proteins), was reported to be associated with epileptic encephalopathy. GNAO1-associated neurological phenotypes remain insufficiently characterized and have been reported to include epileptic encephalopathy (*n*=5), epileptic encephalopathy with movement disorder (*n*=6), and only movement disorders (*n*=3) (Nakamura *et al.*, 2013; Euro *et al.*, 2014; Law *et al.*, 2015; Talvik *et al.*, 2015; Kulkarni *et al.*, 2016; Marce-Grau *et al.*, 2016; Saitsu *et al.*, 2016).

We describe a patient with GNAO1-associated epilepsy and chorea who is unique in being the first male with this phenotype. Additionally, we propose that the particular genetic variant (c.607G>A, p.Gly203Arg) found in our patient probably represents a mutation hotspot and characterizes a distinct phenotype.

Case study

This 5.2-year-old boy presented with seizures, abnormal movements, and developmental delay. Seizures started at around 1 month of age and consisted of behavioural arrest and staring, lasting for a few seconds, and increased drooling, above baseline. At around 1 year of age, his seizure semiology changed to eyes being wide open, unresponsiveness, facial suffusion, and a drop in SpO_2 detected by home oximetry. He also developed episodes of abnormal movements at around 5 months of age, consisting of kicking and/or thrashing movements of the arms and legs which flowed from one body part to another, sometimes associated with heavy breathing (*see video sequence*). The episodes typically lasted 1.5-2 hours and occurred daily. Parents distinguished these episodes from seizures based on a longer duration and no decrease in SpO_2.

He was delivered by emergency C-section at 37 weeks of gestation done for poor progress of labour, but did not have any problems at birth. His development had always been globally delayed. He had never been able to lift his head while supine, though at around 3-4 months age, he might reach out for toys if they were brought within his visual field and could partially track the objects horizontally. Also, before the age of 3 months, he was able to swallow liquids and semi-solids, to some extent. Subsequently, his swallowing progressively worsened which necessitated placement of a feeding tube. He was diagnosed with choreo-athetoid cerebral palsy elsewhere. He is the second child born to non-consanguineous parents of African descent. His older sibling is healthy and the family history generally unremarkable. According to the parents, his seizures were acceptably controlled for a year on zonisamide, clobazam, and ketogenic diet, having failed four other medications in the past. He was also receiving clonazepam for his movement disorder.

Examination revealed a boy of thin build in a wheelchair who did not vocalize or visually fixate, though he did seem to orient to his mother's voice. A bilateral horizontal nystagmus at extreme abduction, with a fast component towards the side of

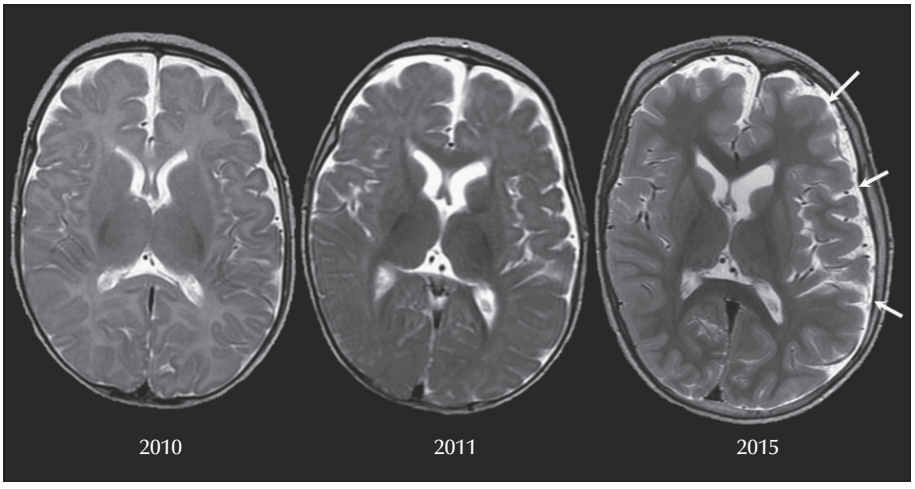

Figure 1. Serial brain magnetic resonance imaging performed over four years demonstrating interval progressive volume loss involving cerebral hemispheres (more prominent in the left hemisphere). There was also volume loss in the cerebellum (more pronounced on the left), minimal non-specific T2/FLAIR hyper-intense foci in the white matter, and normal spectroscopy (not shown).

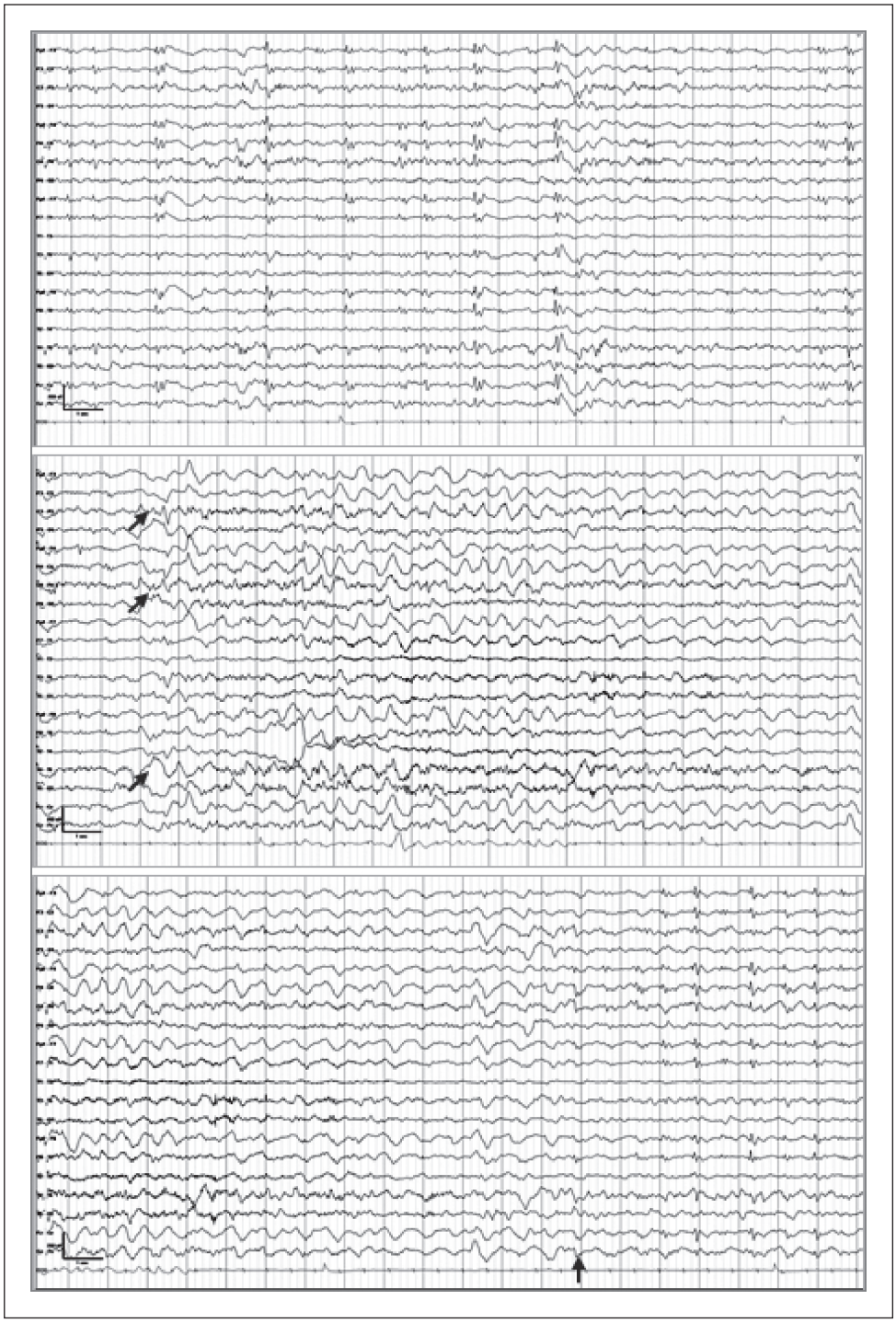

Figure 2. EEG (AP bipolar montage; LFF: 1 Hz; HFF: 70 Hz; sensitivity: 10 μV) epochs showing interictal (upper panel) and ictal (middle and lower panel) findings. Interictal epileptiform discharges can be seen asynchronously in the left frontal, left anterior temporal, right frontal, and right anterior temporal head regions, as well as diffusely bilaterally. Low-amplitude paroxysmal fast activity can also be seen in the right posterior temporal head region. Ictal onset (middle panel; arrows) was characterized by a diffuse sharp wave with central/parietal amplitude emphasis, followed by diffuse attenuation and superimposed low-amplitude fast activity, followed by 2-3 Hz sharply contoured polymorphic delta activity in a similar distribution. The seizure ends abruptly after 22 seconds (lower panel; arrow) with return of the interictal pattern. Clinically, the patient opened his eyes, had tonic extension of all four extremities (predominantly the legs), and a few clonic jerks of the arms towards the end of the seizure.

abduction, was noted on spontaneous eye movement. Features of pseudo-bulbar palsy (stiff tongue, brisk jaw, and gag reflexes) were also noted. Muscle bulk and tone were diffusely decreased, though spontaneous anti-gravity movements in all extremities were noted. He also had dystonia of both upper extremities and perioral dyskinesia.

Serial brain imaging performed at four-year interval showed progressive diffuse atrophy involving the cerebral hemispheres (more prominent in the left hemisphere) and cerebellum, non-specific T2/FLAIR hyperintense foci in white matter, and normal spectroscopy (*figure 1*). EEGs performed at the time of onset of initial seizures were reported to show multifocal interictal discharges. EEG monitoring at the time of presentation also showed a slow background and bilateral multi-focal interictal epileptiform discharges. Also, multiple daily seizures were identified, occurring predominantly during sleep, with eye opening, tonic stiffening of all four limbs (sometimes asymmetric), and semi-rhythmic jerking of arms (left>right). Ictal onset was characterized by generalized 1.5-2-Hz discharges with frontal amplitude emphasis. The seizures were brief, lasting 20-25 seconds, and were unrecognized by parents (*figure 2*). Metabolic testing, including serum lactate, pyruvate, amino acids; urine organic acids; CSF biochemistry, and neurotransmitter profile were unremarkable. A phenotype-driven gene panel utilizing a whole-exome platform was performed with specific sub-panels chosen based on specific clinical features including seizures, encephalopathy, and movement disorder. Detailed clinical information about the proband and parental samples were used for variant filtering. A heterozygous *de novo* mutation in the *GNAO1* gene (c.607G>A; p.Gly203Arg; NM_020988.2; rs587777057; Chr16: 56336744 [on Assembly GRCh38]) was found and classified as pathogenic.

Discussion

Pathogenic variants in *GNAO1* were recognised as a cause of epileptic encephalopathy based on testing of a large cohort of individuals with epilepsy and structural modelling to demonstrate molecular consequences of genetic variants (Nakamura *et al.*, 2013). Based on available reports, it is possible to classify the clinical presentations into three somewhat overlapping phenotypes with considerable genetic heterogeneity (*table 1*). These phenotypes include: epileptic encephalopathy (typically early infantile onset with suppression-burst pattern on EEG), drug-resistant epilepsy (with/without epileptic encephalopathy) with movement disorders (chorea, athetosis, dystonia, stereotypies), and chorea/athetosis without seizures.

Although our patient is the first male with GNAO1-associated epileptic encephalopathy and chorea, given the small number of patients reported with this phenotype it is unclear if there is a female preponderance or merely a sampling bias. On reviewing this patient together with other published cases, it appears that the c.607G>A variant probably represents a mutation hotspot and may characterize a distinct phenotype. This genetic variant (c.607G>A) has been documented in three individuals, whereas all other reported patients with GNAO1-associated epilepsy had distinct private variants. The c.607G>A variant presents with multiple seizure types including partial tonic-clonic

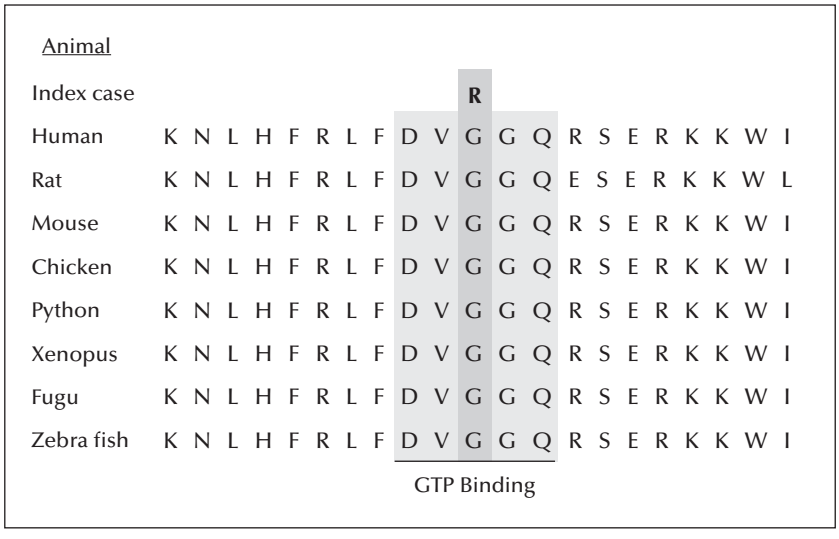

Figure 3. Alignment of amino acid sequences from vertebrate orthologs of *GNAO1* demonstrating the evolutionary conservation of the *GNAO1* variant G203R. The variant occurs in a region predicted to be a GTP binding site based on the sequence.

Table 1. Clinical phenotype of patients with *GNAO1* variants.

Genotype	Inheritance	Sex	Age at onset	Presenting feature	Epilepsy Syndromic Dx	Movement Disorder	Initial EEG findings (age)	Brain MRI	Course	Reference
With seizures but no movement disorder										
c.118G>A, (p.Gly40Arg)	*de novo*	F	4 days	Clonic seizures	Epileptic encephalopathy	None	Disorganized background with multi-focal epileptiform discharges without definite burst-suppression pattern	Normal at presentation	Epilepsy*, developmental delay	Law *et al.*, 2015
c.521A>G (p.Asp174Gly)	*de novo*, somatic mosaic	F	29 days	Tonic seizures in cluster	EIEE	None	Suppression-burst pattern at 29 days of age	Delayed myelination and thin corpus callosum at 10 months	DRE, developmental delay	Nakamura *et al.*, 2013
c.808A>C (p.Asn270His)	*de novo*	F	3 months	Seizure(s)	Epileptic encephalopathy	None	Hypsarrythmia	N/A	Seizure-free since 5 months of age, off medications	EuroEPINOMICS-RES Consortium *et al.*, 2014
c.824T>C (p.Phe278Ser)	*de novo*	F	3 days	Seizure(s)	EIEE	None	Burst suppression pattern	N/A	DRE	EuroEPINOMICS-RES Consortium *et al.*, 2014
c.836T>A (p.Ile279Asn)	*de novo*	F	4 days	Tonic seizure	EIEE	None	Suppression-burst pattern at 4 days of age	Normal at 1 month; cerebral atrophy at 5.5 years	DRE, severe developmental delay	Nakamura *et al.*, 2013
With both seizures and movement disorders										
c.572_592 del (p.Thr191_Phe197 del)	*de novo*	F	2 weeks	Tonic seizures in cluster	EIEE	Dystonia	Suppression-burst pattern at 2 weeks of age	Normal at 3 months	DRE, death at 11 months	Nakamura *et al.*, 2013

Table 1. (*Continued*)

Genotype	Inheritance	Sex	Age at onset	Presenting feature	Epilepsy Syndromic Dx	Movement Disorder	Initial EEG findings (age)	Brain MRI	Course	Reference
c.996T>C (p.Leu199Pro)	*de novo*	F	3 days	Tonic seizures	EIEE	Oral-facial dyskinesia	Background slowing, multi-focal discharges	Delayed myelination, thin corpus callosum	At 21 months: DRE; developmental delay; spastic quadriparesis	Marcé-Grau *et al.*, 2016
c.607G>A (p.Gly203Arg)	*de novo*	F	7 months	Opisthotonic posture, developmental delay	Epileptic encephalopathy	Severe chorea, athetosis	Diffuse irregular spike-and-slow-wave dischargesat 5 years	Delayed myelination at 1.3 years; reduced cerebral white matter, thin corpus callosum at 4.8 years	Focal tonic seizure(s) at 5 years; DRE; developmental delay	Nakamura *et al.*, 2013
c.607G>A, p.(Gly203Arg)	*de novo*	F	7 days	Tonic-clonic seizures	Epileptic encephalopathy	Severe chorea	Slow-wave bursts, migrating focal epileptiform discharges	Normal at 20 days; progressive cerebral atrophy with delayed myelination at 14 months	Focal epilepsy*, developmental delay	Saitsu *et al.*, 2016
c.607G>A, (p.Gly203Arg)	*de novo*	M	1 month	Partial seizures	Epileptic encephalopathy	Severe chorea, athetosis	Multi-focal epileptiform discharges at 1 month	Progressive cerebral and cerebellar atrophy	DRE; developmental delay	This case
c.625C4T, p.(Arg209Cys)	*de novo*	F	7 months	Developmental delay	Partial seizures at 10 and 11 years	Severe chorea	Diffuse low activity (age: not mentioned)	Progressive cerebral and cerebellar atrophy, brainstem atrophy, thin corpus callosum	Movement disorder, intellectual disability	Saitsu *et al.*, 2016

Table 1. (*Continued*)

Genotype	Inheritance	Sex	Age at onset	Presenting feature	Epilepsy Syndromic Dx	Movement Disorder	Initial EEG findings (age)	Brain MRI	Course	Reference
c.680C4T, p.(Ala227Val)	*de novo*	F	2 months	Infantile spasms	EIEE	Hand stereotypies	Hypsarrhythmia at 2 months	Progressive cerebral atrophy, thin corpus callosum at 10 months	Focal epilepsy*, severe developmental delay	Saitsu *et al*., 2016, 2015
c.692A>G (p.Tyr231Cys)	*de novo*	F	1 month	Myoclonic jerks	Epileptic encephalopathy	Stereotypic spasm-like movements	Modified suppression-burst pattern at 3 months	Progressive WM volume loss, thin corpus callosum at 2.5 years	DRE	Talvik *et al*., 2015
With movement disorders but no seizures										
c.626G>A (p.Arg209His)	Two siblings with *de novo* mutation, presumed germline mosaic in parent	M	34 months	Choreo-athetoid movements	None	Severe chorea, athetosis	Diffuse slowing	Normal at 3 years	Multiple admissions for increased involuntary movements, improved by bilateral pallidal DBS; motor developmental delay	Kulkarni *et al*., 2016
c.626G>A (p.Arg209His)	Sibling of above	M	18 months	Hypotonia	None	Severe chorea, athetosis	Diffuse slowing	Normal at 3 years	Same as above, non-ambulatory	Kulkarni *et al*., 2016
c.736G4A, p.(Glu246Lys)	*de novo*	F	4 months	Developmental delay	None	Severe athetosis	x	Normal at 4 and 12 years	Movement disorder, intellectual disability	Saitsu *et al*., 2016

*unclear if drug-resistant.
DBS: deep brain stimulation; DRE: drug-resistant epilepsy; EIEE: early infantile epileptic encephalopathy; F: female; M: male.

seizures that are drug-resistant and associated with multi-focal and diffuse epileptiform discharges on EEG, which potentially contribute to the epileptic encephalopathy (*table 1*). All reported patients with this variant have had severe chorea/athetosis and progressive cerebral atrophy on brain MRI. In contrast, a majority of epilepsy patients with other *GNAO1* genetic variants have presented in the neonatal period with features of Ohtahara syndrome or early-onset spasms and variable abnormal movements.

GNAO1 encodes for a class of guanine binding proteins (G proteins) called $G\alpha_{o,}$ which is the most abundant type in brain tissue (Huff *et al.*, 1985). The substituted residue corresponding to Gly203 of human $G\alpha_o$ is located within the highly conserved switch II region (*figure 3*), responsible for activation of downstream effectors upon GTP binding. Nakamura *et al.* showed that p.Gly203Arg substitution causes steric hindrance between the arginine side chain and the switch I region and/or GTP, destabilizing the $GDP^+AlF_4^-$bound Gα effector complex (Nakamura *et al.*, 2013). This was shown to affect norepinephrine (NE)-induced calcium currents in NG108-15 neuronal cells (a hybrid cell line formed by Sendai virus-induced fusion of the mouse neuroblastoma clone N18TG-2 and the rat glioma clone C6 BV-1) (Nakamura *et al.*, 2013). Under conditions of impaired GABAergic inhibition, activation of G-protein coupled α_2 adrenergic receptors (AR) on pyramidal cells by NE was reported to decrease the frequency of spontaneous epileptiform bursts in the rat hippocampal CA3 region (Jurgens *et al.*, 2007). This effect has been shown to use a $\alpha_{2A}AR/G\alpha_o$ protein-mediated pathway under strong inhibitory control by regulators of G-protein signalling (Goldenstein *et al.*, 2009). Alterations in this mechanism resulting from $G\alpha_o$ variants has been proposed as a potential basis for seizures in GNAO1-associated epilepsy. This also led to speculation that AEDs with calcium channel modulating properties may have particular efficacy in GNAO1-associated epilepsy (Nakamura *et al.*, 2013). However, zonisamide, which has a T-type calcium channel blocking property, was ineffective in our patient (Kito *et al.*, 1996).

Epileptic encephalopathies starting in infancy have been traditionally classified into certain electroclinical syndromes based on age of onset and other features (Donat, 1992). In recent years, advances in imaging, metabolic, and genetic investigations have elucidated the aetiology for some patient subgroups within these syndromes. We would emphasize co-existent movement disorders, particularly choreoathetosis, as a clinical clue for GNAO1-associated syndromes. However, the phenotype of seizures and movement disorders presenting in infancy is also seen with intra-uterine stroke, creatine biosynthesis defects, glutaric acidaemia type I, certain other organic acidurias, and rarely in patients with cerebral folate deficiency, *STXBP1* variants, or some mitochondrial defects. Often, these disorders can be suspected by history, imaging, and conventional biochemistry.

In summary, this case report highlights a probable association of c.607G>A with a clinical syndrome characterized by early-onset encephalopathy, drug-resistant epilepsy, and chorea. Though the ubiquity of G-protein coupled mechanisms is probably responsible for multiple neurological manifestations, given the small number of patients, this association should only be recognized as putative and further genotype-phenotype correlations will have to await more data. □

Disclosures.
None of the authors have any conflicts of interest to disclose.

Legend for video sequence

Video showing arrhythmic, asynchronous, and jerky movements of all four extremities and face, which appear to "flow" from one body part to another. Subtle, low-amplitude, tremulous movements are also noticeable, particularly in distal upper extremities. Overall, these features are consistent with choreoathetosis.

Key words for video research on www.epilepticdisorders.com

Phenomenology: non-epileptic paroxysmal event.
Localisation: not applicable.
Syndrome: not applicable.
Aetiology: genetic.

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