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Homozygous B4GALNT1 mutation and biochemical glutaric acidemia type II: A case report

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1. Introduction

The beta-1,4-*N*-acetylgalactosaminyl transferase-1 (B4GALNT1) gene on chromosome 12q13.3 encodes the GM2/GD2 synthase protein, which is responsible for the synthesis of complex gangliosides found predominantly in the central nervous system. Mutations in the B4GALNT1 gene lead to the loss of GM2 synthetase activity and thus affect important glycosphingolipid biosynthesis and signaling pathways of the central nervous system [1]. *B4GALNT1* mutations are inherited in an autosomal recessive manner in Hereditary Spastic Paraplegia Subtype 26 (SPG26). It is proposed that the loss of function mutation results in dysfunctional ganglioside biosynthesis, leading to the accumulation of globotriaosylceramide and simple gangliosides (GM3, GD3, and GT3). Predominant features of SPG26 include lower extremity spasticity and muscle weakness causing abnormal gait, as well as intellectual disability, dysarthria, and extrapyramidal and cerebellar signs. Concomitant glutaric acidemia or other metabolic abnormality has not been reported as an associated feature of SPG26 to our best knowledge [2,3].

Glutaric acidemia type II (GAII), or multiple acyl-CoA dehydrogenase deficiency [2], is a seemingly unrelated autosomal recessive disorder caused by mutations in one or more genes responsible for electron transfer in the mitochondrial respiratory chain: electron transfer flavoprotein subunit alpha (ETFA), subunit beta (ETFB), and electron transfer flavoprotein dehydrogenase (ETFDH). Such deficiency results in the excess excretion of glutaric acid and dysfunctional metabolism of fatty acids, amino acids, and choline. The late onset form of GAII is characterized by wide variability in symptoms and age of presentation. The most common features of late onset GAII include episodes of lethargy, vomiting, hypoglycemia, and metabolic acidosis in the setting of metabolic stress. These patients may also experience muscle weakness and pain. Organic aciduria is commonly intermittent and only evident during periods of stress. We report a case of a patient found to have SPG26 as well as concomitant glutaric acidemia diagnosed biochemically.

2. Case report

Our patient is a 37 year old female with a history of intellectual disability, temporal lobe epilepsy, and progressive muscle weakness and spasticity who was referred to the genetics clinic at age 31 years.

She was born vaginally at term to a 19 year old mother who had two prior normal pregnancies and deliveries. The pregnancy was unremarkable without perinatal complications. The patient had difficulty as an infant with feeding, and began to show significant developmental delays by age 3 months. Though her motor milestones were delayed, she had no apparent gait issues in early childhood. Language development was also delayed; she currently has a vocabulary of approximately 15 words. At 7 years she was diagnosed with severe intellectual disability and at this time also developed seizures. Seizures are characterized by perioral movements, eye deviation, hand automatisms, urinary incontinence, and unresponsiveness, consistent with left temporal lobe epilepsy. At 18 years she developed atrophy of the hand muscles and leg weakness, poor balance and spastic gait associated with falls. Electromyography to demonstrate peripheral neuropathy was unable to be performed due to the patient's preference. She has no obvious dysmorphic features (Fig. 1).

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Fig. 1. Facial characteristics of patient, age 37. No obvious dysmorphic features noted.

At age 31 years, extensive metabolic workup showed abnormal blood acylcarnitine levels suggestive of glutaric acidemia type 2 (Fig. 2). The method utilized was electrospray ionization tandem mass spectrometry, and the patient had five profiles over the years that all showed abnormally elevated acylcarnitines, even during periods of clinical stability and following treatment with riboflavin and coenzyme Q10. We performed skin fibroblast testing and obtained normal values for electron transfer flavoprotein (ETF) activity and electron transfer flavoprotein dehydrogenase (ETFDH) activity.

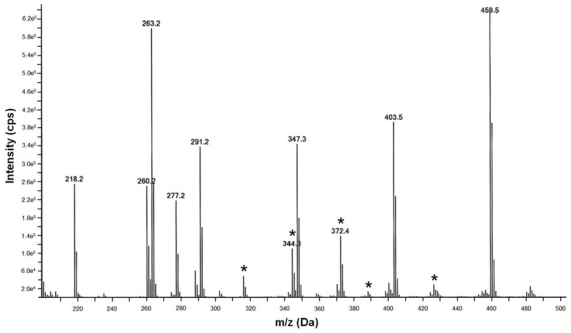
Urine oligosaccharide analysis by matrix-assisted laser desorption/ionization time of flight (MALDI TOF) mass spectrometry was performed to detect elevated GM3 and low GM2 since that would be consistent with B4GALNT1 deficiency. However, no abnormalities were detected, possibly because the assay may not have the sensitivity to detect abnormalities when the urine sample is dilute. Urine acylglycine labs showed elevated ethylmalonic acid and hexanoylglycine with no increased excretion of other acylglycines. Plasma total and free carnitine, lactate, and pyruvate levels were normal.

Analysis of the EFTA, EFTB, and ETFDH genes did not reveal any significant alterations. Thus whole exome sequencing was pursued, which revealed a homozygous, biparental frameshift mutation in exon 3 of the B4GALNT1 gene, specifically resulting in nucleotide change c.263dupG and protein change p.L89Pfs*13. The duplicated G results in a translational frameshift and a stop codon after 13 amino acids. Co-segregation studies showed that this genetic alteration is heterozygous in the patient's sister, mother, and father.

Regarding methods, exome sequencing was performed following published methology [4]. Genomic DNA was isolated from the patient's whole blood. DNA samples were sheared, adaptor ligated, PCR-amplified, and incubated with exome baits. Final quantified libraries were seeded onto an Illumina flow cell and sequenced, then initial data processing, base calling, alignments and variant calls were generated. Identified variants were annotated with HGVS nomenclature, population frequency, and predicted functional impact. Common alterations and alterations outside of analytical range were removed. Variants were then filtered further based on applicable inheritance models. Interpretation was based on clinical, family, and test information provided by the referring provider and data analysis was focused on small insertions and deletions, canonical splice site alterations, and non-synonymous alterations. Multiple online databases were used to search for previously described gene mutations and polymorphisms. All relevant findings underwent confirmation by Sanger sequencing.

3. Discussion

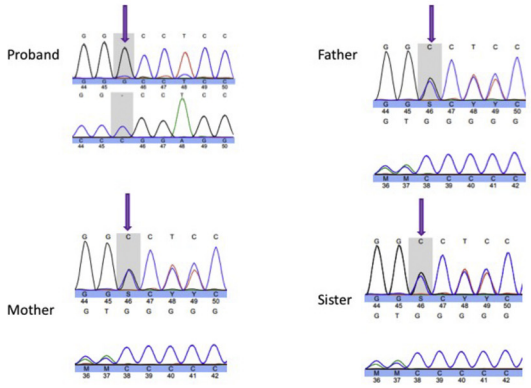
The patient's genetic results of homozygous B4GALNT1 provide a



a: Plasma acylcarnitine profile demonstrating multiple abnormally elevated species (asterisks) suggestive of a biochemical diagnosis of glutaric acidemia type II, including, from left to right: hexanoylcarnitine (C6), octanoylcarnitine (C8), decanoylcarnitine (C10), glutarylcarnitine (C5-DC), tetradecenoylcarnitine (C14:1), and myristoylcarnitine (C14).

Acylcarnitines	Quantitative Plasma Level	Reference value
Iso-/Butyrylcarnitine, C4	1.43 nmol/mL	<0.83
Isovaleryl-/2Methylbutyrycarn, C5	0.73 nmol/mL	<0.51
Hexanoylcarnitine, C6	0.94 nmol/mL	<0.17
Octanoylcarnitine, C8	2.35 nmol/mL	<0.78
Decenoylcarnitine, C10:1	0.54 nmol/mL	<0.47
Decanoylcarnitine, C10	3.37 nmol/mL	<0.88
Glutarylcarnitine, C5-DC	0.12 nmol/mL	<0.11
Dodecanoylcarnitine, C12	0.43 nmol/mL	<0.26
Tetradecenoylcarnitine, C14:1	0.38 nmol/mL	<0.24
Tetradecanoylcarnitine, C14	0.13 nmol/mL	<0.12
Hexadecenoylcarnitine, C16:1	0.13 nmol/mL	<0.10

b: Patient's original acylcarnitine profile, including all elevated levels



c: Chromatogram of patient and her parents reveals frameshift mutation in exon 3 of the B4GALNT1 gene, resulting in nucleotide change c.263dupG which she inherited from each parent.

Fig. 2. a Plasma acylcarnitine profile demonstrating multiple abnormally elevated species (asterisks) suggestive of a biochemical diagnosis of glutaric academia type II, including, from left to right: hexanoylcarnitine (C6), octanoylcarnitine (C8), decanoylcarnitine (C10), glutarylcarnitine (C5FDC), tetradecenoylcarnitine (C14:1), and myristoylcarnitine (C14). b Patient's original acylcarnitine profile, including all elevated levels. c Chromatogram of patient and her parents reveals frameshift mutation in exon 3 of the B4GALNT1 gene, resulting in nucleotide change c.263dupG which she inherited from each parent.

Table 1
Phenotypic Characteristics of B4GALNT1 mutations[Δ].

Characteristics	Patient	Wakil et al (2014) [1]: 2 families	Harlalka et al (2013) [7] : 3 families	Boukhris et al (2013) [3] : 7 families
Country of Origin	Mexico (Durango)	Bedouin	Kuwait, Italian-Canadian, Old Order Amish	Tunisia, Spain, Brazil, France, Portugal, Germany, Algeria
Number of individuals	1	9	12 (including 5 described in Wakil et al)	18
LE spasticity and progressive weakness (LE), gait abnormalities	+	+	+	+
Hyperreflexia, clonus	–	NA*	+	+
Babinski sign (extensor plantar response)	+	NA	+	+
Lower motor neuron involvement, distal amyotrophy (LE muscle wasting)	+	+	+	+
UE involvement (e.g. weakness, atrophy, spasticity, hyperreflexia)	+	+	+	+
Early disease onset (range 2–19)	+	+	+	+
Intellectual disability	+	+	+	+
Language impairment/delay	+	+	+	+
Slow progression	+	+	+	+
Cerebellar Ataxia/Cerebellar Signs	+	+	+	+
Peripheral neuropathy	+	+	+	+
Extrapyramidal features	+	+	+	+
Autism features	+	+	+	NA
Pseudobulbar dysarthria	+	+	+	+
Epilepsy	+	NA	+	NA
Stereotypies	+	NA	+	NA
Emotional lability, behavior problems, psychiatric illness	+	+	+	+
Cataracts and/or strabismus	+	NA	+	+
Pes cavus	+	NA	+	+
Decreased peripheral sensation	+	NA	+	+
Dysmorphic features	–		+	+
Osteopenia	+	NA	NA	NA
Glutaric Acidemia	+	NA	–	–

[Δ] At least 1 patient or 1 family presenting with feature as listed. Not all individuals in case reports presented with symptoms.
* NA: not available.

potential explanation for her clinical findings, in that many of her features resemble the phenotype described in the SPG26 literature. Such characteristics include early disease onset, intellectual disability, ataxia, peripheral neuropathy, and behavioral difficulties [1]. Additional features consistent with SPG26 seen in our patient include muscle wasting and pseudobulbar dysarthria [3].

The c.263dupG mutation has only been reported in one other individual: a 24-year-old male patient born to consanguineous Algerian parents [3]. That individual was reported to have moderate intellectual disability, lower extremity spasticity and weakness, cerebral atrophy with white matter hyperdensities on MRI, brisk ankle reflexes and Babinski sign in addition to ocular telangiectasia, nystagmus with saccadic pursuit, and convergent strabismus. Our patient shares a similar phenotype with the exception of telangiectasia and brain changes on MRI. Additional findings seen only in our patient include distal extremity wasting, pseudobulbar dysarthria and temporal lobe epilepsy. Additional reported cases of B4GALNT share features similar to our patient (Table 1).

To our knowledge, no B4GALNT1 reports have described metabolic abnormalities such as glutaric acidemia. We hypothesize that this patient's biochemical findings of GAII suggest a mitochondrial function of B4GALNT1, such that loss of function of the gene leads to metabolic abnormalities. Though not reported in SPG26, proteins encoded by genes mapped to various SPG loci have apparent roles in mitochondrial function; literature on SPG28 and SPG49 suggests dysfunction in phospholipid metabolism affecting the composition of the mitochondrial membrane and mitochondrial bioenergetics, which may impact corticospinal tract nerve function [3,6]. Similarly, ST3GAL5 variants, another ganglioside biosynthesis defect, result in the absence of plasma GM3 due to deficiency of GM3 synthase [3]. Fragaki et al. (2013) demonstrated that in patients with homozygous *ST3GAL5* mutations, fibroblasts lacking GM3 ganglioside demonstrated reduced mitochondrial membrane potential, suggestive of respiratory chain dysfunction [8]. Thus GM3 synthase deficiency may cause mitochondrial dysfunction due to accumulation of globotrialosylceramide found in fibroblasts [8]. The mechanism by which this occurs in B4GALNT1 deficiency remains unclear, though it is predicted to resemble that of GM3 synthase [3] or other enzymes involved in fatty-acid metabolism resulting in HSP. The pathophysiology includes alteration of mitochondrial architecture and bioenergetics with increased oxidative stress [6]. Hereditary spastic paraplegias are known to result from alterations in fatty acid metabolism, and glutaric acidemia results from impaired fatty acid conversion in mitochondria.

While our patient did not exhibit symptoms such as lethargy, vomiting, or metabolic acidosis, she presented with progressive muscle weakness in the setting of glutaric acidemia. She has no history of rhabdomyolysis, hypoketotic hypoglycemia, or liver dysfunction. She has been taking riboflavin for several years which has not affected her laboratory abnormalities; however, her seizure activity has improved. A deficiency in riboflavin transporter genes could also present as a GAII-like disease, however no mutations in SLC52A1, SLC52A2, and SLC52A3 were found. Muscle biopsy to evaluate activity of acyl-CoA dehydrogenases has been considered to confirm diagnosis of GAII, however not performed due to patient's preference. Flavin adenine dinucleotide synthase deficiency may present with similar manifestations as GAII, however our patient was negative for the FLAD1 mutation [5]. An alternative explanation is that spastic paraplegia and glutaric acidemia have coincidentally segregated together. Future subject investigations should include metabolic studies to elucidate the role the B4GLANT1 gene may play in electron transfer function in the mitochondrial respiratory chain.

4. Conclusion

To our knowledge, this is the first case of SPG26 associated with a biochemical diagnosis of GAII, possibly suggesting a novel mechanism for GAII. Alternatively, this is a case of spastic paraplegia and glutaric acidemia coincidentally segregating together. We expect this report will lead to additional cases in the literature which will clarify a potential new role of B4GALNT1 in mitochondrial function.

Declaration of Competing Interest

All authors report no conflicts of interest.

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