Case Report

Whole exome sequencing reveals a dual diagnosis of *BCAP31*-related syndrome and glutaric aciduria III

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ABSTRACT

Background: Biochemical testing is a common first-tier approach in the setting of genetic evaluation of patients with unexplained developmental delay. However, results can be unclear, and a plan for second-tier analysis must be determined based on the patient's biochemical results and clinical presentation - in many cases, triggering a diagnostic odyssey.

Case presentation: A male patient from the United States presenting with unexplained developmental delay, microcephaly, hypotonia, and feeding difficulties was referred for clinical genetic evaluation at age 8 months. Biochemical testing revealed an isolated marked elevation of glutaric acid on urine organic acid profile, without elevations of related metabolites. Further testing included *GCDH* sequencing, a neurometabolic gene panel, chromosomal microarray, Prader Willi/Angelman testing, and lysosomal disease enzyme panel, all of which were non-diagnostic. The patient had persistent developmental delay and hypotonia, dystonia, sensorineural hearing loss, and abnormal brain myelination on magnetic resonance imaging. Whole exome sequencing (WES) was performed and revealed a dual diagnosis of glutaric aciduria III (GA III) and *BCAP31*-related disorder, an X-linked intellectual disability syndrome, caused by a novel pathogenic variant.

Conclusions: GA III has historically been considered clinically benign, with few reported cases. This patient's presenting symptoms were similar to those commonly seen in GA I and GA II, however the biochemical abnormalities were not consistent with these disorders, prompting additional molecular and biochemical testing. Ultimately, WES confirmed a diagnosis of *BCAP31*-related syndrome, a rare neurological disorder, which explained the patient's presenting symptoms. WES also identified a secondary diagnosis of GA III. We present a patient with two rare genetic conditions, highlighting the importance of deep phenotyping and the utility of WES in the setting of a patient with dual genetic diagnoses.

1. Background

BCAP31, previously known as *DXS1357E*, is located at Xq28. It encodes a chaperone protein, B-cell receptor associated protein 31 (BAP31) that is abundantly expressed in the endoplasmic reticulum (ER) membrane and involved in programmed cell death and transfer of ER proteins to the Golgi apparatus [1,2]. First described in 1994 [3], this gene was initially identified as part of a microdeletion syndrome known as contiguous *ABCD1 DXS1357E* deletion syndrome (CADDS). Patients with CADDS have a severe presentation similar to peroxisomal biogenesis disorders, characterized by marked growth failure, developmental delay, liver dysfunction, movement disorder, and early death [4]. *BCAP31* is located between *SLC6A8*, (associated with X-linked creatine transporter deficiency) and *ABCD1* (associated with X-linked adrenoleukodystrophy). Contiguous gene deletion syndromes involving these three genes have been described with varying phenotypes dependent on the size and location of the deletion [5–7]. Loss-of-function (LOF) intragenic variants in *BCAP31* have been more recently associated with

an X-linked intellectual disability (XLID) syndrome, characterized by deafness, dystonia, and central hypomyelination (DDCH; OMIM: 300475). A cohort of seven male patients from Italy was described in 2013, including three males from two families with maternally inherited intragenic variants and four patients in a single family with deletion of exon 8 along with 248 base pairs of the 3′ untranslated region of *SLC6A8* (though the latter was not thought to impact the phenotype). Patients presented with severe motor delay (7/7), deafness (6/7), facial dysmorphia (6/7), and strabismus (5/7) [8].

While disease prevalence is unknown, approximately 50 cases have been reported worldwide including both male and female patients (Table 1). Aside from DDCH, other features have been reported including symptoms resembling mitochondrial encephalopathy, with choreoathetosis and dyskinesia [9,10]. One male patient had a hemizygous truncating variant that was inherited from his mother, who was reported to have sensorineural hearing loss but normal cognition [9]. There have also been reports of de novo variants causing *BCAP31*-related syndrome [10,11]. A female patient in Taiwan with deafness, dystonia, and seizures was found to have *BCAP31*-related syndrome caused by skewed X-inactivation [12]. Recently, the largest report of patients with *BCAP31*-related syndrome to date described 17 cases, 14 of which were caused by intragenic missense or truncating variants; overall, these patients tend to have milder presentations compared to those with microdeletions, such as CADDS [13].

Glutaric aciduria III (GA III; OMIM: 231690) is an autosomal recessive condition characterized by isolated elevation of urinary glutaric acid. It is caused by variants in *SUGCT* (previously known as *C7orf10*), which is highly expressed in the kidneys. *SUGCT* encodes succinate-hydroxymethylglutarate-CoA transferase, a mitochondrial enzyme that catalyzes the succinyl-CoA-dependent conversion of glutarate to glutaryl-CoA in the lysine and tryptophan catabolic pathway [14]. It is generally considered clinically benign, with only a biochemical phenotype and no confirmed clinical manifestations. The first reported individual with GA III was published in 1991 [15], and there have been scarce reports in the literature since (Table 2). It was described in a cohort of Amish patients while screening for GA I; three healthy children were found to have abnormally elevated urinary glutaric acid but without increased 3-hydroxyglutaric acid, and were ultimately found to be homozygous for a missense variant, NM_001193313.2(*SUGCT*):c.985 > T (p.Arg329Trp) [16], previously reported as NM_024728(*SUGCT*): c.895C > T(p.Arg299Trp) and NM_001193311.1(*SUGCT*): c.1006C > T(p.Arg336Trp). This and other *SUGCT* missense variants have been identified in patients with GA III, most of whom were diagnosed incidentally during the workup for another genetic condition or via urine-based newborn screening studies [17,18]. A rare contiguous gene deletion syndrome including *SUGCT* and *MPLKIP* has also been described in two patients, causing both trichothiodystrophy type 4 and GA III [19,20]. There is some evidence of possible gastrointestinal disturbances related to GA III, with some reported patients presenting with cyclic vomiting [15,17], including one patient whose symptoms improved with antibiotic treatment to reduce bacterial GA production [17]. Non-specific white matter changes on brain magnetic resonance imaging (MRI) have also been reported [17,18], though it is unclear if these are related to GA III or another underlying genetic condition. Given the paucity of reported cases and lack of an established phenotype, it is likely that GA III is underdiagnosed and thus underreported. The literature reveals inconsistent phenotypic data that is challenging to interpret given that most patients identified have a secondary diagnosis or are asymptomatic.

Herein, we report another case of dual diagnosis in a patient presenting with abnormal muscle tone, failure to thrive, sensorineural hearing loss, and developmental delay. He underwent multiple biochemical, cytogenetic, and molecular tests over a year-long diagnostic odyssey and was ultimately found to have two distinct rare conditions.

Table 1
Literature review of reported cases of *BCAP31*-related syndrome. M = male, F = female.

Author (Year) [PMID]	Number of patients (families) / Sex	Race/ Ethnicity	Genetic Etiology	Inheritance
Corzo (2002) [11992258] [4]	3 (3) / M	French Canadian (n = 2) Vietnamese (n = 1)	*CADDS* (*BCAP31* and *ABCD1*)	De novo (n = 2) Maternal (n = 1)
Anselm (2006) [16601897] [27]	2 (2) / M	Middle Eastern (n = 1) Hispanic (n = 1)	*SLC6A8* exon 8–13 deletion (n = 1) *SLC6A8* complete coding region deletion (n = 1)	De novo
Osaka (2012) [22472424] [6]	1 (1) / M	Japanese	19 kb deletion including exons 5–13 of *SLC6A8* and exon 5–8 of *BCAP31*	De novo
Cacciagli (2013) [24011989] [8]	7 (3) / M	French	*BCAP31* intragenic truncating variant (n = 5)	Maternal
			BCAP31 exon 8 deletion +248 bp of the 3'UTR of *SLC6A8* (n = 2)	Maternal
Calhoun (2014) [25044748] [5]	1 (1) / M	Unknown	*CADDS* (full *BCAP31* and *SLC6A8* deletion, partial *ABCD1* deletion)	Maternal
Vittal (2015) [30713915] [28]	2 (1) / M	Italian	*BCAP31* intragenic 6 bp deletion in exon 3 resulting in an indel	Maternal
Albanyan (2017) [28332767] [9]	1 (1) / M	Unknown	*BCAP31* intragenic 4 bp duplication resulting in frameshift	Maternal (mother had sensorineural hearing loss)
Rinaldi (2019) [31330203] [11]	1 (1) / M	Belgian	*BCAP31* intragenic 19 bp deletion in exon 8 resulting in frameshift	De novo
Shimizu (2020) [31953925] [10]	1 (1) / M	Japanese	*BCAP31* intragenic single nucleotide change resulting in stop codon	De novo
Kao (2020) [32652807] [12]	1 (1) / F	Taiwanese	*BCAP31* intragenic single nucleotide change resulting in missense change predicted to result in	De novo

Table 1 (continued)

Author (Year) [PMID]	Number of patients (families) / Sex	Race/ Ethnicity	Genetic Etiology	Inheritance
Louie (2020) [32681719] [29] and Schimke (1984) [6538752] [30]	4 (1) / M	Unknown	skipping of exon 2	
			BCAP31 intragenic two bp deletion resulting in frameshift	Maternal (three mothers with sensorineural hearing loss)
Whalen (2021) [33 603160] [13]	25 (17) / M and F	Unknown	BCAP31 intragenic LOF or missense variants (n = 25)	Maternal/de novo
			CADDS deletion (n = 1)	
			CADDS deletion + SLC6A8 (n = 1)	De novo

2. Case Report

The patient is a white male born in the United States. He was conceived via intrauterine insemination using anonymous donor sperm without other assistive reproductive technology. He was born at full term via vaginal delivery to a G2P1001 mother who was 36 years old at time of birth. He had normal Apgar scores and normal biochemical and hearing newborn screens. At birth, his weight was in the 50th percentile. By age two months, his weight had decreased below the third percentile due to feeding issues, which improved with increased caloric intake. At four months old, he had a persistent head lag and difficulty holding up his head. He also had light sensitivity and had difficulty being in sunlight or bright rooms. Initial workup was non-diagnostic and included creatine kinase, lactic acid, ammonia, thyroid function panel, plasma organic acids, plasma acylcarnitine profile, a complete metabolic panel, and a complete blood count. Urine organic acid (UOA) profile was not ordered at this time; it is possible plasma organic acids were ordered in error in lieu of UOA. He was referred for medical genetics evaluation at age eight months, and was noted to be non-dysmorphic and developmentally delayed. He was unable to roll over or sit independently and was not appropriately responding to sounds. A chromosomal microarray and methylation studies were ordered to rule out Prader-Willi and Angelman syndromes; both were non-diagnostic. Additional biochemical testing was performed including plasma free and total carnitine, very long chain fatty acids, and a qualitative UOA profile. The UOA profile revealed markedly elevated glutaric acid without elevation of other metabolites seen in glutaric acidemia I (GA I). The finding was suggested to be secondary to dietary factors or gut bacterial metabolism, but GA I could not be ruled out. Single-gene sequencing for GCDH, associated with GA 1, did not reveal any variants. A larger next-generation sequencing (NGS) panel of 144 genes related to neurometabolic disorders was performed. This panel was non-diagnostic, revealing three heterozygous variants of uncertain significance in ALDH5A, PNPO, and GALC along with three benign pseudodeficiency variants in GALC. Follow-up lysosomal enzyme panel in blood was normal.

At age nine months, the patient's head circumference had decreased from the eighth percentile to below the first percentile. At age 11 months, he underwent brain magnetic resonance imaging (MRI) which revealed generous extra-axial cerebrospinal fluid space and slightly generous appearance of the lateral ventricle and third ventricle with slightly diminished white matter, suggesting overall diminished supratentorial cerebral volume. There was increased signal intensity on fluid-attenuated inversion recovery (FLAIR) in T2 weighted sequence in the periventricular white matter, representing abnormal myelination. By age one year, the patient was making some developmental gains with physical therapy and was rolling over and beginning to sit unsupported. His weight remained below the third percentile, despite steadily gaining weight on his own curve. Given his history of lack of response to sound, including his name, he underwent sedated audiologic evaluation which revealed bilateral profound sensorineural hearing loss.

Whole exome sequencing (WES) with mitochondrial genome sequencing was performed as a trio with his mother and a healthy full sister (conceived via the same donor sperm) and aligned to GRCh37/ UCSC hg19 human genome build. WES revealed a hemizygous nonsense variant NM_001139441.1(BCAP31):c.526 A > T (p.Lys176Ter). This variant had not been previously reported in BCAP31-related disorder or in healthy populations. Based on this information and its predicted effect of protein truncation or nonsense-mediated decay in a gene for which LOF is a known mechanism of disease, this variant was classified as pathogenic by the testing laboratory and thought to be diagnostic of his symptoms. WES also revealed apparent homozygosity for a pathogenic variant, NM_001193313.2(SUGCT):c.985C > T(p.Arg329Trp). This variant was observed in approximately 0.5% of alleles in large population cohorts [21] and had been reported previously in individuals with GA III [16,17]. There is an entry for this variant in the ClinVar database (Variation ID: 1849) and has seven submissions with conflicting interpretations of pathogenicity (pathogenic - 5; benign – 1; not provided – 1). Previously published functional studies supported LOF effect [14]. Based on the patient's unexplained elevation in urinary glutaric acid without other abnormal metabolites, this finding was diagnostic of GA III. In support of this diagnosis, a repeat UOA profile was collected at age 2.7 years, which demonstrated a persistent elevation of glutaric acid, without elevation of other metabolites. No additional nuclear or mitochondrial variants were identified.

The BCAP31 variant was identified in 1/160 sequencing reads in the patient's mother, suggesting low-level mosaicism; however, this was not able to be confirmed via Sanger sequencing, and thus mosaicism could not be confirmed without additional testing. Because the family was not planning future pregnancies, testing of multiple tissues was not pursued. The variant was not detected in his sister. His mother was also a carrier of the SUGCT variant, which was not detected in his sister. The patient's mother reported no medical concerns for herself and reported normal hearing, though she had not had a formal audiologic evaluation. She reported two full brothers who are healthy adults. There is otherwise no known family history of developmental delay or deafness in the mother's family. At the time of donation, the sperm donor reported no personal health issues and no known family history of genetic disorders. The donor underwent carrier screening including of approximately 175 autosomal recessive genetic conditions, which included GA I, but not GA II or III.

The patient continued to gain milestones and had improvements in muscle tone with continued physical therapy. At age 17 months, he underwent awake and asleep electroencephalogram which did not reveal any seizure activity. He was found to have mild optic atrophy, explaining his light sensitivity, but otherwise normal vision for age. At age 20 months, bilateral cochlear implants were placed. He had persistent mildly elevated liver transaminases, a finding reported as part of the phenotypic spectrum of BCAP31-related syndrome, with normal liver imaging. Workup to rule out alpha-1-antitrypsin deficiency was completed and was negative, with normal serum alpha-1-antitrypsin and MM genotype. He receives about 50% of solid foods and 100% of liquids via gastric tube due to ongoing difficulty with maintaining weight, especially during periods of illness. He was also diagnosed with dystonic quadriplegia and established care in a cerebral palsy clinic,

Table 2
Summary of Glutaric Aciduria Type 3 Case Reports.

Author (Year) [PMID]	*SUGCT* Variant 1	*SUGCT* Variant 2	Co-Occurring Genetic Condition	Clinical Features	Urinary glutaric acid (mmol/moL creatinine)
Bennett (1991) [1909402] [15] Sherman (2008) [18926513] [16]	c.322C > T, p. Arg108Ter	c.322C > T, p.Arg108Ter	β-thalassemia	Failure to thrive, post-prandial vomiting; right lower motor neuron facial palsy at 2.5 years, which slowly resolved; hematologic evidence of β-thalassemia	500
Knerr (2002) [12555941] [31]	Unknown	Unknown	Monosomy 6q26-qter	Birth: dysmorphic features (microcephaly, micrognathia, prominent nasal bridge and hypertelorism), and generalized muscular hypotonia 6 months: psychomotor retardation and stereotypical movements 2.5 years: episode of convulsions during fever	106–500 (normal <10)
Knerr (2002) [12555941] [31]	c.985C > T, p. Arg329Trp *	c.985C > T, p. Arg329Trp	Autoimmune hyperthyroidism	Gastroenteritis, goiter, arterial hypertension, hypoglycemia, metabolic acidosis	85–1460 (normal <10)
Knerr (2002) [12555941] [31]	Unknown	Unknown	None reported	Asymptomatic; evaluated due to family ketotic hypoglycemia in brother	18–290 (normal <10)
Sherman (2008) [18926513] [16]	c.985C > T, p. Arg329Trp	c.985C > T, p. Arg329Trp	None reported	Asymptomatic healthy individuals (3 children, 1 adult) in the Amish population**	Mean ± SD: 78.5 ± 97 (controls: 0.9 ± 0.5)
Sherman (2008) [18926513] [16]	c.985C > T, p. Arg329Trp	c.424C > T, p. Arg142Ter	None reported	None reported	Unknown
Skaricic (2016) [32]	c.985C > T, p. Arg329Trp	c.985C > T, p. Arg329Trp	None reported	Recurrent vomiting, hypotonia, ataxia, left abducens paresis, white matter changes on MRI; recovered completely	39–98
Skaricic (2016) [32]	c.985C > T, p. Arg329Trp	c.985C > T, p. Arg329Trp	None reported	12 months: agitation and unresponsiveness 3 years: ADHD	90–133
Waters (2017) [28766179] [17]	c.985C > T, p. Arg329Trp	c.826G > A, p. Val276Ile	None reported	Global developmental delay, wheezing episodes, lethargy, unexplained ketonuria/ketoacidosis, nonspecific periventricular white matter signal at 2 years and still present at 12–16 years; mild cognitive delays, learning disability and attention and behavioral issues.	65
Waters (2017) [28766179] [17]	c.985C > T, p. Arg329Trp	c.625G > A, p. Ala209Thr	None reported	Asymptomatic	151–701
Waters (2017) [28766179] [17]	c.985C > T, p. Arg329Trp	c.985C > T, p. Arg329Trp	Aminoacylase 1 deficiency diagnosed by identification of *N*-acetyl-amino acids in urine samples; no confirmatory genetic testing reported	Cyclic vomiting from 4 months age, gross motor delay, deafness, axial hypotonia, single palmar crease, inverted nipples, frontal bossing, left torticollis, right plagiocephaly, and increase in head circumference (90th percentile) at 6 months from 5th percentile at birth	61–145
La Serna-Infantes (2018) [29421601] [20]	125 kb microdeletion at 7p14.1	125 kb microdeletion at 7p14.1	Trichothiodystrophy type 4 caused by homozygous deletion of MPLKIP	Short stature, microcephaly, prematurely aged and asymmetric face, development delay, intellectual disability and trichorrhexis nodosa	Unknown
Dorum (2020) [32779420] [33]	c.322C > T, p. Arg108Ter	c.322C > T, p. Arg108Ter	Homozygous NM_147196(*TMIE*): c.250C > T, p.Arg84Trp. Consistent with autosomal recessive deafness-6	1 year: Sensorineural hearing loss, intellectual disability, speech impairments; seizure 2 years: global developmental delay	92 (normal: < 4.3)
Dorum (2020) [32779420] [33]	c.964C > T, p. Arg322Trp	c.964C > T, p. Arg322Trp	None reported	Neuromotor developmental delay, tremor, head circumference < 3rd percentile, axial hypotonia and bilateral clonus and spasticity; periventricular and deep cerebral white matter abnormalities on brain MRI	56 (normal: < 4.3)
Demir (2023) [37064336] [34]	83 kb microdeletion at 7p14	83 kb microdeletion at 7p14	Trichothiodystrophy type 4 caused by homozygous deletion of MPLKIP	Hypotonia, failure to thrive, microcephaly, dysmorphic features, brittle hair, hypertransaminasemia, and recurrent lower respiratory tract infections	7.3× higher than upper limits of normal
Bu (2023) [37920852] [35]	c.286del, p. V96Lfs*28	c.286del, p.V96Lfs*28	Complete paternal isoUPD (7)	Excessive weight from birth	69; normal <8.4;
Unpublished case***	c.985C > T, p. Arg329Trp	7p14.1 34.2 kb microdeletion involving SUGCT exons 10 and 11	None reported	Epilepsy, autistic features, developmental delay, intellectual disability	Unknown
Current case	c.985C > T, p. Arg329Trp	c.985C > T, p. Arg329Trp	BCAP31-related disorder	Developmental delay, microcephaly, hypotonia, feeding difficulties; bilateral profound sensorineural hearing loss.	Markedly elevated

[*] NM_001193313.2(*SUGCT*):c.985C > T (p.Arg329Trp) has been previously reported as NM_024728(*SUGCT*): c.895C > T (p.Arg299Trp) and NM_001193311.1 (*SUGCT*):c.1006C > T(p.Arg336Trp). For consistency in this summary table, this variant is referred to as c.895C > T (p. Arg299Trp).
[**] Two other individuals from the Amish population were mentioned in relation to urinary glutaric acid measurements (6 Amish individuals with glutaric acidemia type 3); no description of clinical status was provided.
[***] Internal case. Epilepsy panel negative; WES not performed.

where he is followed regularly and receives mobility support. At age 4.5 years, he uses a manual wheelchair independently and is working on taking steps using a gait trainer. He has mild facial dysmorphia consistent with other reported cases of *BCAP31*-related syndrome. He communicates primarily with some sign language and an assistive communication device. He has not had formal neurodevelopmental testing, and attends school full time with an aid. He is described by his family as outgoing, friendly, and loving.

3. Discussion

We present a case of a patient with two distinct rare diagnoses identified via WES, one of which is thought to fully explain the patient's full phenotype (*BCAP31*-related syndrome) and another which is not clinically well-understood (GA III). WES was an appropriate diagnostic tool given the patient's multisystemic manifestations and multiple non-diagnostic molecular, cytogenic, and biochemical evaluations. Additional panel testing may not have yielded a complete diagnosis. Outside of single-gene sequencing or WES, *SUGCT* is primarily found on panels for peroxisomal disorders or specific GA panels including genes for all three types of the condition. Further, if additional genetic testing had been ordered based on subsequent symptom identification, a hearing loss panel may have been considered given the patient's diagnosis of bilateral SNHL; however, *BCAP31* is on very few major hearing loss and/or deafness panels. There are, of course, no panels on which both *SUGCT* and *BCAP31* are included.

The patient's symptoms seem to all be explained by the pathogenic variant in *BCAP31*; however, potential contributions from the *SUGCT* variants cannot be completely ruled out. There are published cases of individuals with *BCAP31* variants demonstrating variable expressivity, even among those with intragenic variants. A review of the literature (Table 1) reveals a variety of symptoms expanding on the core DDCH phenotype. Patients with large contiguous gene deletions involving *BCAP31*, primarily including *ABCD1* or both *ABCD1* and *SLC6A8*, appear to have a more severe phenotype; patients with deletions that include only *BCAP31* and *SLC6A8* appear to have similar phenotypes to those with only *BCAP31* intragenic variants [7]. The symptoms reported in our patient have all been reported in other cases of *BCAP31*-related syndrome. While the variant identified in our patient had not been previously reported in affected or healthy individuals, other truncating and missense variants in nearby nucleotide positions have been reported in those with intragenic variants in *BCAP31* with similar clinical presentations.

GA III has long been considered clinically benign since the first published patient, despite inconsistent reports in the literature of varying phenotypes. Notably, most of the patients evaluated for various clinical presentations were found to have a second genetic diagnosis, making it difficult to determine which symptoms, if any, could be attributed to the GA III. There are some reports of gastrointestinal disturbance in patients with GA III[17], supported by evidence of gut microbiota dysbiosis in *SUGCT* knockout mice [22]. One study identified *SUGCT* p.Arg299Trp as a potential autism spectrum disorder (ASD) susceptibility variant [23]. Of note, although our patient has not had a formal neurodevelopmental evaluation, his parents reported no features or clinical concern for ASD. Although there is a relatively high allele frequency (0.5%) of *SUGCT* p.Arg299Trp in large population cohorts, there are limited opportunities to ascertain individuals with GA III without co-morbidities, given the absence of glutarylcarnitine production secondary to the deficiency of glutaryl-CoA synthesis. Unique screening programs, such as urine organic acid analysis in the Amish

population [16] and the Quebec urine spot newborn screening program [17] have identified individuals from birth who have remained asymptomatic. In contrast, the standard newborn screening methods that measure dried blood spot acylcarnitine species will be unable to identify individuals with GA III. Hence, GA III is likely to remain an underdiagnosed condition despite an overall prevalence that has been estimated at 1 in 27,000 [17]. The lack of a phenotype associated with GA III has been explained by the proposed limited toxicity of non-conjugated glutaric acid. This is in contrast to GA I, where the accumulation of glutaryl-CoA and its downstream metabolites are proposed to underlie the pathogenesis of this disorder [24]. It is not clear whether this variant would have been reported on the patient's WES if the finding of glutaric aciduria had not been reported.Although the variant was classified as pathogenic by the testing laboratory, it is not known whether it would have passed the laboratory's internal protocols for variant reporting, which is influenced by clinical and biochemical information provided at the time of testing.

Our case adds phenotypic information to the literature for two rare diseases. With next-generation sequencing through WES and WGS at the forefront of medical genetics, it is likely more patients with multiple genetic diagnoses, including rare diseases, will be identified. Previous studies suggest as many as 2–4% of individuals with one genetic diagnosis may have a secondary genetic diagnosis [25,26], thus supporting the use of WES as a diagnostic tool in the setting of non-diagnostic biochemical and cytogenetic evaluation of a patient with multi-system features. We emphasize the importance of providing complete and well-documented clinical information when ordering broad-scale genetic testing such as WES or WGS in order to increase the likelihood of identifying causative variants in an undiagnosed patient. Finally, we acknowledge that further work is needed to determine whether GA III is indeed clinically benign in all patients, which will require testing in healthy populations or through urine-based newborn screening.

Ethics approval and consent to participate

Written informed consent was obtained from the patient/legally authorized representative and/or guardians of all patients for publication of this case report.

CRediT authorship contribution statement

Erin Huggins: Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **David G. Jackson:** Writing – original draft, Visualization, Conceptualization. **Sarah P. Young:** Writing – review & editing, Visualization, Conceptualization. **Priya S. Kishnani:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors have no competing interests as defined by MGM, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Data availability

Not applicable (this manuscript does not report data generation or

analysis).

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