Glutaric Aciduria Type 3: Three Unrelated Canadian Cases, with Different Routes of Ascertainment

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Abstract Glutaric aciduria type 3 (GA3) is associated with decreased conversion of free glutaric acid to glutaryl-coA, reflecting deficiency of succinate-hydroxymethylglutarate coA-transferase, caused by variants in the *SUGCT* (*C7orf10*) gene. GA3 remains less well known, characterised and understood than glutaric aciduria types 1 and 2. It is generally considered a likely “non-disease,” but this is based on limited supporting information, with only nine individuals with GA3 described in the literature. Clinicians encountering a patient with GA3 therefore still face a dilemma of whether or not this should be dismissed as irrelevant.

We have identified three unrelated Canadian patients with GA3. Two came to clinical attention because of symptoms, while the third was identified by a population urine-based newborn screening programme and has so far remained asymptomatic. We describe the clinical histories, biochemical characterisation and genotypes of these individuals. Examination of allele frequencies underlines the fact that GA3 is underdiagnosed. While one probable factor is that some GA3 patients remain asymptomatic, we highlight other plausible reasons whereby this diagnosis might be overlooked.

Gastrointestinal disturbances were previously reported in some GA3 patients. In one of our patients, severe episodes of cyclic vomiting were the major problem. A trial of antibiotic treatment, to minimise bacterial GA production, was followed by significant clinical improvement.

At present, there is insufficient evidence to define any specific clinical phenotype as attributable to GA3. However, we consider that it would be premature to assume that this condition is completely benign in all individuals at all times.

Introduction

Three distinct types of glutaric aciduria have been described. Glutaric aciduria type 1 (GA1; OMIM 231670; Goodman and Frerman 2001; Boy et al. 2017) is caused by deficiency of glutaryl-CoA dehydrogenase (EC 1.3.8.6), an intramitochondrial flavoprotein required for metabolism of lysine, hydroxylysine and tryptophan. As well as accumulation and increased excretion of glutaric acid (GA), biochemical markers of GA1 include elevations of 3-hydroxyglutaric acid (3HGA), glutaconic acid and glutaryl-carnitine. The natural clinical course of GA1 is characterised by encephalopathic crises, dystonia and dyskinesia, reflecting neuronal degeneration of the striatum.

Glutaric aciduria type 2 (GA2; OMIM 231680; Frerman and Goodman 2001) is caused by decreased activities of multiple acyl-CoA dehydrogenases, reflecting a primary defect in transfer of electrons from these flavoprotein enzymes to the respiratory chain. GA2 is recognised biochemically by characteristic patterns of elevation of several organic acids, acylglycines and acylcarnitines, derived from the various accumulating substrates. Clinical presentations of GA2 reflect, in part, a deficiency of energy generation. However, the pathogenesis of GA2 largely reflects toxicity of accumulated acyl-CoAs and/or of toxic acids derived from alternative metabolism of these acyl-CoAs. The pathogenesis of GA1 likewise reflects toxic consequences of an accumulation of glutaryl-coA and its conversion to toxic acids (Goodman and Frerman 2001). Thus, GA1 and GA2 both represent examples of “CASTOR” (Coenzyme A sequestration, toxicity or redistribution) disorders (Mitchell et al. 2008).

Glutaric aciduria type 3 (GA3, OMIM 231690) is fundamentally distinct from GA1 and GA2, and it is much less well known, characterised and understood than those two conditions. Only nine individuals with GA3 have been described (Bennett et al. 1991; Knerr et al. 2002; Sherman et al. 2008), five asymptomatic, with no single consistent clinical presentation evident in the other four. The observed biochemical phenotype was persistent elevation of GA, notably without elevation of any other markers of GA1 or GA2, and increased GA excretion following lysine loading. The enzyme deficient in GA3 corresponds to an intramitochondrial succinate-hydroxymethylglutarate CoA-transferase (EC 2.8.3.13), encoded by *SUGCT* (originally called *C7orf10*), which converts free GA to glutaryl-CoA (Sherman et al. 2008; Marlaire et al. 2014). While this is probably its main physiological role, several other dicarboxylic acids are good alternative CoA acceptors *in vitro*, glutaryl-CoA and succinyl-CoA are interchangeable CoA donors, and the reaction is easily reversible; together suggesting potential complexity *in vivo* under certain conditions.

GA3 is generally considered a likely “non-disease.” However, there is only limited supporting information available. Clinicians encountering a patient diagnosed with GA3 still face the dilemma of whether or not to dismiss this as irrelevant. We therefore describe three unrelated cases, ascertained by different routes, and contribute to the clinical, biochemical and molecular characterisation of this condition.

Patient Descriptions

Patient 1

Patient 1 is an 18-year-old male, born in the province of Ontario, Canada, to non-consanguineous parents of British ancestry. He came to clinical attention at age 22 months with global developmental delay. He had recurrent wheezing episodes in early childhood and admissions for lethargy and unexplained ketonuria/ketoacidosis. There was no history of acute encephalopathy, regression, vomiting episodes or hypoglycemia. MRI at age 2 years showed non-specific periventricular white matter signal change. Growth parameters were unremarkable. He had low-set posteriorly rotated ears, almond shaped eyes, a smallish midfacies with thin upper lip and a sacral dimple.

Qualitative urine organic acid analysis at age 22 months indicated isolated elevation of GA, which was persistent. (Analysis of urine metabolite profiles in recent samples from all patients is described in more detail in the following section, with results in Tables 1 and 2). Results of other metabolic testing, including plasma acylcarnitine and amino acid profiles, were normal. GA1 and GA2 were further excluded by fibroblast studies, respectively by direct enzyme assay and by beta oxidation profiling.

At various times between ages 2 and 7 years, before the diagnosis of GA3 was established and also before the underlying biochemical basis of GA3 was defined, dietary interventions were introduced on an empiric basis, attempting to improve or prevent the recurrent ketotic episodes. A trial of low-fat diet with riboflavin supplementation was changed (following the exclusion of GA2) to a trial of low-protein diet with carnitine supplementation. All such interventions were subsequently discontinued, except carnitine, for which the dose was decreased.

GA3 was diagnosed with the finding of compound heterozygous sequence variants in *SUGCT* (NCBI Reference Sequence NM_001193313.1/NP_001180242.1). The c.1006C>T, p.Arg336Trp variant (previously called c.895C>T, p.Arg299Trp, Sherman et al. 2008) has been reported in other GA3 patients and is deleterious to the protein (Marlaire et al. 2014). The other variant identified

Table 1 Urine organic acids and acylcarnitines

Patient	Sample	Age	Organic acid profile ^a GC/MS method							Organic acids ^a LC MS/MS		Acylcarnitines MS/MS		
			GA ^b	3HGA ^c	HMG	Ket.	Dic.	Gut	SNS	GA ^d	3HGA ^e	C5DC ^f	C5DC/ C5OH ^g	C5DC/ C5 ^h
1	1-1	16 years	<u>45</u>	5	—	—	—	—	—	<u>65</u>	5.1	0.6	2.1	1.7
2	2-1 ⁱ	4 months	<u>940</u>	7	+	—	—	—	—	NC	NC	NC	2.4	2.5
2	2-2 ⁱ	6 months	<u>309</u>	0	+	—	—	—	—	NA	NA	NA	NA	NA
2	2-3	9 months	NA	NA	NA	NA	NA	NA	NA	<u>701</u>	4.1	0.4	2.0	1.7
2	2-4	1 year	<u>153</u>	0	++	—	—	—	—	<u>151</u>	4.7	0.4	2.6	0.2
		10 months												
2	2-5	1 year	NA	NA	NA	NA	NA	NA	NA	<u>241</u>	5.2	0.5	2.1	0.2
		10 months												
3	3-1 ⁱ	4 weeks	<u>9</u> ^j	0	—	—	—	—	—	NC	NC	NC	1.9	1.8
3	3-2	5 months	<u>91</u>	6	+	—	+	+	+	<u>61</u>	3.0	0.3	3.4	1.8
3	3-3	6 months	<u>48</u>	4	—	—	+	++	+	<u>41</u>	1.2	0.3	3.1	1.2
3	3-4	7 months	<u>144</u>	0	++	++	+	++	+	<u>110</u>	4.3	0.1	2.0	0.9
3	3-5	7 months	<u>166</u>	4	++	++	+	+	+	<u>107</u>	2.1	0.2	4.1	1.7
		3 weeks												
3	3-6	8 months	<u>393</u>	2	++	+++	+	++	+	NA	NA	NA	NA	NA
		2 weeks												
3	3-7	1 year	<u>158</u>	0	—	—	—	—	+	<u>145</u>	4.5	0.7	5.1	1.2
		2 months												
3	3-8	1 year	<u>38</u>	0	—	—	—	—	—	NA	NA	NA	NA	NA
		4 months												
3	3-9	2 years	<u>89</u>	0	+	—	—	k	+	<u>111</u>	2.9	0.5	2.6	0.7
		9 months												

GA glutaric acid, 3HGA 3-hydroxyglutaric acid, HMG 3-hydroxy-3-methylglutaric acid, Ket ketones, Dic dicarboxylic acids, Gut acids of probable intestinal origin (including methylmalonic, 3-hydroxypropionic, 4-hydroxyphenyllactic), SNS other acids considered secondary or non-specific, NA not analysed (specimen not available), NC could not be calculated

Concentrations of organic acids other than GA and 3HGA are summarised as follows: — denotes within reference range; + denotes 1×–3× reference limit; ++ denotes 3×–10× reference limit; +++ denotes >10× reference limit

^a All organic acids are expressed in mmol/mol creatinine. Underlined figures indicate concentrations above reference range

^b Age-related reference limits for glutaric acid by GC/MS: 12 (age 0–5 months), 22 (5–24 months), 4 (2–12 years), 2 (>12 years)

^c Age-related reference limits for 3HGA by GC/MS: 10 (0–5 months), 7 (5–24 months), 4 (2–12 years), 5 (>12 years)

^d Ref. limit: 16

^e Ref. limit: 7.1

^f Ref. limit: 5.2 mmol/mol creatinine (Tortorelli et al. 2005; Al-Dirbashi et al. 2011)

^g Ref. limit (ratio): 10

^h Ref. limit (ratio): 10

ⁱ Urine specimen received on filter paper

^j Retrospective analysis of stored newborn screening filter paper sample

^k Elevation of lactic acid seen in this specimen only, without elevation of other “gut” markers and without elevation of plasma (L-)lactate, attributed to probable production by gut bacteria

in Patient 1, c.826G>A, p.Val276Ile, is predicted to be deleterious.

Brain MRI, repeated at ages 12 and 16 years, again showed non-specific changes of white matter signal, which were stable from age 12 to age 16. He continues to have attention and impulsivity issues and learning disability, but his cognitive delay is relatively mild and not progressive. He has not had any further ketotic episodes since age 10 years. There are no other clinical concerns.

Patient 2

Patient 2 is a 3-year-old boy, born in the province of Quebec, Canada, to non-consanguineous French-Canadian parents. He came to clinical attention through population urine-based newborn screening. In the Quebec Neonatal Urine Screening Programme (Auray-Blais et al. 2007), an infant's urine sample is collected on filter paper and submitted by parents. Screening is performed on eluted

Table 2 Urine acylglycines

Patient	Sample	Age	Acylglycines ^a			
			Acetylglutamine ^b	Propionylglutamine ^c	Crotonylglutamine ^d	Butyrylglutamine ^e
3	3-1 ^f	4 weeks	<u>56.51</u>	<u>2.91</u>	<u>11.17</u>	<u>2.74</u>
3	3-2	5 months	<u>50.57</u>	<u>2.27</u>	<u>10.60</u>	<u>3.72</u>
3	3-3	6 months	<u>51.81</u>	<u>3.75</u>	<u>10.39</u>	<u>3.85</u>
3	3-4	7 months	<u>66.83</u>	<u>7.22</u>	<u>18.28</u>	<u>4.90</u>
3	3-5	7 months 3 weeks	<u>71.36</u>	<u>3.56</u>	<u>15.18</u>	<u>3.86</u>
3	3-6	8 months 2 weeks	NA	NA	NA	NA
3	3-7	1 year 2 months	<u>105.95</u>	<u>9.65</u>	<u>2.91</u>	<u>7.22</u>
3	3-8	1 year 4 months	<u>156.45</u>	<u>9.72</u>	<u>2.44</u>	<u>9.49</u>
3	3-9	2 years 9 months	<u>148.00</u>	<u>10.75</u>	<u>6.72</u>	<u>8.27</u>
Ctrl pos ACY1 ^g		12 years	<u>81.35</u>	<u>9.66</u>	<u>2.37</u>	<u>3.41</u>

NA not analysed (specimen not available)

Acylglycine profile analysis was performed by an LC-MS/MS method (Bherer et al. 2015) which includes quantitative analysis of 15 acylglycines and semi-quantitative analysis of 6 others. Glutarylglutamine analysis is not included in this method

^a All acylglycines are expressed in mmol/mol creatinine. Underlined figures indicate concentrations above reference range

^b Age-related reference limits for acetylglutamine: 2.55 (age <9 months); 3.13 (age 9 months–8 years)

^c Age-related reference limits for propionylglutamine: 0.15 (age <9 months); 0.15 (age 9 months–8 years)

^d Age-related reference limits for crotonylglutamine: 0.94 (age <9 months); 1.46 (age 9 months–8 years)

^e Age-related reference limits for butyrylglutamine: 0.16 (age <9 months); 0.22 (age 9 months–8 years)

^f Urine specimen received on filter paper; retrospective analysis of stored newborn screening filter paper sample

^g “Ctrl pos ACY1” is a positive control urine sample from an individual with aminoacylase 1 deficiency; “Sample 240” provided by the ERNDIM qualitative organic acids (Heidelberg) external quality assurance scheme

samples by thin-layer chromatography, then any samples with apparent substantial elevations of certain organic acids are re-analysed by more quantitative gas chromatography-mass spectrometry (GC-MS) in the biochemical genetics laboratory of CHU Sherbrooke. In two successive screening samples from this infant, GA was elevated, without elevation of markers suggesting GA1 or GA2.

The infant was referred to a metabolic specialist for evaluation. No clinical concerns were identified. Analyses of subsequent urine samples again showed elevation of GA (Table 1). Acylcarnitine profiles were normal in plasma and urine. Analysis of *SUGCT* revealed compound heterozygosity for the known deleterious variant c.1006C>T, p. Arg336Trp, in *trans* with c.625G>A, p. Ala209Thr, which is predicted to be deleterious.

This patient continues to show normal development, without any signs or symptoms of disease, and without any treatment.

Patient 3

Patient 3 is a 4-year-old girl, born in Quebec to French-Canadian parents, who are first cousins. Her urine newborn screening results were negative. She was followed by metabolic specialists because of cyclic vomiting episodes, which began at age 4 months while on breastfeeding, in a

context of gross motor delay, deafness and axial hypotonia. She had a single palmar crease, inverted nipples, frontal bossing, left torticollis and right plagiocephaly. Her head circumference at 6 months had increased to the 90th percentile from the 5th percentile at birth. MRI at age 6 months (Supplementary Fig. 1) showed minimal delayed myelination at the genu of the corpus callosum, and axial CSF space enlargement which raised concern about possible GA1. MR spectroscopy was normal. A repeat MRI at age 1 year was normal.

She continued to have severe episodes with intractable vomiting (10–12 times within a few hours) and dehydration, accompanied by lethargy, metabolic acidosis and ketonuria, with hypoglycemia documented during one episode. These crises occurred at intervals of 2 weeks to 2 months. She was started on L-carnitine (100 mg/kg/day) and riboflavin (100 mg bid).

Plasma acylcarnitine and amino acid profiles were essentially unremarkable. Urine organic acid profiles were somewhat variable; at first they were considered probably non-specific and partly related to gastro-intestinal sources or nutritional status, but GA elevation was noted to be a persistent feature. Profiles were not suggestive of GA1 or GA2. Genetic testing for GA1 (analysis of the *GCDH* gene) was negative. Sequencing of *SUGCT* showed homozygosity for c.1006C>T, p. Arg336Trp, establishing the diagnosis of GA3.

Over time the patient's parents noticed that she had changes in stool quality together with decreased energy and general unwellness in the days immediately prior to each acute episode. This temporal association, recognisable as a recurrent prodrome, prompted clinical suspicion that the episodes could reflect an interplay between the metabolic changes due to GA3 and gut bacterial metabolism. This hypothesis seemed plausible particularly because it is known that free GA can be produced by gut bacteria (Wendel et al. 1995; Kumps et al. 2002). Treatment with the antibiotic metronidazole, to "sterilise" the gut, was therefore introduced on a trial basis (10 mg/kg/day, divided tid, given 10 days per month).

We later noted elevations of several *N*-acetyl-amino acids in urine samples (data not shown). This observation was persistent, being confirmed retrospectively in samples from 5 months of age onwards, and is characteristic of aminoacylase 1 (ACY1) deficiency (Sass et al. 2006; Gerlo et al. 2006). This represented a second unrelated diagnosis for this patient, independent of her diagnosis of GA3. ACY1 deficiency, like GA3, is a condition of uncertain clinical significance; widely considered benign or likely benign, but observed in some patients with non-specific neurological findings (Sass et al. 2007; Tytki-Szymanska et al. 2010). However, it has not been reported in association with episodic metabolic disturbance or cyclic vomiting, therefore did not provide an explanation for this patient's major symptoms.

Other metabolic and genetic testing, for a possible other disorder which could explain the symptoms, especially the cyclic vomiting, has thus far been normal. This has included extensive biochemical workup, mitochondrial DNA sequencing and deletion/duplication testing, molecular analysis for channelopathies and sequencing of the HMG CoA lyase (*HMGCL*) and synthase (*HMGCS2*) genes.

The patient has been doing much better, with only one crisis over the last 18 months, since metronidazole was started. She has sensorineural hearing loss, for which she wears hearing aids. She still has developmental delay, but this is mild and consists of learning difficulties only. There are no other neurological or clinical concerns.

Metabolite Profiles in Urine

Quantitative urine organic acid profiling, by GC-MS following trimethylsilyl derivatisation, was performed at CHUS during clinical investigations of patients 2 and 3. Other investigations for patient 2, at Children's Hospital of Eastern Ontario (CHEO), included analysis of dried urine spots for GA and 3HGA by a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay (Al-Dirba-

shi et al. 2011), with quantification of glutarylcarntine (C5DC) and associated acylcarntine ratios by MS/MS (Tortorelli et al. 2005). Patient 3 also had urine acylglycine profiling performed at CHUS by LC-MS/MS (Bherer et al. 2015). Exchanges of available samples later provided results from all methods for all three patients.

Table 1 summarises urine organic acid and acylcarntine results. Elevation of GA was observed in all urine samples from all patients, except the original newborn screening sample (3-1) from Patient 3. GA values from the GC/MS organic acid profile method and from the dedicated LC-MS/MS method were broadly similar. No sample showed elevation of 3HGA, by either method, nor was 2HGA elevated in any organic acid profile (data not shown). Urine C5DC (expressed relative to creatinine concentration and also as ratios to C5OH and to C5) was consistently normal. These findings were in accord with GA3.

Several samples from Patient 3 showed elevations of organic acids other than GA, including products of gut floral metabolism as well as ketones, dicarboxylic acids and mild elevations of some citric acid cycle metabolites, all supposed secondary to a catabolic state related to vomiting episodes. Her GA levels showed some apparent correlation with variations in her condition, as relatively modest GA elevations were observed at times when the profile was otherwise unremarkable. 3-hydroxy-3-methylglutaric acid (HMG) was elevated in several samples from Patient 3, again tending to correlate with catabolic state. HMG was also somewhat elevated in samples from Patient 2, while his organic acid profiles were otherwise unremarkable.

Acylglycine profiling of urine samples from Patient 3 showed persistent elevations of acetylglutamine, propionylglutamine, crotonylglutamine and butyrylglutamine in all specimens, including the original newborn screening filter paper sample (Table 2). Mild elevations of isobutyrylglutamine and valerylglutamine were also seen in some samples, while all other acylglycines assayed were within reference range (data not shown). No obvious correlations were identified between acylglycine concentrations and concentrations of organic acids in the corresponding samples. Subsequent acylglycine analysis of available specimens from Patients 1 and 2 (samples 1-1, 2-1 and 2-3), by the same method, gave essentially normal results (data not shown). However, analysis of a control sample from an individual with ACY1 deficiency gave a similar profile to those of Patient 3.

Sequence Variants in *SUGCT* (*C7orf10*)

Table 3 summarises the variants identified in our patients, and all previously published variants, with population frequencies from the ExAC database (<http://exac.broadinstitute.org>).

Discussion

We present three cases of GA3; two investigated because of clinical symptoms, the other identified through population screening. There are very few published reports of GA3 patients. Considering observed allele frequencies (Table 3), frequency of GA3 is estimated at ~1/11,000 (European) to ~1/27,000 (Global). This condition is clearly underdiagnosed.

One reason is that some GA3 individuals, perhaps most, remain asymptomatic. GA3 is not detectable by bloodspot acylcarnitine-based newborn screening, as GA3 does not result in accumulation of glutaryl-coA, hence glutarylcarnitine is not elevated. Very few jurisdictions perform urine newborn screening for organic acidemias. In Quebec, only one case of GA3 has been identified by this route in the 16 years (1,161,000 infants screened) since addition of GA1 as a target of the provincial urine screening programme (Auray-Blais et al. 2007); probably because GA was below the detection threshold in most cases.

Some GA3 individuals could have symptoms which are insufficiently specific, severe or persistent to prompt consideration of an inherited disorder. There are other potential reasons why GA3 could be missed, even when “metabolic work-up” is performed. GA excretion levels are variable and often moderate. GA3 is described as presenting with “isolated glutaric aciduria,” however this was not always the case with our patients (Table 1); in Patient 3 other acids were often more prominently elevated than GA. Moderate GA elevations are often seen in clinical laboratories; causes or associations (Kumps et al. 2002; Boy et al. 2017) include gut bacterial metabolism and primary or secondary mitochondrial dysfunction. With no known biomarker other than GA, GA3 has been a “diagnosis of exclusion,” especially while no genetic or enzymatic test was available. Even with the relevant gene now known, DNA testing is normally only initiated following suspicion based on recognised clinical manifestations or metabolite profiles.

Patient 3 presented with cyclic vomiting. This was true of the first reported GA3 patient (Bennett et al. 1991), while another presented with acute gastroenteritis (Knerr et al. 2002). Both were possibly attributable to, or influenced by, co-existing conditions. A recent abstract (Skaricic et al. 2016) mentions two new patients, one with recurrent vomiting. Intracellular accumulation of free GA, a direct result of GA3, could contribute directly to gastrointestinal disturbances. Our experience with Patient 3 suggests that antibiotic treatment, to minimise bacterial GA production, may be helpful in this context. The significance of interplay between human metabolism and gut microbiome is becoming increasingly recognised, including in patients with inborn errors (Gertsman et al. 2015).

We note that gastrointestinal disturbances are not typical features of either GA1 or GA2, although both conditions are associated with increased GA in body fluids. However, the biochemical basis of GA3 is fundamentally different from that of GA1 or GA2: only in GA3 is the free acid itself a primary substrate of the enzyme, liable to direct accumulation within cells, whereas the elevations of GA observed in blood and urine in GA1 or GA2 arise by a more indirect route via accumulation of glutaryl-coA.

Clinical concerns for Patients 1 and 3 included lethargy, hypotonia and developmental issues. Both had MRI anomalies, albeit minimal and non-specific or transient. Neurological and developmental problems have been described in other GA3 patients (Bennett et al. 1991; Knerr et al. 2002; Skaricic et al. 2016), although some were transient or potentially explicable by other factors.

Patient 3 also has evidence of ACY1 deficiency. We cannot exclude a small possibility that this contributed to her mild developmental delay, but there is no obvious association between ACY1 deficiency and her other symptoms.

There is presently insufficient evidence to define any clinical phenotype as attributable to GA3. However, it would be premature to assume that GA3 is completely benign in all patients at all times. An international collaboration to compile histories of known patients might elucidate common threads, or provide stronger evidence for a lack thereof. Similar considerations apply to ACY1 deficiency.

The biochemical characterisation of GA3 also remains incomplete. For example, elevations of 3-hydroxy-3-methylglutaric acid (HMG) in several samples from two patients (Table 1) were initially discounted as non-specific. HMG is in fact an alternative substrate for C7orf10 *in vitro* (Marlaire et al. 2014), but its *in vivo* significance (if any) in GA3 patients is unknown. Specificity studies with other possible CoA donors and acceptors examined only a few candidate molecules.

The persistently abnormal urine acylglycine profile of Patient 3 was intriguing. There was no obvious link with what is known of the biochemical basis of GA3, and we did not observe similar profiles in our two other GA3 patients. We have, however, seen similar patterns in some other patients in decompensated states, with or without an underlying primary disorder of energy metabolism. It later became apparent that the profile could be attributed in this case to co-existing ACY1 deficiency. N-acetylglycine is a known marker of ACY1 deficiency. It is reasonable to suppose that propionylglycine, crotonylglycine and butyrylglycine (Table 2) are also substrates of ACY1. Broader metabolomic studies on larger groups of GA3 and ACY1-deficient individuals could be worthwhile, particularly in an era where many metabolic enzymes are newly recognised to have additional diverse and significant “moonlighting” roles in other cellular processes (Zschocke 2012; Vilardo and Rossmann 2015; Boukouris et al. 2016).

Table 3 Sequence variants in *SUGCT* (*C7orf10*) and their frequencies

Variant designations ^a					Allele frequencies ^b	
cDNA	Protein	dbSNP	Identified in this study?	Previously published?	European	Global
c.1006C>T	p.Arg336Trp	rs137852860	Patients 1, 2, 3	Yes ^{c-e}	0.009295	0.005642
c.826G>A	p.Val276Ile	rs750657344	Patient 1	No	0.00004132	0.00006313
c.625G>A	p.Ala209Thr	rs781200920	Patient 2	No	Not found	0.000008293
c.322C>T	p.Arg108Ter	rs137852862	No	Yes ^c	0.0002758	0.0003234
c.535C>T	p.Arg179Ter	rs137852861	No	Yes ^{c,e,f}	0.00001498	0.00001656
All five variants (totals of allele frequencies)					0.009627 (1/104)	0.006053 (1/165)

^a Variant designations (cDNA and protein) used in the present study are based on NCBI Reference Sequence NM_001193313.1/NP_001180242.1

^b The quoted allele frequencies were obtained from the ExAC (Exome Aggregation Consortium) database (<http://exac.broadinstitute.org>), which includes 60,706 individuals, of whom approximately half are “European (non-Finnish)”

^c Sherman et al. (2008)

^d Variant originally referred to as c.895C>T, p.Arg299Trp

^e Differences in numbering of some variants, versus the original descriptions by Sherman et al. (2008), are related to the use of a newer reference sequence (also discussed by Marlaire et al. 2014)

^f Variant originally referred to as c.424C>T, p.Arg142Ter

In summary, we have described three individuals with GA3, two of whom showed clinical symptoms. We propose that this condition may have some clinical significance, if only in a subset of patients or in combination with other factors, and that this possibility warrants investigation.

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Synopsis

Glutaric aciduria type 3 may have some clinical significance, if only in a subset of patients or in combination with other factors.

Compliance with Ethics Guidelines

Author Contributions

PJW reviewed and compiled laboratory data and literature, co-ordinated communications with all authors, and wrote much of the manuscript. TMK and WAH initiated a preliminary report of Patients 1 and 3 (poster presentation 204, SSIEM 2014, Kitzler et al., JIMD 37 Suppl. 1:S97), which served as a starting point. TMK, AF, MTG, KS, YT, CBG and WAH (physicians) contributed patient data and clinical descriptions. OAD, PB, CAB and NI (scientists/analysts) generated and contributed biochemical laboratory results. SG reviewed and discussed molecular genetic data

and population genetic aspects. All authors critically reviewed the first draft and also approved the final manuscript for submission.

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Conflict of Interest Statements

Paula J. Waters, Thomas M. Kitzler, Annette Feigenbaum, Michael T. Geraghty, Osama Al-Dirbashi, Patrick Bherer, Christiane Auray-Blais, Serge Gravel, Nathan McIntosh, Komudi Siriwardena, Yannis Trakadis, Catherine Brunel-Guitton and Walla Al-Hertani declare that they have no conflict of interest.

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Ethics Approval

This article does not contain any experimental studies with human or animal subjects performed by any of the authors. Ethics approval was not required for this study.

Patient Consent Statement

Informed consent for publication was obtained from the parents of all patients for whom any identifying information is included in this article.

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