Two patients with unexplained raised glutamine concentrations

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Case 1 presentation

- 3y old girl presented to BCH
- Global developmental delay
  - Talking and crawling within first year, but stopped by 12 months
  - Hypotonia with tremor and ataxia
  - Speech delay

Lab investigations

- LFTs normal
- Ammonia normal
- Lactate normal
- Urine Organic acids normal
- Carnitines/VLCFA normal

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Plasma amino acids – Case 1 at presentation

Plasma aa	µmol/L	
Glutamine	1843	305-700
Alanine	505	119-325
Glycine	386	95-332
Proline	397	83-384
Threonine	332	36-171
Lysine	299	63-245
Citrulline	13	5-44
Glutamate	77	26-134
Ornithine	78	21-103
Arginine	158	17-105
ASA (Arginosuccinic acid)	Not detected	

Case 2 Presentation

- 2y old boy (unrelated to case 1) presented around the same time as case 1
- Delayed motor milestones, not able to walk and delayed speech
- Poor balance, falls frequently
- LFTs normal
- Other metabolic investigations normal

Plasma QAA at presentation – Case 2

- Glutamine was grossly increased and suggests hyperammonaemia
- Increased alanine and proline suggests lactic acidosis
- Ammonia – normal (referral lab)

Plasma aa	µmol/L	
Glutamine	2021	305-700
Alanine	933	119-525
Glycine	371	95-332
Proline	693	83-384
Threonine	464	36-171
Citrulline	18	5-44
Glutamate	92	26-134
Ornithine	71	21-103
Arginine	55	17-105
ASA	Not detected	

Metabolic investigations – ?diagnosis

- Both cases grossly raised glutamine (but ammonia normal)
- ?Urea cycle disorder

Disorder	Plasma amino acids raised	Urine orotic acid
Carbomyl Phosphate Synthase (CPS1 deficiency)	Glutamine, alanine	Normal
Ornithine transcarbomylase (OTC defieciency)	Glutamine, arginine	Large increase
Citrullinaemia	Citrulline	Increase
Arginosuccinic aciduria	Arginosuccinic Citrulline	Increase
Hyperarginaemia	Arginine	Increase
N-acetylglutamate synthetase deficiency	Glutamine, alanine	Normal

Metabolic investigations – ?diagnosis

- Urine orotic acid – normal
- Genetics
  - No mutation in urea cycle genes (CPS, NAG, OTC)
- Liver biopsy
  - enzymes normal CPS, NAG, OTC
- Urea cycle disorder excluded

Treatment

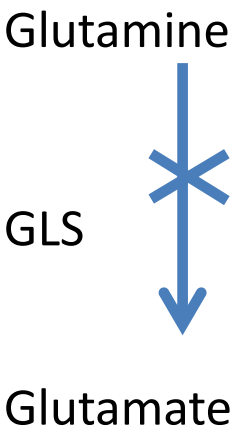
- Initially given sodium phenylbutyrate (ammonia scavenger)
- Glutamine levels did not change significantly
- No significant clinical improvement. So stopped.
- Avoid high protein foods

Further investigations

- Monitoring over following 3 years
  - Continued unexplained high glutamine >1000µmol/L
  - Clinically severe ataxia
- 100K genome project and TIDEX study in Vancouver (treatable causes intellectual disability)

Diagnosis? – unexplained raised glutamine

Based on biochemical findings, Glutaminase deficiency considered. But no reports of glutaminase deficiency in literature



- Glutamate major excitatory neurotransmitter in brain
- Enzyme important for neurotransmission and neurogenesis

Glutaminase deficiency

- Glutaminase activity in skin fibroblasts (Amsterdam) - markedly reduced activity in both cases
- CSF glutamate and glutamine: Case 1
    - CSF snap frozen
- Glutamate 0.7 µmol/L (<8.3)
- Glutamine 714.4 (<658.4) : slight elevation

Genetic Testing	Case 1	Case 2
Glutaminase Gene (*GLS*)	Pathogenic mutation from father	No variants detected
Whole genome sequencing	Identified a novel trinucleotide GCA repeat expansion around promoter region GLS from her mum, resulting in reduced gene expression	homozygous for GCA Trinucleotide repeat expansion around promoter region (GLS)

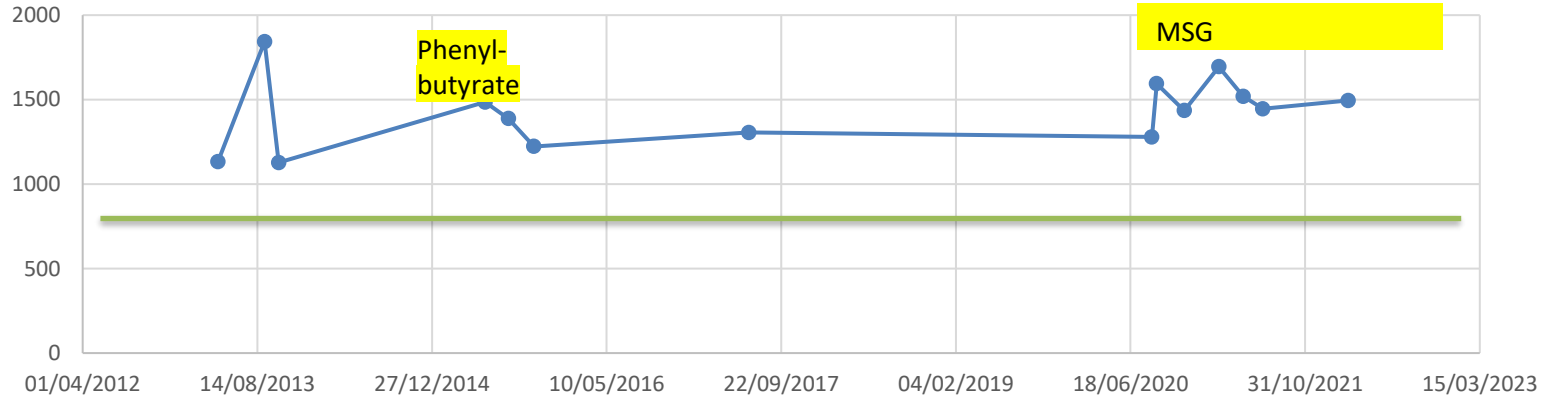
Further treatment – both cases

- Parents and siblings – all had plasma glutamine levels within normal range
- Both cases persistent raised glutamine >1000 umol/L
- Both progressive ataxia – dependent on walking frame/wheelchair
- Treatment with sodium phenylbutyrate ineffective in both patients
- ?Pathophysiology related to glutamate deficiency rather than high glutamine

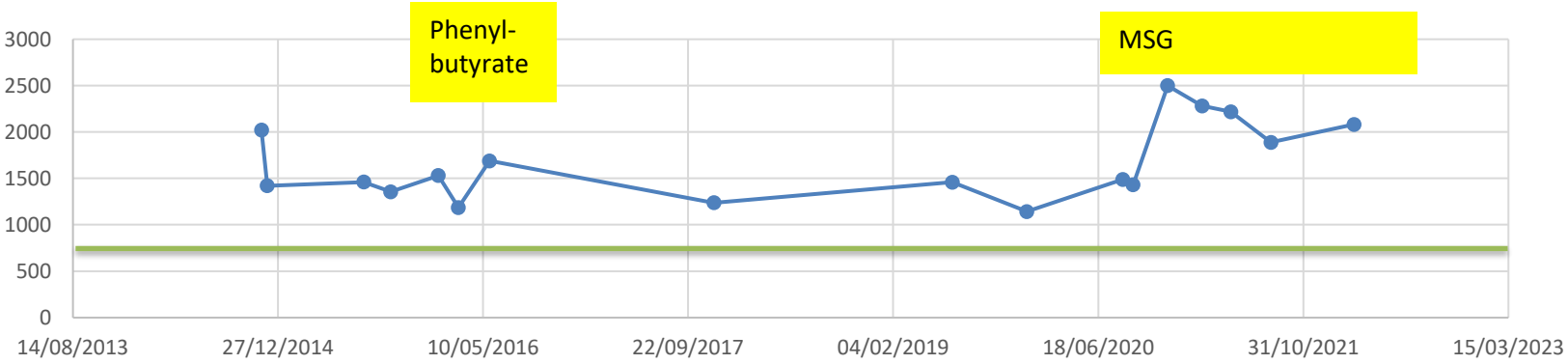
Treatment trial

- Pre-clinical work suggests intracellular glutamate deficiency (since glutamate levels in blood and CSF are normal)
- Potential treatment with glutamate supplementation
  - Approval for clinical trial in Summer 2020
  - Monosodium glutamate will be gradually increased to a total dose of 2g/kg/day in three divided doses
  - Study is designed to assess changes in biochemical parameters, clinical measures of ataxia/mobility, neurological function and development and quality of life over a 2 year period.
  - Preliminary results are encouraging

Glutamine levels case 1



Glutamine levels case 2



Summary

- 2 cases presenting with early-onset motor delay and delayed speech, progressive ataxia
- Persistent raised plasma glutamine despite normal ammonia levels
- Urea cycle defects excluded
- Whole genome sequencing identified a novel trinucleotide GCA repeat expansion around promoter region GLS, resulting in reduced expression
- Novel inherited metabolic disorder
- Potential for treatment with glutamate supplementation (MSG)

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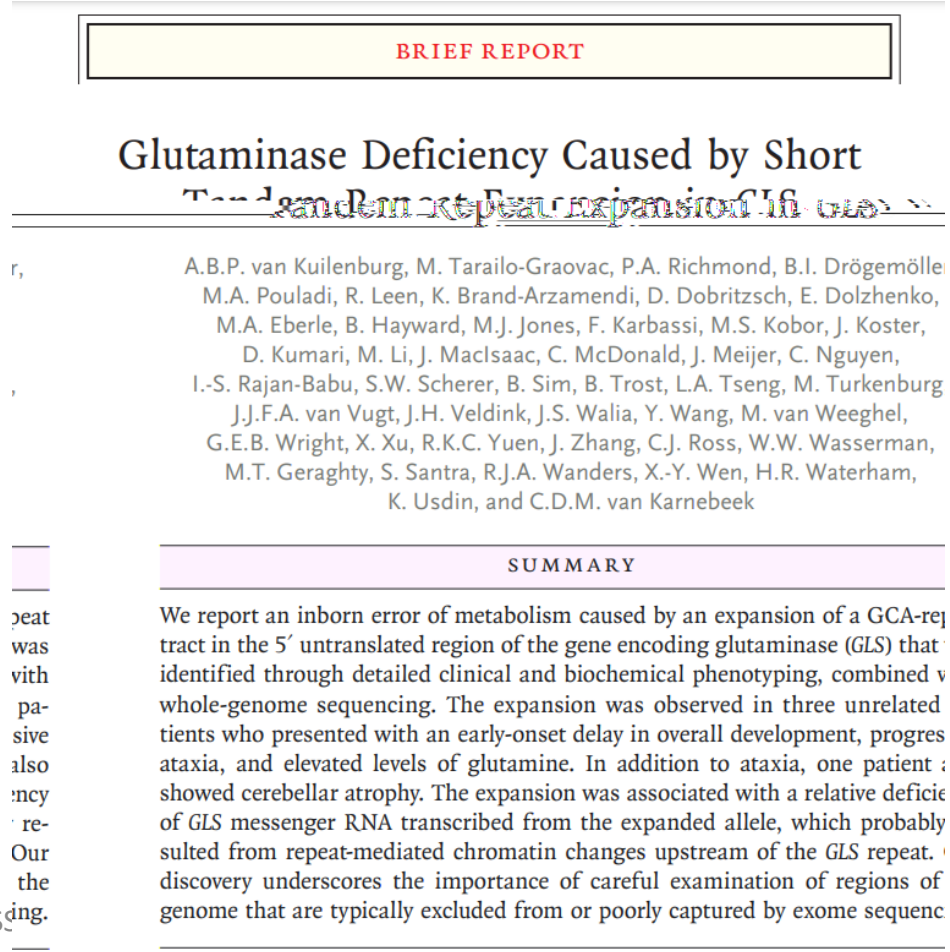

BRIEF REPORT

Glutaminase Deficiency Caused by Short Tandem Repeat Expansion in GLS

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SUMMARY

We report an inborn error of metabolism caused by an expansion of a GCA-rep
tract in the 5′ untranslated region of the gene encoding glutaminase (*GLS*) that
identified through detailed clinical and biochemical phenotyping, combined v
whole-genome sequencing. The expansion was observed in three unrelated
tients who presented with an early-onset delay in overall development, progres
ataxia, and elevated levels of glutamine. In addition to ataxia, one patient
showed cerebellar atrophy. The expansion was associated with a relative deficie
of *GLS* messenger RNA transcribed from the expanded allele, which probably
sulted from repeat-mediated chromatin changes upstream of the *GLS* repeat.
discovery underscores the importance of careful examination of regions of
genome that are typically excluded from or poorly captured by exome sequenc