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¹H MRS identifies symptomatic and asymptomatic subjects with partial ornithine transcarbamylase deficiency

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Abstract

Objective—To evaluate brain metabolism in subjects with partial ornithine transcarbamylase deficiency (OTCD) utilizing ¹H MRS.

Methods—Single voxel ¹H MRS was performed on 25 medically-stable adults with partial OTCD, and 22 similarly aged controls. Metabolite concentrations from frontal and parietal white matter (FWM, PWM), frontal gray matter (FGM), posterior cingulate gray matter (PCGM), and thalamus (tha) were compared with controls and IQ, plasma ammonia, glutamine, and disease severity.

Results—Cases ranged from 19–59 years; average 34 years; controls ranged from 18–59 years; average 33 years. IQ scores were lower in cases (full scale 111 vs. 126; performance IQ 106 vs. 117). Decreased myoinositol (mI) in FWM (p=0.005), PWM (p<0.001), PCGM (p=0.003), and tha (p=0.004), identified subjects with OTCD, including asymptomatic heterozygotes. Glutamine (gln) was increased in FWM (p<0.001), PWM (p<0.001), FGM (p=0.002), and PCGM (p=0.001). Disease severity was inversely correlated with [mI] in PWM (r= -0.403; p= 0.046) and [gln] in PCGM (r= 0.548; p=0.005). N-acetylaspartate (NAA) was elevated in PWM (p=0.002); choline was decreased in FWM (p=0.001) and tha (p=0.002). There was an inverse relationship between [mI] and [gln] in cases only. Total buffering capacity (measured by [mI/mI + gln] ratio, a measure

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of total osmolar capacity) was inversely correlated with disease severity in FWM ($r = -0.479$; $p = 0.018$), PWM ($r = -0.458$; $p = 0.021$), PCGM ($r = -0.567$; $p = 0.003$), and tha ($r = -0.345$; $p = 0.037$).

Conclusion—Brain metabolism is impaired in partial OTCD. Depletion of mI and total buffering capacity are inversely correlated with disease severity, and serve as biomarkers.

Keywords

ammonia; glutamine; hyperammonemia; heterozygote; metabolic; myoinositol; ornithine transcarbamylase deficiency; magnetic resonance imaging spectroscopy

Introduction

The urea cycle disorders (UCDs) represent one of the most common groups of inborn errors of metabolism, with an estimated incidence of 1 in 30,000 [1,2]. Clinical syndromes involving deficiencies of five urea cycle enzymes and three related cofactors and transporters have been described [3–5]. Ornithine transcarbamylase deficiency (OTCD) is the only X-linked disorder of ureagenesis. The estimated incidence is 1 in 14,000 [6]. Over 240 mutations have been identified in the OTCD gene [7]. About 60% of hemizygous males have a mutation around the enzyme active site and present with hyperammonemic coma in the newborn period [8,9]. The remaining 40% of patients demonstrate more peripheral mutations in the gene, with a less severe phenotype, often with later onset of symptoms [10].

The majority of children with complete OTCD have substantial cognitive and motor deficits associated with neonatal hyperammonemic episodes [8,11–14]. In males with partial deficiencies, disease onset is later and outcome is better, although many individuals manifest cognitive, motor and/or psychiatric sequelae [15–17]. Females heterozygous for OTCD show a broad phenotype due to both allelic heterogeneity and differential X-inactivation patterns. Based on history, approximately 85% of heterozygous females are considered to be asymptomatic; the remainder show symptoms ranging from behavioral and learning disabilities and protein intolerance to cyclical vomiting, stroke-like episodes and hyperammonemic coma [18–22].

Gyato et al demonstrated evidence of specific neurocognitive deficits in females with partial OTCD [23]. Despite IQ scores in the average range, these women displayed a specific neurobehavioral phenotype characterized by strengths in verbal intelligence, verbal learning/memory and reading, but weaknesses or greater variability in nonverbal intelligence, fine motor/dexterity/speed, visual memory, attention and executive skills, and math. This neurocognitive profile was found in symptomatic and asymptomatic females, and supports a nonverbal learning disability, typically associated with white matter or subcortical dysfunction.

In this study, using ^1H MRS, we sought to investigate biochemical changes that may underlie neuronal damage in individuals with late-onset or partial OTCD, imaged in stable medical condition, and to assess brain biochemical changes that may exist in asymptomatic female carriers of OTCD gene mutations. If present, these changes might provide a possible explanation for cognitive deficits previously noted in symptomatic and asymptomatic female heterozygotes. This study represents the largest series of patients with partial OTCD studied by ^1H MRS, and includes the largest number of asymptomatic OTCD individuals studied by this method.

Material and Methods

Participants

Twenty-five adult patients with partial OTCD (20 females, 5 males) and 22 adult control subjects (12 females, 10 males) without UCDs or neurological symptoms participated in this study. Subjects were recruited as part of an NIH-funded Rare Diseases Clinical Research Center established to study the natural history of UCDs. The protocol was approved by local IRBs and all subjects provided informed consent. We included males with late-onset OTCD as well as both asymptomatic and symptomatic females heterozygous for OTCD, with varying ages of diagnosis and metabolic control who had IQ scores above 80 as measured by the Wechsler Abbreviated Scales of Intelligence, (WASI). Patients ranged in age from 19–59 years (mean 34 years); controls ranged in age from 18–59 years (mean 33 years). Patients and healthy normal volunteers were excluded if there was a history of another neurological or psychological condition. Patients were rated by a UCD severity score adapted from a previously reported scoring system [13]. Patients were classified as being symptomatic or asymptomatic based on clinical symptoms and results of previous stable isotope studies, if available. Patient demographics are listed in Table 1.

Biochemical studies

Blood and urine were collected from subjects on the day of spectroscopy imaging studies. All blood samples were collected while subjects were in a post-absorptive state. Plasma amino acids were deproteinized and analyzed by ion exchange chromatography (Beckman or Toshiba), with detection of ninhydrin metabolites. Urine organic acids were extracted in ether acetate, dried, derivitized with BSTFA 10% TMCS and washed and analyzed by electrospray ionization used for GC/MS of organic acids (Agilent Technologies). Ammonia levels were measured using a Dade-Behring RXL analyzer. Data are presented in Table 1.

MRI acquisition and processing

Data Acquisition—MRI was performed using a 3T whole-body MRI scanner (Siemens MAGNETOM Trio, Erlangen, Germany) and an eight-channel phased array head coil. Head movement was minimized by padding fitted to hold the subject's head firmly and comfortably in the coil.

Structural MRI

High-resolution T1-weighted, MPRAGE scans were collected and were used for MRS voxel positioning as well as to provide the basis for tissue type estimates used in partial volume correction during subsequent data processing. The structural protocol consisted of three MPRAGE scans acquired during the same scanning session with the following parameters: TR = 1600ms, TE = 4.38ms, TI = 640ms, flip angle 15°, averages = 1, 160 slices with a 1.0mm thickness, FOV = 256 × 256mm², effective resolution = 1.0×1.0×1.0mm³, total scan time = 6:51 minutes. A clinical sequence of conventional T1 and T2 Axial and FLAIR images was also obtained.

Magnetic Resonance Spectroscopy

We used short echo time PRESS MRS (TE 30ms), to look at distribution of glutamine as well as glutamine plus glutamate (Glx) and myoinositol (mI), compounds that have been shown to be altered in OTCD [24, 25] and are not seen clearly on long TE scans. All spectroscopy measurements were acquired using a manufacturer-supplied eight-channel phased array head coil. To optimize signal-to-noise we employed single-voxel spectroscopy using 2×2×2 cm³ volumes of interest (VOIs) in parietal white matter (PWM), posterior cingulate gray matter (PCGM), thalamus (tha), frontal gray matter (FGM), and frontal white

matter (FWM). We chose these areas because they represent regions that display possible abnormalities in neuroimaging studies of patients with OTCD. Voxel locations are shown in Figure 1. A point-resolved spectroscopy (PRESS) sequence with a TE 30ms, TR 2000ms, and 200 averages was used. Automatic and manual shimming was performed before each acquisition. A full-width at half maximum of 0.1ppm was used as the upper cut-off for acceptable shim results. An additional 16 second reference spectrum was obtained from the same voxel location without water suppression for eddy current correction and absolute metabolite concentration estimates. In this study, we investigated metabolite concentrations of tNAA (representing the total pool of NAA+NAAG), tCreatine (reflecting creatine and phosphocreatine), choline, lactate, mI, gln, glu, and glx.

Estimates of metabolite concentrations

The contribution of individual metabolites to the in vivo spectrum was quantified using the analysis program 'LCModel' (Provencher Inc., Oakville, Canada) [26]. The method is fully automatic, with no subjective inputs needed for phasing, referencing and initial estimates. The in vivo spectrum is modeled as a linear combination of adequately line-broadened individual metabolite spectra. A standard basis set was used for this analysis, which was acquired on an equivalent Siemens 3T Trio scanner. Metabolites that are known to contribute to brain spectra are provided in the basis set, which in this study included the following compounds: Alanine (Ala), aspartate (Asp), glycerolphosphoryl-choline (GPC), creatine (Cr), γ -aminobutyric acid (GABA), glucose (Glc), glutamine (Gln), glutamate (Glu), lactate (Lac), N-acetylaspartate (NAA), N-acetyl-aspartyl-glutamate (NAAG), myoinositol (mI), lipids, and macromolecules. The intensity of the in vivo spectrum was calibrated using the water signal from the same voxel as an internal reference, assuming water content of 43300mM for gray matter and 35880mM for white matter [27]. A full-width half maximum of metabolites greater than 0.071-0.1ppm coupled with CRMVB >20% for large peak metabolites and >30% for glutamate and glutamine were used as criteria for rejection of spectra or individual metabolite spectra. Unexplainable features in the spectra such as unusual residuals, grossly asymmetrical line-shape, split peaks – usually motion-induced, and outer volume ghosting were also taken into account for exclusion [28].

Partial volume correction

A high-resolution anatomic scan was used for graphical positioning of the MRS voxels. These structural data were used to determine tissue composition of the sampling voxel for use in partial volume correction using a voxel-based morphometry method that involves voxel-wise comparison of the local concentration of gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) between groups of subjects, and involves spatially normalizing high-resolution images from all subjects in the study into the same stereotactic space followed by segmenting the GM, WM, and CSF spaces from the spatially-normalized images. Voxel-wise parametric statistical tests that compared the smoothed images from the two groups were performed. Corrections for multiple comparisons were made using the theory of Gaussian random fields. The voxels from the different locations were transformed onto GM, WM, and CSF maps to obtain voxel tissue content, used for voxel CSF content correction.

Each spectroscopic voxel was co-registered with the segmented images to derive gray matter, white matter, and cerebrospinal fluid tissue composition. Varying CSF content was corrected for 'LCModel' concentration estimates using the formula:

$$C_{\text{corrected}} = \frac{C_{\text{uncorrected}}}{\left(\frac{100 - \% \text{CSF}}{100}\right)}$$

The result from the tissue segmentation processing was used for correction of VOI CSF content.

Reliability estimation

Assessment of the reliability of the metabolite concentrations was based on the Cramér-Rao lower bounds (CRBs), from Cramér-Rao inequality, estimates of standard deviations of the fit for each metabolite given in percent SD [29]. Use of a model of complete spectra allows the incorporation of prior knowledge, which can significantly reduce the CRBs.

Statistical analysis

After partial volume correction, and quantitation by 'LCModel', absolute metabolite concentrations were compared using a two-tailed t-test in subjects versus controls. Regression analysis against other clinical variables was performed with all statistics performed using SPSS (version 16). For each of the seven metabolite measurements (NAA, choline, creatine, glutamine, glutamate, myoinositol, and glx) two tailed t-tests of significance ($p=0.05$) were retested using a Bonferroni corrected Type I error rate of $0.05/7 = 0.007$ in order to account for multiple comparisons.

Results

Clinical features

Patient demographics are reported in Table 1. Fifteen subjects were clinically symptomatic, ten were asymptomatic. FSIQ scores were in the low-average to above-average range. Mutations had been identified in 21/25 subjects (2 are pending; 2 unknown) and were associated with neonatal ($n=11$) or late-onset disease ($n=10$). There was no correlation between genotype alone and imaging phenotype.

Neuroimaging

High-resolution Sagittal T1, Axial T1, T2, FLAIR, and coronal images were reviewed by a neuroradiologist blinded to clinical history. No structural abnormalities were found in gray or white matter on T1 and T2 weighted images, despite a history of hyperammonemia in many subjects (data not presented). FLAIR imaging disclosed periventricular and white matter hyperintensities in several subjects who had had recent hyperammonemic episodes (data not shown).

Metabolite differences distinguish subjects from controls

Myoinositol was decreased in patients in FWM ($p=0.005$), PWM ($p<0.001$), PCGM ($p=0.003$), and tha ($p=0.004$) (Figure 2a–e) in both symptomatic and asymptomatic individuals. Although subjects were imaged in a medically-stable state, glutamine concentration was elevated in subjects, including many self reported asymptomatic females in FWM ($p<0.001$), PWM ($p<0.001$), FGM ($p=0.002$), and PCGM ($p=0.001$) with trends towards increased gln in thalamus. (Figure 2a–e).

The degree of mI decrease was inversely correlated with disease severity score, a measure reflecting total number of hyperammonemic episodes and coma (PWM $r=-0.403$, $p=0.046$ (data shown in Figure 3); PCGM $r=-0.430$, $p=0.032$; FWM $r=-0.397$, $p=0.054$) as well as gln concentrations (PCGM $r=0.548$, $p=0.005$ (data shown in Figure 3); PWM $r=0.511$, $p=0.009$; FWM $r=0.502$, $p=0.013$; FGM $r=0.561$, $p=0.024$; tha $r=0.461$, $p=0.031$). There was a positive correlation between plasma and brain gln in FWM, PWM, and PCGM (FWM $r=0.464$; $p=0.022$; PWM $r=0.408$; $p=0.043$; PCGM $r=0.445$; $p=0.026$) (Figure 4). When subjects with OTCD were divided into symptomatic and asymptomatic subgroups, based on

either clinical history or disease severity scores, the asymptomatic subgroup had a smaller magnitude of change in metabolites than the symptomatic subgroup in all regions of interest (data not shown). For example, in subjects who had ever had an episode of hyperammonemia, the mI level was about half that seen in age matched controls. However, asymptomatic subjects with OTCD also showed decreased mI compared to age-matched control subjects. Choline was decreased in subjects in thalamus ($p=0.002$) and FWM ($p=0.001$) (Figure 2). NAA was elevated in subjects in PWM ($p=0.002$). There was an inverse relationship between brain gln and mI in subjects but not controls (Figure 5) ($r=-0.608$; $p=0.002$). Total buffering capacity as measured by mI/mI + gln ratio (which accounts for the total measured osmolar capacity) was inversely correlated with disease severity in FWM ($r=-0.479$; $p=0.018$); PWM ($r=-0.458$; $p=0.021$); PCGM ($r=-0.567$; $p=0.003$); tha ($r=-0.345$; $p=0.037$) (Figure 6a showing all brain regions; Figure 6b data shown for PCGM). Overlapping spectroscopic metabolite differences between an affected subject and an age-matched control are displayed in Figure 7 for PWM.

Biochemical correlations

Sixteen subjects had normal blood ammonia levels (normal range determined by our laboratory is 11–32 $\mu\text{mol/L}$) and nine subjects had elevations of ammonia (mild: range 35–68 $\mu\text{mol/L}$; with one subject having an ammonia level of 152 $\mu\text{mol/L}$) (Table 1). We found no consistent correlations between blood ammonia and glutamine levels. In subjects with OTCD, plasma levels of gln but not ammonia correlated with brain gln in specific brain regions (Figure 4).

Discussion

We demonstrated brain biochemical differences by ^1H MRS in medically-stable adults with partial OTCD, even in individuals who were considered asymptomatic with normal anatomic MRI findings. We found a decrease in mI in PWM, FWM, PCGM, and tha as well as elevated gln in PCGM, PWM, FGM, and FWM in many asymptomatic subjects, and elevated NAA in PWM of subjects. The concentration of mI was inversely correlated with disease severity score (Figure 3a, b, c) and plasma gln levels. Glutamine concentration was elevated in subjects, both in symptomatic and many asymptomatic females. Choline was decreased in thalamus and in FWM. Myoinositol and gln exhibit an inverse relationship in subjects with partial OTCD reflecting the presumed osmotic compensation with mI depletion in the face of increased gln production due to limitations of the astrocyte to continue to provide nitrogen buffering in the face of hyperammonemia in OTCD (Figure 5).

This observation suggests unrecognized biochemical disturbances that may also underlie previous findings of cognitive impairments in a pattern indicating a white matter injury model. It may also serve as a biomarker to identify carriers of a gene mutation in OTCD, and those who may have low clinical reserve in the face of triggers for hyperammonemia. Subjects with repeated hyperammonemic episodes or neonatal mutations revealed the most striking reductions in mI.

The decrease of mI is supported by other studies [24, 25]. The role of mI in the brain is uncertain, but it plays a role as an osmolyte with importance in cell volume in astrocytes. The mI component in MRS is a composite signal with the bulk of the contributions coming from mI itself. Previous theories posit that decreases in mI are due to volume regulatory mechanisms with astrocytic mI release as a direct response to ammonia-induced astrocytic accumulation of gln [30]. The finding of decreased mI also occurs in hepatic encephalopathy associated with the effects of hyperammonemia [31]. In astrocyte cultures, mI is released through volume-sensitive anion channels in response to osmotic stress [30].

Elevations of NAA in the white matter of subjects were unexpected. The possibility of either another compound resonating at the same ppm or increased axonal density has not been evaluated. It has been suggested that NAA may play a role as a molecular water pump or in brain nitrogen balance [32].

The choline resonance contains contributions from phosphocholine and glycerophosphorylcholine, cell membrane precursors and degradation products. The observed reduction may reflect alterations in phospholipid metabolism, membrane alterations, membrane fluidity, or secondary changes in water content, glycerophosphorylcholine being a cerebral osmolyte.

There are several possible limitations of our methodologic approach that others experience in similar studies. First, overlap of the spectra of metabolites is a challenge with one-dimensional MR spectroscopy. The spectra for glutamine, *N*-acetylaspartate, γ -aminobutyric acid, and aspartate overlap in the 2–3 ppm region, whereas mI, glutamate/glutamine, glucose, and aspartate spectral peaks overlap in the 3.5–4 ppm region. To account for and overcome these phenomena, we used time-domain fitting with ‘LCModel’ which is more accurate for quantitation of overlapping peaks. Although voxel placement was designed to maximize tissue homogeneity, the relatively large size of our voxel ($2 \times 2 \times 2$ cm³) resulted in inclusion of both gray and white matter in some voxel locations. By using segmentation analysis as we describe, the contributions from gray versus white matter minus CSF is more accurately reflected in the total metabolite concentrations that are reported. Previous autopsy and neuroimaging studies show that OTCD results in white matter injury. Many previous studies reported small series of patients. The majority of these subjects had a history of hyperammonemic coma and were studied at different stages of disease, and emphasized CT which is not comparable to MRI [33] in delineating white matter pathology.

When ammonia is not adequately detoxified by the urea cycle, there is an increase in scavenger amino acids, including glutamine [34]. Ammonia entering the brain is rapidly incorporated into the formation of glutamine by glutamine synthetase, present in the astrocyte, which serves to rapidly detoxify and buffer excess ammonia [35]. As a result, glutamine concentrations increase in hyperammonemic states [34]. Previous ¹H magnetic resonance spectroscopy studies of patients with UCDs demonstrated elevations of the glutamine/glutamate complex and depletion of mI that may be detected non-invasively in various stages of disease.

Glutamine has been implicated in hyperammonemic encephalopathy [36]. Previous studies demonstrated that ¹H MRS can be used to non-invasively detect elevated brain glutamine in chronic hepatic encephalopathy [37] as well as experimentally-induced hyperammonemia [38]. A rise in plasma glutamine levels precedes hyperammonemia [38, 39]. The role of glutamine in this process has been demonstrated by the temporal relationship between hyperammonemia, neurological dysfunction, and glutamine concentrations in CSF observed in patients with hepatic encephalopathy. A triad of biochemical changes that can be observed by ¹H MRS include increased Glx, decreased mI, and decreased choline, which is the reverse order of changes seen in hepatic encephalopathy (decreased choline, mI depletion, and increased Glx) [24].

Inhibition of glutamine synthetase in hyperammonemic rats by treatment with enzyme inhibitors prevents a rise in cortical glutamine levels and cortical water content [40]. Acute ammonia toxicity is mediated by excessive activation of the NMDA type of glutamate receptors that mediate glutamate neurotoxicity. Clearance of synaptic glutamate by glial cells is required for the normal function of excitatory synapses and to prevent further neurotoxicity. This process occurs in the astrocyte, which takes up glutamate from the

synapse and returns it to the neurons in the form of glutamine, a non-toxic amino acid. Neurons subsequently reconvert glutamine to glutamate via the action of mitochondrial phosphate-dependent glutaminase.

Biochemical changes such as altered energy and membrane metabolism and intercellular metabolite trafficking (glutamate-glutamine shuttle) may be detected prior to structural MRI changes. Given the normal structural MRI scans in the majority of our subjects, these data suggest that biochemical changes precede structural changes, thus providing a window of opportunity for intervention before irreversible damage ensues.

We demonstrate that brain biochemistry differs in subjects with partial OTCD and may explain neurocognitive differences between these groups. These data are in agreement with previous studies, and support white matter pathology. We hypothesize that mI decrements mark prior hyperammonemic episodes and represent a marker of vulnerability. We observed elevations of glutamine in several brain regions despite normal plasma ammonia and normal plasma glutamine levels in some subjects. Further work is ongoing to clarify these aspects of brain metabolism in subjects with OTCD. Our findings have implications for clinical practice and dietary management to prevent cognitive sequelae of hyperammonemia or its effects. Future investigations are directed towards understanding elevation of NAA in white matter as well as the extent to which dietary factors may play a role in effects on brain function and biochemistry.

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Abbreviations

Cho	choline
Cr	creatinine
FGM	frontal gray matter
FWM	frontal white matter
gln	glutamine
glu	glutamate
glx	glutamine + glutamate
lac	lactate
mI	myoinositol
MRS	magnetic resonance spectroscopy
ms	milliseconds
NAA	N-acetylaspartate
OTCD	ornithine transcarbamylase deficiency
PCGM	posterior cingulate gray matter
PWM	parietal white matter

TE	echo time
tha	thalamus
UCD	urea cycle disorder

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APPENDIX

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Figure 1.

Depiction of voxel locations from which metabolism was sampled: a) PWM= parietal white matter ; b) PCGM= posterior cingulate gray matter; c) tha= thalamus; d) FWM= frontal white matter; e) FGM= frontal gray matter.

Figure 2.

a–e: These bar graphs demonstrate the concentrations of the major measured metabolites in brain in subjects and controls. Regions of significance between subjects carrying a mutation in the OTCD gene and controls is shown as a P value

Figure 3.

a–c: Disease severity and metabolite trends for several brain regions studied

Figure 4.
Correlations between plasma and brain glutamine levels

Figure 5.

This figure shows the inverse relationship between brain gln and mI in subjects with OTCD but not controls, implicating impaired osmoregulation.

Figure 6.

Osmotic index is indicated by ratio of mI divided by total osmolytes (gln + mI).

Figure 7.

This overlapping spectrum obtained from the parietal white matter in a subject and age matched control illustrates differences in the concentration of several key metabolites.

Table 1

Demographics of patients with partial OTCD; plasma glutamine and ammonia concentration in subjects

Subject #	Age	Gender	Clinical/ Familial History	Symptoms	Age at Onset; Diagnosis	Genotype	FSIQ VIQ/PIQ	Highest Ammonia	Ammonia (normal: 11–32 μmol/L)	Plasma Gln (normal: 414– 863 μmol/L)
1	59	F	symptomatic, son neonatal onset	vomiting, off balance, milk and meat intolerance fatigue,	Early childhood; 35	c. 959 G > T p. Arg320Leu neonatal	110 119/100	774	27	1017 ^{**}
2	20	M	symptomatic	vomiting, coma, lethargy, abdominal pain	8; 8	c. 673 C > A p.Pro225Thr late onset	110 98/124	379	54 ^{**}	706
3	36	M	symptomatic	vomiting, confusion, dizziness, slurred speech	18, 34; 35	c.1033 T > C p. Tyr 345 His late onset	97 97/96	128	65 ^{**}	884 ^{**}
4	20	F	symptomatic	vomiting, coma	2 1/2; 20	c.77+5G>A neonatal	94 92/96	400	16	681
5	19	F	symptomatic	Vomiting, lethargy, slurred speech, picky eating, nausea	Early childhood; 2 1/2	c.256 G>T p. Gly256X neonatal	103 98/108	423	37 ^{**}	758
6	19	F	symptomatic, mother symptomatic, sister non-symptomatic, brother neonatal onset	Vomiting, lethargy, confusion, picky eating	Early childhood; 2	c.421C>T p.Arg141X neonatal	83 88/81	90	14	717
7	22	F	symptomatic	Vomiting, lethargy, light-headedness, memory lapses, picky eating	Early childhood; 7	c.322 C>G p.Leu322Val neonatal	109 116/100	100	29	946 ^{**}
8	37	M	symptomatic	Nausea, numbness, confusion, slurred speech, coma	35; 35	c.1033T>C p.Tyr345His late onset	134 130/131	654	24	574
9	54	M	symptomatic	Vomiting, protein intolerance, confusion, personality changes, coma	Early childhood, coma at 30; 30	unknown	113 114/109	600	30	707
10	45	F	symptomatic, late onset father, affected son	Nausea, confusion, headaches, vomiting	Late 20s; 37	c. 156 A>T p.Glu52Asp late onset	124 122/119	25 [*]	25	499
11	27	F	symptomatic, carrier mother, neonatal onset brother	Lethargy, weakness, confusion, vomiting, hyperactive as child	Infancy; 2	c. 422 G>A p. Arg 141Gln neonatal	122 117/121	400s	152 ^{**}	654
12	35	F	symptomatic, carrier daughter, 2 sons neonatal onset	Vomiting, protein intolerance, picky eating, headaches	Childhood; 28	c. 958 C>T p.Arg320X neonatal	111 98/124	22 [*]	22	637
13	34	F	Symptomatic daughter neonatal onset	Protein intolerance, vomiting, headaches	Childhood; 30	c.77+1G>A female mutation	124 119/123	23 [*]	50 ^{**}	884 ^{**}
14	38	F	symptomatic, symptomatic daughter	Migraine-like episodes, fatigue, confusion, irritability	Early childhood; 35	c. 1063delT neonatal	110 108/110	85	30	823
15	30	F	symptomatic	Fatigue, weakness, slurred speech, confusion, vomiting, protein aversion	Infancy; 5	c. 520G>C p.Ala174Pro female	87 92/83	215	19	1367 ^{**}
16	20	M	symptomatic	Nausea, vomiting, confusion	15; 15	c. 829 C>T p.Arg277Trp late onset	112 108/114	320	16	814
17	28	F	symptomatic, son neonatal onset,	Abdominal pain, protein aversion	26; 26	pending	101 100/101	>100	67 ^{**}	771

Subject #	Age	Gender	Clinical/ Familial History	Symptoms	Age at Onset; Diagnosis	Genotype	FSIQ VIQ/PIQ	Highest Ammonia	Ammonia (normal: 11–32 μmol/L)	Plasma Gln (normal: 414– 863 μmol/L)
18	53	F	daughter non-symptomatic	n/a	n/a; 43	c.673 C > A p.Pro225Thr late onset	124 115/127	35 [*]	35 ^{***}	470
19	50	F	Non-symptomatic, son late onset	n/a	n/a; 35	unknown	114 121/104	68 [*]	68 ^{***}	431
20	49	F	Non-symptomatic, son neonatal onset	n/a	n/a; 30	del OTC gene eonatal	111 107/114	42	21	467
21	24	F	Non-symptomatic, 4 sons neonatal onset	n/a	n/a; 6	c. 421C>T p. Arg 141X neonatal	114 110/114	20 [*]	20	559
22	22	F	Non-symptomatic, brother neonatal onset, symptomatic mother and sister	n/a	n/a; 18	pending	125 119/124	65 [*]	65 ^{***}	818
23	29	F	Non-symptomatic, carrier mother and grandmother, late onset male cousin	n/a	n/a; 25	c. 533 C>T p. Thr178 Met neonatal	111 106/115	31 [*]	31	599
24	54	F	Non-symptomatic, 2 sons neonatal onset	n/a	n/a; 49	c. 829 C>T p.Arg 277Trp late onset	113 109/115	30 [*]	30	654
25	26	F	Non-symptomatic, son late onset	n/a	n/a; 21	c. 829 C>T p.Arg 277Trp late onset	109 106/112	21 [*]	21	835

* only ammonia level reported is the one from our testing
*** indicates elevated level

FSIQ=full scale IQ; VIQ= verbal IQ; PIQ= performance IQ