Progressive myoclonic epilepsy type 1 (EPM1) patients present with abnormal ^{1}H MRS brain metabolic profiles associated with cognitive function

Jelena Hyppönen [a,b], Vili Paanila [b,c], Marja Äikiä [d], Päivi Koskenkorva [b,c], Mervi Könönen [c], Ritva Vanninen [b,c], Esa Mervaala [a,b], Reetta Kälviäinen [b,d], Juhana Hakumäki [b,c,*]

[a] *Department of Clinical Neurophysiology, Epilepsy Center, Diagnostic Imaging Center, Kuopio University Hospital, Full Member of ERN EpiCARE, Kuopio, Finland*
[b] *Institute of Clinical Medicine, University of Eastern Finland, Kuopio, Finland*
[c] *Department of Clinical Radiology, Diagnostic Imaging Center, Kuopio University Hospital, Full Member of ERN EpiCARE, Kuopio, Finland*
[d] *Epilepsy Center, Neurocenter, Kuopio University Hospital, Full Member of ERN EpiCARE, Kuopio, Finland*

A B S T R A C T

Purpose: Progressive myoclonic epilepsy, type 1A (EPM1, Unverricht-Lundborg disease), is a rare neurodegenerative autosomal recessive disorder characterized by stimulus-sensitive and action myoclonus and tonic-clonic epileptic seizures. Patients develop neurological symptoms, including ataxia, intention tremor, and dysarthria, over time, with relatively limited and nonspecific MRI atrophy findings. The effects of the disease on brain metabolism are largely unknown.
Method: Eighteen EPM1 patients (9 M, 9F) underwent clinical evaluation and neuropsychological testing, which included the assessment of intellectual ability, verbal memory, and psychomotor and executive functions. Magnetic resonance spectroscopy (MRS) and imaging (MRI) were performed on a 1.5 T MRI system. 2D MRS chemical shift imaging (CSI) maps (TE = 270) were obtained from the following regions of the brain: basal ganglia, thalamus, insula, splenium, and occipital white and gray matter, and N-acetyl-aspartate (NAA)-, choline (Cho)-, and lactate (Lac)-to-creatine (Cr) ratios were analyzed. Ten healthy age-and sex-matched subjects (5M, 5F) were used as controls for MRS.
Results: We found significant brain metabolic changes involving lactate, NAA, and choline, which are widespread in the basal ganglia, thalamic nuclei, insula, and occipital areas of EPM1 patients. Changes, especially in the right insula, basal ganglia, and thalamus, were associated with intellectual abilities and impairment of the psychomotor and executive functions of EPM1 patients.
Conclusion: Multiple brain metabolic alterations suggest the presence of neurodegeneration associated with EPM1 progression. The changes in metabolite ratios are associated with the neurocognitive dysfunction caused by the disease. However, the role of MRS findings in understanding pathophysiology of EPM1 warrants further studies.

1. Introduction

Progressive myoclonic epilepsy, type 1A (EPM1, Unverricht-Lundborg disease, OMIM #254800) is a rare neurodegenerative autosomal recessive disorder characterized by stimulus-sensitive and action myoclonus and tonic-clonic epileptic seizures. Disease onset occurs in late childhood or adolescence (6–18 years) (Kälviäinen et al., 2008). As EPM1 progresses, patients develop neurological symptoms, including ataxia, intention tremor and dysarthria, while their verbal memory remains largely intact (Kälviäinen et al., 2008; Canafoglia et al., 2017; Äikiä et al., 2021; Ferlazzo et al., 2009).

EPM1 is caused by a pathogenic variant in the gene encoding cystatin B (*CSTB*), a cysteine protease inhibitor. The 12-nucleotide dodecamer repeat expansion pathogenic variant in the *CSTB* accounts for approximately 90% of cases worldwide and 99% of EPM1 cases in Finland (Joensuu et al., 2008). Based on previous mouse model studies, cystatin B is involved in biological processes such as neurogenesis and apoptosis, oxidative stress response and inflammation, cell cycle regulation, and

synapse physiology. However, the precise mechanism of cystatin deficiency leading to clinical disease is still uncertain in mouse models and humans. A full cystatin B deficiency causes neonatal-onset encephalopathy that manifests with severe developmental delay, progressive microcephaly, and hypomyelination in MRI (Mancini et al., 2016).

In contrast, in dodecamer expansion homozygous EPM1 patients' imaging findings rarely show abnormalities at the time of EPM1 diagnosis. However, as the disease progresses, cerebral atrophy may evolve. Moreover, compound heterozygous patients reported to have a more severe clinical course of the disease were shown to have mild to moderate cerebellar and cerebral atrophy by visual MRI assessment (Koskenkorva et al., 2011). Recent studies have examined EPM1 patients with voxel-based morphometry and show gray matter volume loss in the motor cortex (including premotor and primary motor areas and supplementary motor cortex), precuneus, and thalami, which are affected bilaterally. The changes, especially those found in motor areas, parallel the motor symptoms characteristic of EPM1 (Koskenkorva et al., 2009). Cortical thickness analysis has demonstrated widespread cortical thinning in EPM1 patients, for instance, in the sensorimotor cortex, visual cortex, Broca's area, and primary auditory cortex, as well as in premotor and supplementary motor cortices (Koskenkorva et al., 2012). Cortical thinning correlated significantly with the severity of the myoclonus.

Magnetic resonance spectroscopy (MRS) imaging can provide in vivo information on the N-acetyl-aspartate (NAA), choline (Cho), creatine (Cr), lactate (Lac), glutamine (Glu), and gamma-aminobutyric acid (GABA) levels in different areas of the brain. Previously, in EPM1 patients, metabolite peak ratios have been measured only in the pons and dentate nucleus (Mascalchi et al., 2002). However, more extensive cerebral metabolic changes in EPM1 remain unknown. This study aimed to examine possible metabolic changes in the supratentorial brain regions of EPM1 patients via MRS, evaluate analogies between this and earlier radiologic studies of EPM1, and provide additional information on causative and co-associated factors in the clinical picture of EPM1.

2. Subjects and methods

2.1. Subjects

Eighteen genetically verified adult EPM1 patients (9 males, 9 females) homozygous for the dodecamer expansion pathogenic variant in the *CSTB* gene participated in the study. The patients are a part of the large EPM1 cohort reported previously (Hyppönen et al., 2015; Äikiä et al., 2021; Danner et al., 2013; Koskenkorva et al., 2012). Ten age- and sex-matched healthy volunteers (5 males, 5 females) were selected as controls for MRS. The main demographic characteristics of the EPM1 patients and controls are presented in Table 1. All patients and control subjects were right-handed. The Ethical Committee of Kuopio University Hospital approved the study protocol, and informed consent was obtained from all participants.

2.2. Clinical and neuropsychological assessment

Detailed clinical and neuropsychological characteristics of the cohort have been published earlier (Hyppönen et al., 2015; Äikiä et al., 2021). Previously collected clinical histories, including age at disease onset and antiseizure medication (ASM) history, were used in this study. The results of the video-recorded unified myoclonus rating scale (UMRS) test panel (Frucht et al., 2002), including stimulus sensitivity (SS), myoclonus with action (AM), and functional tests (FT), were used for the analyses. The patients were classified based on their myoclonus and action scores (max. score = 160) into three myoclonus severity groups: mild myoclonus (score = 1–30), moderate myoclonus (score = 31–59), and severe myoclonus (score > 60). Neuropsychological assessments were performed by the same experienced neuropsychologist (M.Ä.). The battery of tests included intellectual ability, verbal learning and memory, and psychomotor and executive functions. Six subtests of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (David, 1981)— information, similarities, digit span, digit symbol, picture completion, and block design—were used to calculate the patients' verbal intelligence quotients (VIQ) and performance intelligence quotients (PIQ). A detailed protocol for evaluating verbal memory, executive function and psychomotor function has been described previously (Äikiä et al., 2021). WAIS-R digit symbol, picture completion, and block design tests were used to evaluate patients' perceptional reasoning and processing speed. List learning, story recall, and delayed recall were used to evaluate patients' verbal learning and memory.

2.3. Imaging and spectroscopy

The MRI and MR spectroscopy were conducted at Kuopio University Hospital with a Siemens Avanto 1.5 T MRI system (Siemens AG, Erlangen, Germany). 2D MR chemical shift imaging (CSI) spectral maps were obtained, as indicated by Fig. 1 (based on an MR spectroscopy protocol with TE = 270 ms, TR = 1500, NA = 4). The area was chosen to encompass as much of the basal ganglia as possible, together with the insular cortices and occipital white matter, including the dorsal splenium and even occipital gray matter. Spectroscopy analysis was performed using vendor workstation software in research mode, with baseline correction and frequency domain fitting using Lorentzian line shapes. The 270 ms echo time was chosen for reliable fitting and spectral clarity. The identified and analyzed metabolite groups were NAA, creatine (Cr), choline (Cho), and lactate (Lac). Metabolite concentrations are expressed according to standards in relation to the Cr signal calculated from the 2D CSI images and analyzed in the following regions of the brain: caudate nucleus, putamen, globus pallidus, anterior thalamus, dorsomedial thalamus, lateral thalamus, splenium, insula, occipital white matter, and occipital gray matter (visual cortex).

2.4. Statistical analysis

Statistical analyses were performed with the IBM Statistical Package for the Social Sciences (SPSS) version 26 (SPSS Inc., Chicago IL, USA). All variables were tested for normality of distribution using the Shapiro–Wilk test. Independent samples t-tests or non-parametric Kruskal-Wallis tests were used in comparisons between metabolite concentrations of patients and control subjects where appropriate. Pearson or Spearman correlation tests were carried out to investigate the relationship between metabolite levels and clinical variables. To ensure the clinical utility of the findings, only correlations of moderate effect size (r^2 of 0.30) or large effect size (r^2 of 0.50) (Akoglu, 2018) were interpreted as evidence of a significant association between variables. The interhemispheric deviation was evaluated with a non-parametric test (Wilcoxon signed-rank test).
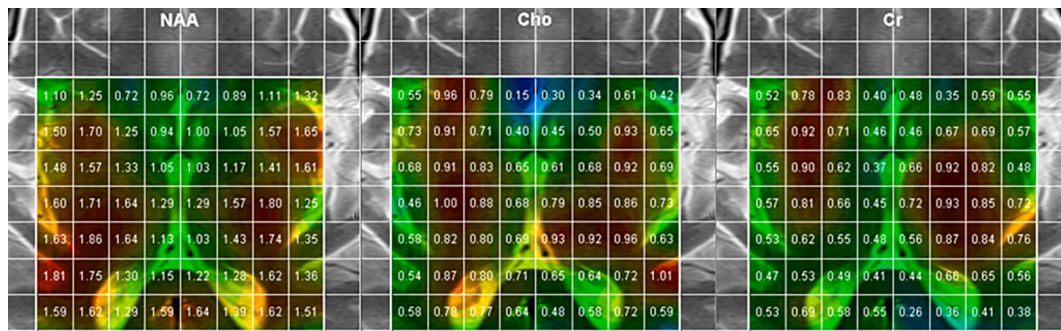
Table 1
Demographic data, including the ambulatory and functional status of the EPM1 patients. The numbers indicate the mean (SD) [range] and the number of subjects where applicable.

	EPM1	Control
Age (years)	32 (10) [19–49]	31 (11) [16–48]
Sex (F/M)	9F; 9 M	5F; 5 M
Age at onset (years)	10 (2) [6–16]	
Disease duration (years)	22 (10) [8–41]	
UMRS SS: total score	2 (2) [0–8]	
UMRS Myoclonus with action: total score	46 (26) [12–90]	
UMRS FT: total score	10 (7) [1–22]	
VIQ	88 (12) [69–109]	
PIQ	78 (13) [55–103]	

Mean (Std. Dev) [Minimum-Maximum]; F, female, M, male; UMRS, unified myoclonus rating scale; SS, stimulus sensitivity; FT, functional tests; VIQ, Verbal Intelligence Quotients; PIQ, Performance Intelligence Quotients.

Fig. 1. Typical TE = 270 ms MR spectroscopic imaging data from a patient, indicating the covered anatomical area, voxels, and intrinsic NAA, Cho, and Cr metabolite signal areas (AU). NAA, N-acetyl-aspartate; Cho, Choline; Cr, Creatine.

3. Results

The main demographic characteristics of the EPM1 patients are presented in Table 1. According to the severity of myoclonus, 6 patients had mild myoclonus, 6 – moderate, and 6 – severe. All patients received treatment with ASMs with individually adjusted combinations and doses. None of the patients were on ASM monotherapy. Valproate was included in the ASM regime in all patients.

3.1. MRS results of EPM1 patients and healthy controls

The main finding was the Lac/Cr ratio increase in EPM1 patients compared to healthy controls bilaterally in the dorsomedial area of the thalamus, splenium, and occipital white and gray matter (Fig. 2). On the left, the Lac/Cr ratio also increased in the insula and globus pallidus.
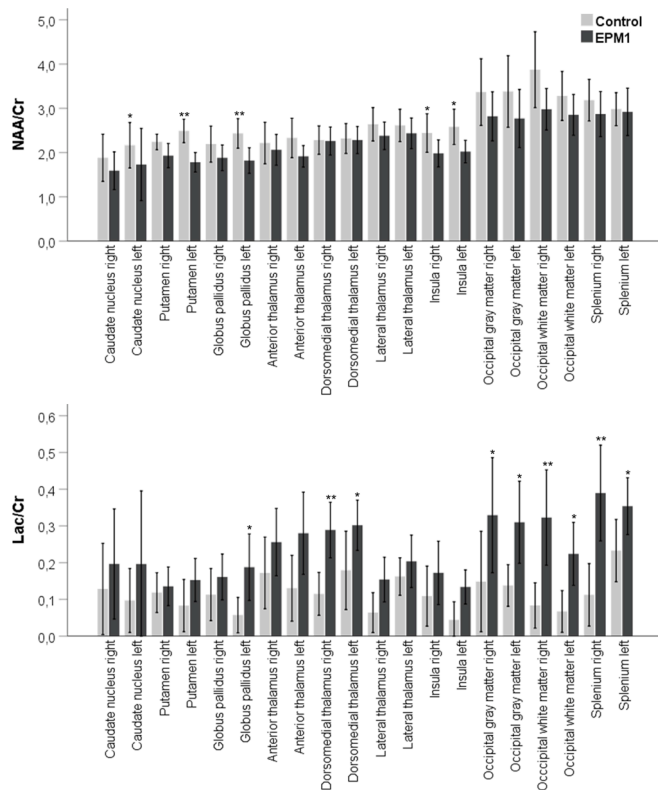

Fig. 2. Metabolite levels in different brain regions of EPM1 patients and healthy controls. Significant differences are marked with an asterisk. The mean average of both hemispheres is presented for each brain region. NAA/Cr, N-acetyl-aspartate-to-Creatine ratio; Lac/Cr, Lactate-to-Creatine ratio; * $p < 0.05$; ** $p < 0.01$.

NAA/Cr levels were significantly lower in EPM1 patients than in controls in the insula bilaterally and left caudate nucleus, globus pallidus, and anterior thalamus. Cho/Cr levels showed no statistically significant difference between the EPM1 patients and the controls. Metabolite ratios compared between EPM1 patients and healthy control subjects for each region are presented separately for both hemispheres in Supplemental Table 1 and Fig. 2.

3.2. MRS and clinical phenotypes of EPM1 patients

We found no global association of metabolic profiles with the age of EPM1 onset or disease duration, or systematic global association with the severity of myoclonus (measured by the AM or FT scores of the UMRS test panel). There were, however, some regional changes, such as in the caudate nucleus associated with the age at onset, as EPM1 patients with the later disease onset had higher Cho/Cr ratio in the right caudate nucleus (r^2 0.615, $p = 0.019$), higher NAA/Cr in the left caudate nucleus (r^2 0.632, $p = 0.020$), and bilateral increases in Lac/Cr ratios (right r^2 0.591, $p = 0.026$, left r^2 0.753, $p = 0.003$). The bilateral Cho/Cr ratio of occipital white matter was moderately associated with AM (right r^2 0.623, $p = 0.006$, left r^2 0.557, $p = 0.016$) and FT (right r^2 0.661, $p = 0.003$, left r^2 0.481, $p = 0.043$). All correlation results of Lac/Cr, NAA/Cr, and Cho/Cr ratios for each brain region with clinical parameters are presented in Supplemental Table 2.

3.3. MRS and neuropsychological findings in EPM1 patients

Higher VIQ and PIQ were correlated with significantly lower NAA/Cr (r^2 −0.482, p = 0.043 for VIQ and r^2 −0.601, p = 0.011 for PIQ) and Lac/Cr ratios (r^2 −0.472, p = 0.048 for VIQ and r^2 −0.677, p = 0.003 for PIQ) in the right insula and significantly lower Cho/Cr ratios bilaterally in the insula (left r^2 −0.477, p = 0.045 and right r^2 −0.731, p = 0.001 for VIQ and left r^2 −0.267, p = NA, right r^2 −0.652, p = 0.005 for PIQ) (Fig. 3). In addition, the Cho/Cr ratio of the right anterior thalamus presented a moderate, negative association with both VIQ and PIQ (r^2 −0.535, p = 0.027 for VIQ and r^2 −0.544, p = 0.029 for PIQ).

A lower Cho/Cr in the left caudate nucleus was associated with better verbal learning (r^2 −0.543, $p = 0.045$ for list learning and r^2 −0.634, $p = 0.015$ for delayed recall). In addition, lower Lac/Cr in the right globus pallidus was associated with better list learning (r^2 −0.585, $p = 0.011$). In addition, lower Lac/Cr in the caudate nucleus (r^2 −0.611, $p = 0.020$) and nucleus pallidus (r^2 −0.529, $p = 0.024$) were associated with delayed recall of words (Supplemental Table 3). Additionally, significantly higher levels of Lac/Cr in the left dorsomedial thalamus and significantly lower levels of NAA/Cr in the right anterior and lateral thalamic nuclei were associated moderately with better immediate and delayed recall of the story (Supplemental Table 3).

Simple psychomotor speed in the tapping task was positively associated with the NAA/Cr ratio bilaterally in the dorsomedial thalamus area (r^2 0.522, $p = 0.038$ for right-hand tapping, and r^2 0.579, $p = 0.019$
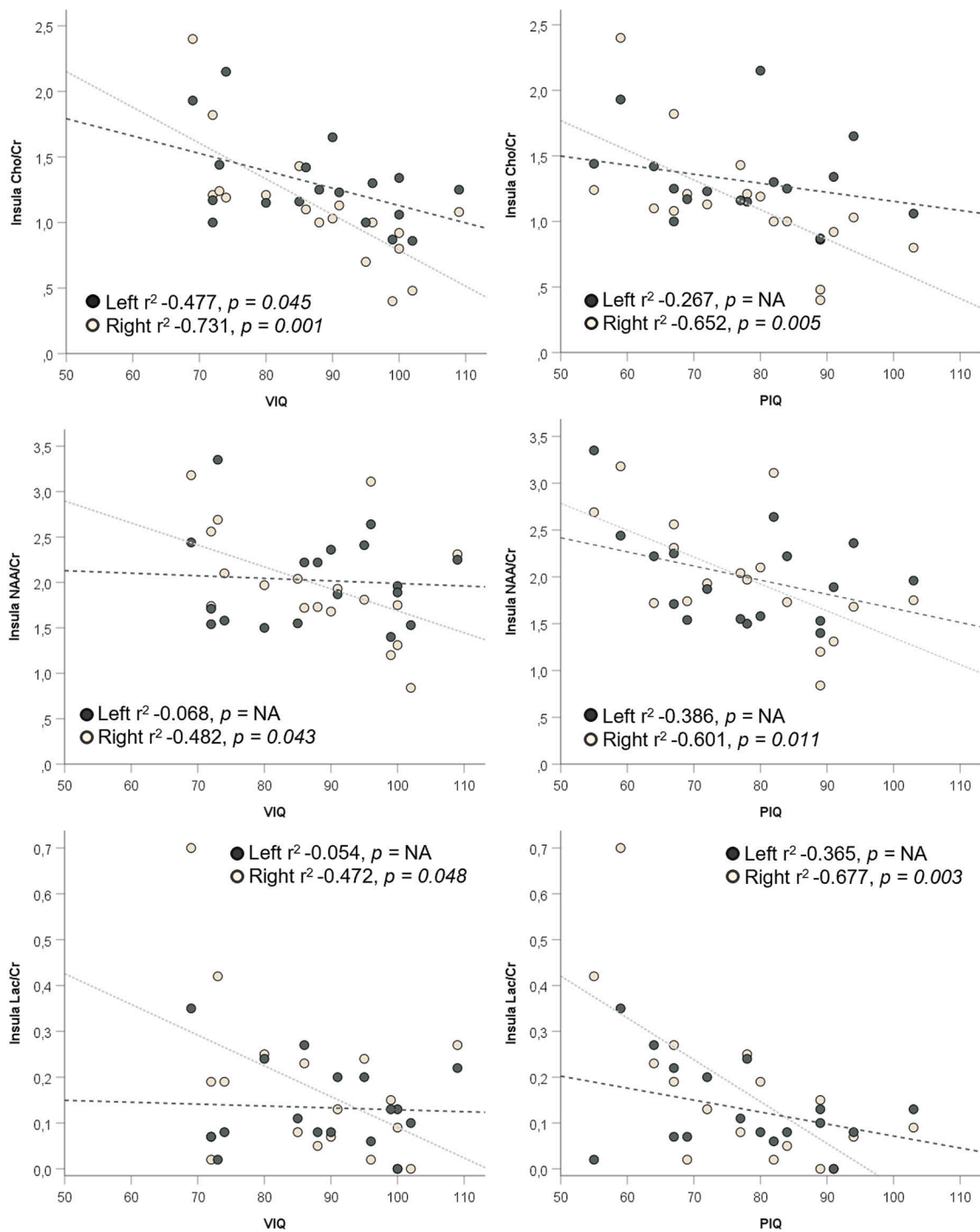
Fig. 3. Association of insular Cho/Cr, NAA/Cr, and Lac/Cr levels with verbal and performance IQs. VIQ, verbal IQ; PIQ performance IQ; Cho/Cr, Choline-to-Creatine ratio; NAA/Cr, N-acetyl-aspartate-to-Creatine ratio; Lac/Cr, Lactate-to-Creatine ratio.

for left-hand tapping). Additionally, non-dominant hand tapping was negatively associated with Cho/Cr and NAA/Cr in the right occipital white matter (r^2 −0.508, $p = 0.037$ for Cho/Cr and r^2 −0.483, $p = 0.050$ for NAA/Cr).

Lower Lac/Cr ratios bilaterally in the globus pallidus, the right anterior and lateral thalamus, and the insula were associated with better performance in the WAIS-R perceptional reasoning and processing speed tests. There was also a significant, moderate association between the lower Cho/Cr of the left occipital white matter with these tests (Table 2 and Suppl Table 3). Lower Lac/Cr levels bilaterally in the putamen, globus pallidus, anterior and lateral thalamus, and insula were

associated with better performance in the Stroop test (Fig. 4). One patient was a strong outlier regarding Stroop test results and high lactate levels; however, this individual also had a significantly lower PIQ than the group average. The associations between metabolite ratios and different cognitive domains are summarized in Table 2 (all detailed correlation results are presented in Supplemental Table 3).

4. Discussion

This is the first clinical MR spectroscopy study of the supratentorial brain in EPM1 patients. We show that EPM1 patients have significantly

Table 2
Overview summary of the metabolite/Cr ratio association with cognitive test outcomes.

Cho/Cr	VIQ	PIQ	List learning	List delayed recall	Story recall	Story delayed recall	WAIS-R: Digit symbol	WAIS-R: Block design	WAIS-R: Picture completion	Tapping	TMT processing	Stroop processing
Basal ganglia*	–	–	↓	↓	–	–	–	↓	–	–	–	↓
Thalamus**	↓	↓	–	–	↓	–	–	–	↓	–	–	–
Insula	↓	↓	–	–	–	–	–	–	↓	–	–	↓
Occipital gray matter	–	–	–	–	–	–	–	–	–	–	↓	–
Occipital white matter/ splenium	–	↓	–	–	–	–	↓	↓	↓	↓	–	–
NAA/Cr												
Basal ganglia*	–	–	–	–	↓	–	–	↓	–	–	–	↓
Thalamus**	–	↓	–	–	↓	↓	–	–	–	↑	–	–
Insula	↓	↓	–	–	–	–	–	–	↓	–	↓	–
Occipital gray matter	–	–	–	–	–	–	–	–	–	–	↓	–
Occipital white matter/ splenium	–	–	–	–	–	–	–	–	–	↓	–	–
Lac/Cr												
Basal ganglia*	–	–	↓	↓	–	–	↓	↓	↓	–	–	↓
Thalamus**	–	–	–	–	↑	↑	↓	–	↓	–	–	↓
Insula	↓	↓	–	–	–	–	–	↓	↓	–	↓	↓
Occipital gray matter	–	↑	–	–	↑	–	↑	–	↑	–	–	–
Occipital white matter /splenium	–	–	–	–	–	–	–	–	–	–	–	↓

↑ High metabolite/Cr ratio is associated with better cognitive test results.
↓ Low metabolite/Cr ratio is associated with better cognitive test results.
Cho/Cr, Choline-to-Creatine ratio; NAA/Cr, N-acetyl-aspartate-to-Creatine ratio; Lac/Cr, Lactate-to-Creatine ratio; * Basal ganglia includes any significant association observed for either putamen or globus pallidus or caudate nucleus; ** Thalamus includes any significant association observed for either anterior or lateral or dorsomedial thalamic nuclei.

higher Lac/Cr concentrations and decreased NAA/Cr ratios than healthy controls in multiple brain regions. However, we did not find significant changes in Cho/Cr compared to the healthy controls. Moreover, widespread changes in Lac/Cr, NAA/Cr, and Cho/Cr were associated with cognitive, verbal, and performance abilities in EPM1 patients.

In MRI studies of EPM1 patients, volume losses have been observed in motor cortices and thalamic areas, along with cortical thinning in sensorimotor, visual, and auditory cortices. However, they are not discernible to the naked eye (Koskenkorva et al., 2009; Koskenkorva et al., 2012). Brain metabolite peak ratios have previously only been measured in the pons and dentate nucleus, demonstrating a lower than normal NAA/Cr ratio in the pons of EPM1 patients (Mascalchi et al., 2002). In our study, the most pronounced NAA/Cr decreases were observed in the insular cortex and basal ganglia, especially of the left dominant hemisphere, and Lac/Cr increases in the thalamus (particularly the dorsomedial part), the insula, white matter of occipital lobe and splenium, and occipital gray matter.

NAA is a free amino acid found in relatively high concentrations in the central and peripheral nervous systems. The NAA concentration in the central nervous system is considered an indicator of neural density. Previous studies have shown changes in NAA/Cr in mesial temporal sclerosis patients in the mesial temporal region (Fernández-Vega et al., 2021). However, it is known that the NAA concentration can vary between different types of neurons, and changes in NAA levels may also reflect neuronal dysfunction instead of actual neuronal loss (Simmons et al., 1991). Decreased NAA concentrations have been found in cortical dysplasia when the cell density has been normal or even elevated (Kuzniecky et al., 1997; Li et al., 1998). In patients with genetic generalized epilepsy (GGE), decreased NAA/Cr levels in the thalamic areas have been reported (Yıldırım et al., 2022). Moreover, in patients with Lafora disease (EPM2), the NAA/Cho ratio decreased in the cerebellum and correlated significantly with myoclonus and ataxia severity scores (Altindag et al., 2009). NAA is metabolized via aminohydrolase,

which is released in cell dysfunction. Elevated aminohydrolase activity, such as that caused by mitochondrial dysfunction and detected as neurodegeneration, can lead to decreased NAA concentration (Goldstein, 1976; Savic et al., 2000). Therefore, it seems plausible that the metabolic changes involving NAA in this study could suggest the presence of neurodegeneration and actual neuronal loss. To support this possibility, a decrease in NAA/Cr ratios may reflect neuronal degeneration, which can eventually be seen in previously mentioned morphometry as a volume loss of the thalamus (Koskenkorva et al., 2009). Previous EPM1 studies on animal models (cstb -/- mice) have also found proof of thalamic atrophy by diffusion tensor imaging (Manninen et al., 2013).

Interestingly, decreased NAA/Cr ratio in the motor cortices together with decreased frontal lobe and thalamic NAA concentrations and NAA/Cr ratios were observed in JME patients (Lin et al., 2009; Haki et al., 2007; Savic et al., 2000; Savic et al., 2004). EPM1 and JME share similarities early in the course of the disease. While JME does not progress and patients do not have neurological symptoms other than epilepsy, EPM1 patients show a large variability in their disease progression. Although EPM1 and JME share some similarities in the early clinical picture, the metabolic changes in subcortical regions show differences in distribution. In contrast to previous JME studies, we found decreased NAA/Cr in the basal ganglia and insular cortex. It is plausible that the involvement of the thalamus and basal ganglia is associated with the presence of myoclonus in both diseases. The basal ganglia and thalamus are known to play essential roles in the integration of motor behavior and motor learning. They also integrate information from multiple brain areas and connect it to the primary motor cortex (McHaffie et al., 2005; Nagano-Saito et al., 2014; Fama and Sullivan, 2015). Metabolic changes in the thalamic area and basal ganglia may account for action-activated myoclonus, which is the most disabling progressive symptom in EPM1 patients. We did not, however, find any global association of myoclonus severity (UMRS score) with the measured metabolites, which implies a more general metabolic imbalance associated with the disease.
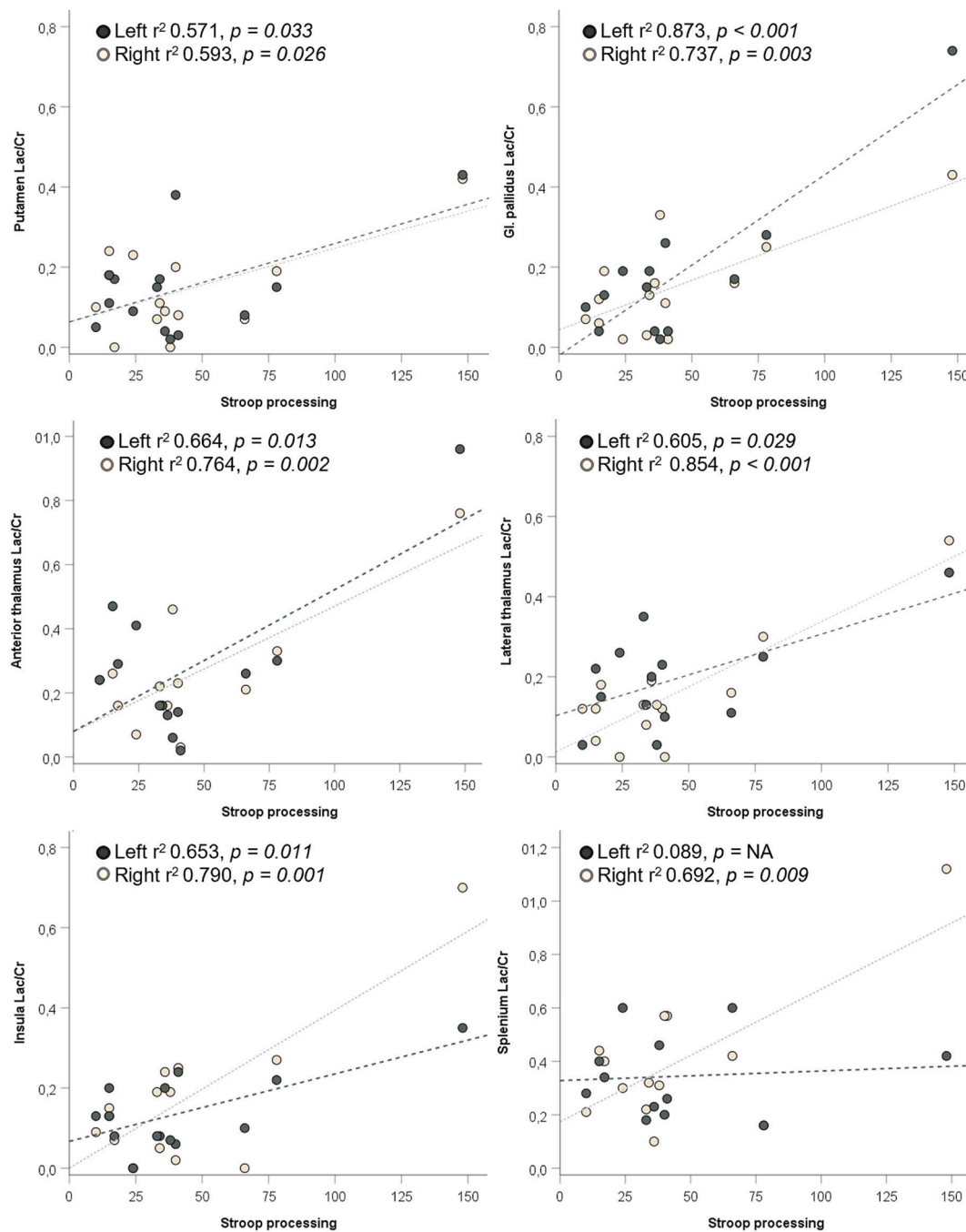
Fig. 4. Association of Lac/Cr in basal ganglia, anterior and lateral thalamus, insula, and splenium with Stroop processing. Lac/Cr, Lactate-to-Creatine ratio.

Choline is the primary substrate for phospholipid synthesis, and changes in its concentrations may be related to the turnover of the cell membrane or changes in cell density. The role of choline as a biomarker of demyelination has also been suggested (Rae, 2014). We did not find significant changes in Cho/Cr in EPM1 patients compared to controls. However, there was some association of Cho/Cr in the white matter with action myoclonus severity and functional test scores. We also showed a significant increase in Lac/Cr in occipital white matter and splenium, as well as in the occipital gray matter. Our research has previously identified cortical thinning in the visual cortex (Koskenkorva et al., 2012). White matter degeneration is observed in both *cstb-/-* mice and EPM1 patients (Manninen et al., 2013). Photosensitivity is a significant early feature of EPM1, but no association between measured metabolites and stimulus sensitivity was observed in our study population. The results,

however, may reflect disturbances in lactate metabolism in occipital gray and white matter and further support the notion that these brain areas are involved in EPM1 pathophysiology.

Lactate has long been considered a marker of hypoxic pathology. However, under physiological conditions, lactate is also a predominant glycolytic substrate in the brain formed in astrocytes and further trafficked to glutamatergic neurons through the astrocyte–neuron lactate shuttle pathway (Magistretti and Allaman, 2018; Patel et al., 2019). Lactate may be involved in neuronal excitability homeostasis. Studies have mainly indicated that lactate influences the excitability of glutamatergic and GABAergic neurons (Bozzo et al., 2013; Patel et al., 2019; Sada et al., 2015). Animal experiments have shown that lactate is a necessary mediator for learning and memory consolidation (Suzuki et al., 2011). We show an association of multiple metabolite changes,

including an increase of Lac/Cr in the insula, with patients' VIQ and PIQ. Moreover, an increase in Cho/Cr and Lac/Cr widely in the basal ganglia, thalamus, and insula was associated with the performance in the neuropsychological tests assessing perceptional reasoning and psychomotor speed. Decreased NAA/Cr in the thalamus and insula were associated with better cognitive performance. In comparison, increased Lac/Cr levels were associated with poorer results in cognitive tests, though verbal learning and memory were inconsistently associated with both an increase of Lac/Cr in the thalamus and a decrease in the basal ganglia.

Our previous study showed that the cognitive function of EPM1 patients is associated with disease severity (Äikiä et al., 2021). In this study population, the cognitive test results also correlated with the severity of myoclonus. However, since we did not find a significant correlation between the measured metabolites and myoclonus severity, the association between the metabolite changes and cognitive performance is likely independent of the patients' motor system symptoms. The basal ganglia, thalamus, and insula are complex structures in various integrative motor and cognitive functions. Each structure consists of multiple substructures and different nuclei with separate connection pathways, structure-specific cytoarchitectonic organization, and neurotransmitter profiles. Therefore, the more precise role of measured metabolites in the cognitive functioning of EPM1 patients remains to be elucidated.

Overall, we observed that Lac/Cr was increased in EPM1 patients and that increased Lac/Cr was associated with poorer performance in multiple cognitive tests. One could speculate that increased Lac/Cr in EPM1 might reflect abnormal glial activation and cell death, complementing the findings previously reported in *cstb-/-* mice (Okuneva et al., 2015). This could also reflect the previously suggested possibility of mitochondrial dysfunction (Kopitar-Jerala, 2015a; 2015b).

A limitation of our study is the lack of MRS data for other cortical regions, such as the primary and supplementary motor and sensory areas. Although we use creatine as an internal reference with adherence to common practice in clinical MRS, theoretical fluctuations in creatine concentrations could occur (Rae, 2014). However, Cr levels have consistently been stable in epilepsies and movement disorders (Govindaraju et al., 2000; Saunders et al., 1999). Our finding that Cho/Cr ratios remain stable compared to control subjects and between the different brain areas further corroborates this assumption. We are also acutely aware of the possibility of drug-induced metabolic effects: the patients were treated with multiple ASMs, and all our patients received sodium valproate, which could theoretically affect brain metabolite levels. There are, however, indications in the literature that, when used at therapeutic concentrations, valproate does not affect the MRS-measured NAA signal in the brain (Seyfert et al., 2003; Silverstone et al., 2003). There is also no evidence that other ASMs would lead to abnormal brain metabolite levels of Cho, Cr, and NAA on ^{1}H MRS (van Veenendaal et al., 2015) and that low NAA/Cr ratios may actually be an indicator of suboptimal medication status or treatment response (Campos et al., 2010). Also, since our study is cross-sectional, we have included patients of different disease severities equally. Although we did not observe any significant changes related to disease duration or myoclonus severity, a larger study size might be needed to show more progressive changes in brain metabolic profiles.

5. Conclusions

To summarize, the findings of this study point to a broad range increase of Lac/Cr and reduction of NAA/Cr in basal ganglia, thalamic nuclei, insula, and occipital white and gray matter, which were previously unknown. These findings are corroborated by previous morphometry, cortical thickness analyses, and similar findings in other myoclonic epilepsies. However, understanding these metabolic changes and assuming neurodegeneration is still inadequate, and further research is needed, particularly regarding the role of energy metabolism. Overall, our findings bring additional evidence that EPM1, like other

progressive myoclonic epilepsies, show structural degeneration and metabolic disturbances in the course of the disease progression.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2023.103459.

References

Äikiä, M., Hyppönen, J., Mervaala, E., Kälviäinen, R., 2021. Cognitive Functioning in Progressive Myoclonus Epilepsy Type 1 (Unverricht-Lundborg Disease, EPM1), 108157 Epilepsy Behav.: E&B 122 (September). https://doi.org/10.1016/j.yebeh.2021.108157.

Akoglu, H., 2018. User's Guide to Correlation Coefficients. Turk. J. Emerg. Med. 18 (3), 91–93. https://doi.org/10.1016/j.tjem.2018.08.001.

Altindag, E., Kara, B., Baykan, B., Terzibasioglu, E., Sencer, S., Onat, L., Sirvanci, M., 2009. MR Spectroscopy Findings in Lafora Disease. J. Neuroimag.: Off. J. Am. Soc. Neuroimag. 19 (4), 359–365. https://doi.org/10.1111/j.1552-6569.2008.00325.x.

Bozzo, L., Puyal, J., Chatton, J.-Y., Amédée, T., 2013. Lactate Modulates the Activity of Primary Cortical Neurons through a Receptor-Mediated Pathway. PLoS One 8 (8), e71721.

Campos, B.A.G., Yasuda, C.L., Castellano, G., Bilevicius, E., Li, L.M., Cendes, F., 2010. Proton MRS May Predict AED Response in Patients with TLE. Epilepsia 51 (5), 783–878. https://doi.org/10.1111/j.1528-1167.2009.02379.x.

Canafoglia, L., Ferlazzo, E., Michelucci, R., Striano, P., Magaudda, A., Gambardella, A., Pasini, E., Belcastro, V., Riguzzi, P., Fanella, M., Granata, T., Beccaria, F., Trentini, C., Bianchi, A., Aguglia, U., Panzica, F., Franceschetti, S., 2017. Variable Course of Unverricht-Lundborg Disease: Early Prognostic Factors. Neurology 89 (16), 1691–1697.

Danner, N., Julkunen, P., Hyppönen, J., Niskanen, E., Säisänen, L., Könönen, M., Koskenkorva, P., Vanninen, R., Kälviäinen, R., Mervaala, E., 2013. Alterations of Motor Cortical Excitability and Anatomy in Unverricht-Lundborg Disease. Movement Disord.: Off. J. Movement Disord. Soc. 28 (13), 1860–2187. https://doi.org/10.1002/mds.25615.

David, W., 1981. *Wechsler Adult Intelligence Scale-Revised. Book, Whole.* Psychological Corporation, San Antonio, TX.

Fama, R., Sullivan, E.V., 2015. Thalamic Structures and Associated Cognitive Functions: Relations with Age and Aging. Neurosci. Biobehav. Rev. 54 (July), 29–37. https://doi.org/10.1016/j.neubiorev.2015.03.008.

Ferlazzo, E., Gagliano, A., Calarese, T., Magaudda, A., Striano, P., Cortese, L., Cedro, C., Laguitton, V., Bramanti, P., Carbonaro, M., Albachiara, A., Fragassi, N., Italiano, D.,

Sessa, E., Coppola, A., Genton, P., 2009. Neuropsychological Findings in Patients with Unverricht-Lundborg Disease. Epilepsy Behav.: E&B 14 (3), 545–549.

Fernández-Vega, N., Ramos-Rodriguez, J.R., Alfaro, F., Barbancho, M.Á., García-Casares, N., 2021. Usefulness of Magnetic Resonance Spectroscopy in Mesial Temporal Sclerosis: A Systematic Review. Neuroradiology 63 (9), 1395–1405. https://doi.org/10.1007/s00234-021-02704-z.

Frucht, S.J., Leurgans, S.E., Hallett, M., Fahn, S., 2002. The Unified Myoclonus Rating Scale. Adv. Neurol. 89. Journal Article): 361.

Govindaraju, V., Young, K., Maudsley, A.A., 2000. Proton NMR Chemical Shifts and Coupling Constants for Brain Metabolites. NMR Biomed. 13 (3), 129–153. https://doi.org/10.1002/1099-1492(200005)13:3<129::aid-nbm619>3.0.co;2-v.

Haki, C., Gümüştaş, O.G., Bora, I., Gümüştaş, A.U., Parlak, M., 2007. Proton Magnetic Resonance Spectroscopy Study of Bilateral Thalamus in Juvenile Myoclonic Epilepsy. Seizure 16 (4), 287–295. https://doi.org/10.1016/j.seizure.2007.02.017.

Hyppönen, J., Äikiä, M., Joensuu, T., Julkunen, P., Danner, N., Koskenkorva, P., Vanninen, R., Lehesjoki, A.-E., Mervaala, E., Kälviäinen, R., 2015. Refining the Phenotype of Unverricht-Lundborg Disease (EPM1): A Population-Wide Finnish Study. Neurology 84 (15), 1529–1536. https://doi.org/10.1212/WNL.0000000000001466.

Joensuu, T., Lehesjoki, A.-E., Kopra, O., 2008. Molecular Background of EPM1-Unverricht-Lundborg Disease. Epilepsia 49 (4), 557–563. https://doi.org/10.1111/j.1528-1167.2007.01422.x.

Kälviäinen, R., Khyuppenen, J., Koskenkorva, P., Eriksson, K., Vanninen, R., Mervaala, E., 2008. Clinical Picture of EPM1-Unverricht-Lundborg Disease. Epilepsia 49 (4), 549–556. https://doi.org/10.1111/j.1528-1167.2008.01546.x.

Kopitar-Jerala, N., 2015a. Innate Immune Response in Brain, NF-Kappa B Signaling and Cystatins. Front. Mol. Neurosci. 8, 73. https://doi.org/10.3389/fnmol.2015.00073.

Kopitar-Jerala, N., 2015b. The Role of Stefin B in Neuro-Inflammation. Front. Cell. Neurosci. 9, 458. https://doi.org/10.3389/fncel.2015.00458.

Koskenkorva, P., Hyppönen, J., Aikiä, M., Mervaala, E., Kiviranta, T., Eriksson, K., Lehesjoki, A.E., Vanninen, R., Kälviäinen, R., 2011. Severer Phenotype in Unverricht-Lundborg Disease (EPM1) Patients Compound Heterozygous for the Dodecamer Repeat Expansion and the c.202C>T Mutation in the CSTB Gene. Neurodegener. Dis. 8 (6), 515–522. https://doi.org/10.1159/000323470.

Koskenkorva, P., Khyuppenen, J., Niskanen, E., Könönen, M., Bendel, P., Mervaala, E., Lehesjoki, A.E., Kälviäinen, R., Vanninen, R., 2009. Motor Cortex and Thalamic Atrophy in Unverricht-Lundborg Disease: Voxel-Based Morphometric Study. Neurology 73 (8), 606–611. https://doi.org/10.1212/WNL.0b013e3181b3888b.

Koskenkorva, P., Niskanen, E., Hyppönen, J., Könönen, M., Mervaala, E., Soininen, H., Kälviäinen, R., Vanninen, R., 2012. Sensorimotor, Visual, and Auditory Cortical Atrophy in Unverricht-Lundborg Disease Mapped with Cortical Thickness Analysis. AJNR Am. J. Neuroradiol. 33 (5), 878–883. https://doi.org/10.3174/ajnr.A2882.

Kuzniecky, R., Hetherington, H., Pan, J., Hugg, J., Palmer, C., Gilliam, F., Faught, E., Morawetz, R., 1997. Proton Spectroscopic Imaging at 4.1 Tesla in Patients with Malformations of Cortical Development and Epilepsy. Neurology 48 (4), 1018–1024. https://doi.org/10.1212/wnl.48.4.1018.

Li, L.M., Cendes, F., Bastos, A.C., Andermann, F., Dubeau, F., Arnold, D.L., 1998. Neuronal Metabolic Dysfunction in Patients with Cortical Developmental Malformations: A Proton Magnetic Resonance Spectroscopic Imaging Study. Neurology 50 (3), 755–779. https://doi.org/10.1212/wnl.50.3.755.

Lin, K., Carrete Jr, H., Lin, J., Peruchi, M.M., de Araújo Filho, G.M., Guaranha, M.S.B., Guilhoto, L.M.F.F., Sakamoto, A.C., Yacubian, E.M.T., 2009. Magnetic Resonance Spectroscopy Reveals an Epileptic Network in Juvenile Myoclonic Epilepsy. Epilepsia 50 (5), 1191–1200.

Magistretti, P.J., Allaman, I., 2018. Lactate in the Brain: From Metabolic End-Product to Signalling Molecule. Nat. Rev. Neurosci. 19 (4), 235–249. https://doi.org/10.1038/nrn.2018.19.

Mancini, G.M.S., Schot, R., Marie, C.Y., de Wit, R.F., de Coo, R., Oostenbrink Heus, K.-d., Berger, L.P.V., et al., 2016. CSTB Null Mutation Associated with Microcephaly, Early Developmental Delay, and Severe Dyskinesia. Neurology 86 (9), 877–888. https://doi.org/10.1212/WNL.0000000000002422.

Manninen, O., Koskenkorva, P., Lehtimäki, K.K., Hyppönen, J., Könönen, M., Laitinen, T., Kalimo, H., Kopra, O., Kälviäinen, R., Gröhn, O., Lehesjoki, A.-E.,

Vanninen, R., 2013. White Matter Degeneration with Unverricht-Lundborg Progressive Myoclonus Epilepsy: A Translational Diffusion-Tensor Imaging Study in Patients and Cystatin B-Deficient Mice. Radiology 269 (1), 232–239.

Mascalchi, M., Brugnoli, R., Guerrini, L., Belli, G., Nistri, M., Politi, L.S., Gavazzi, C., Lolli, F., Argenti, G., Villari, N., 2002. Single-Voxel Long TE 1H-MR Spectroscopy of the Normal Brainstem and Cerebellum. J. Magn. Reson. Imag.: JMRI 16 (5), 532–557. https://doi.org/10.1002/jmri.10189.

McHaffie, J.G., Stanford, T.R., Stein, B.E., Coizet, V., Redgrave, P., 2005. Subcortical Loops through the Basal Ganglia. Trends Neurosci. 28 (8), 401–407. https://doi.org/10.1016/j.tins.2005.06.006.

Nagano-Saito, A., Martinu, K., Monchi, O., 2014. Function of Basal Ganglia in Bridging Cognitive and Motor Modules to Perform an Action. Front. Neurosci. 8, 187. https://doi.org/10.3389/fnins.2014.00187.

Okuneva, O., Körber, I., Li, Z., Tian, L.i., Joensuu, T., Kopra, O., Lehesjoki, A.-E., 2015. Abnormal Microglial Activation in the Cstb(-/-) Mouse, a Model for Progressive Myoclonus Epilepsy, EPM1. Glia 63 (3), 400–411. https://doi.org/10.1002/glia.22760.

Patel, D.C., Tewari, B.P., Chaunsali, L., Sontheimer, H., 2019. Neuron-Glia Interactions in the Pathophysiology of Epilepsy. Nat. Rev. Neurosci. 20 (5), 282–297. https://doi.org/10.1038/s41583-019-0126-4.

Rae, C.D., 2014. A Guide to the Metabolic Pathways and Function of Metabolites Observed in Human Brain 1H Magnetic Resonance Spectra. Neurochem. Res. 39 (1), 1–36. https://doi.org/10.1007/s11064-013-1199-5.

Sada, Nagisa, Lee, Suni, Katsu, Takashi, Otsuki, Takemi, Inoue, Tsuyoshi, 2015. Epilepsy Treatment. Targeting LDH Enzymes with a Stiripentol Analog to Treat Epilepsy. Sci. (New York, N.Y.) 347 (6228), 1362–1367. https://doi.org/10.1126/science.aaa1299.

Saunders, D.E., Howe, F.A., van den Boogaart, A., Griffiths, J.R., Brown, M.M., 1999. Aging of the Adult Human Brain. In Vivo Quantitation of Metabolite Content with Proton Magnetic Resonance Spectroscopy. J. Magn. Reson. Imag.: JMRI 9 (5), 711–776. https://doi.org/10.1002/(sici)1522-2586(199905)9:5<711::aid-jmri14>3.0.co;2-3.

Savic, I., Lekvall, A., Greitz, D., Helms, G., 2000. MR Spectroscopy Shows Reduced Frontal Lobe Concentrations of N-Acetyl Aspartate in Patients with Juvenile Myoclonic Epilepsy. Epilepsia 41 (3), 290–326. https://doi.org/10.1111/j.1528-1157.2000.tb00158.x.

Savic, I., Osterman, Y., Helms, G., 2004. MRS Shows Syndrome Differentiated Metabolite Changes in Human-Generalized Epilepsies. Neuroimage 21 (1), 163–172. https://doi.org/10.1016/j.neuroimage.2003.08.034.

Seyfert, S., Bernarding, J., Braun, J., 2003. Volume-Selective 1H MR Spectroscopy for in Vivo Detection of Valproate in Patients with Epilepsy. Neuroradiology 45 (5), 295–329. https://doi.org/10.1007/s00234-003-0973-5.

Silverstone, P.H., Wu, R.H., O'Donnell, T., Ulrich, M., Asghar, S.J., Hanstock, C.C., 2003. Chronic Treatment with Lithium, but Not Sodium Valproate, Increases Cortical N-Acetyl-Aspartate Concentrations in Euthymic Bipolar Patients. Int. Clin. Psychopharmacol. 18 (2), 73–79. https://doi.org/10.1097/00004850-200303000-00002.

Simmons, M.L., Frondoza, C.G., Coyle, J.T., 1991. Immunocytochemical Localization of N-Acetyl-Aspartate with Monoclonal Antibodies. Neuroscience 45 (1), 37–45. https://doi.org/10.1016/0306-4522(91)90101-s.

Suzuki, A., Stern, S.A., Bozdagi, O., Huntley, G.W., Walker, R.H., Magistretti, P.J., Alberini, C.M., 2011. Astrocyte-Neuron Lactate Transport Is Required for Long-Term Memory Formation. Cell 144 (5), 810–823. https://doi.org/10.1016/j.cell.2011.02.018.

van Veenendaal, T.M., IJff, D.M., Aldenkamp, A.P., Hofman, P.A.M., Vlooswijk, M.C.G., Rouhl, R.P.W., de Louw, A.J., Backes, W.H., Jansen, J.F.A., 2015. Metabolic and Functional MR Biomarkers of Antiepileptic Drug Effectiveness: A Review. Neurosci. Biobehav. Rev. 59, 92–99.

Yıldırım, F., Aydin, Z., Sakcı, Z., Destînâ Yalçın, A.E., 2022. Investigation of Patients With Eye Closure Sensitive Epilepsy With Magnetic Resonance Spectroscopy. Clin. EEG Neurosci. 53 (1), 45–53. https://doi.org/10.1177/15500594211040953.