ADENYLOSUCCINATE LYASE DEFICIENCY: A CASE REPORT

A CURA DI :

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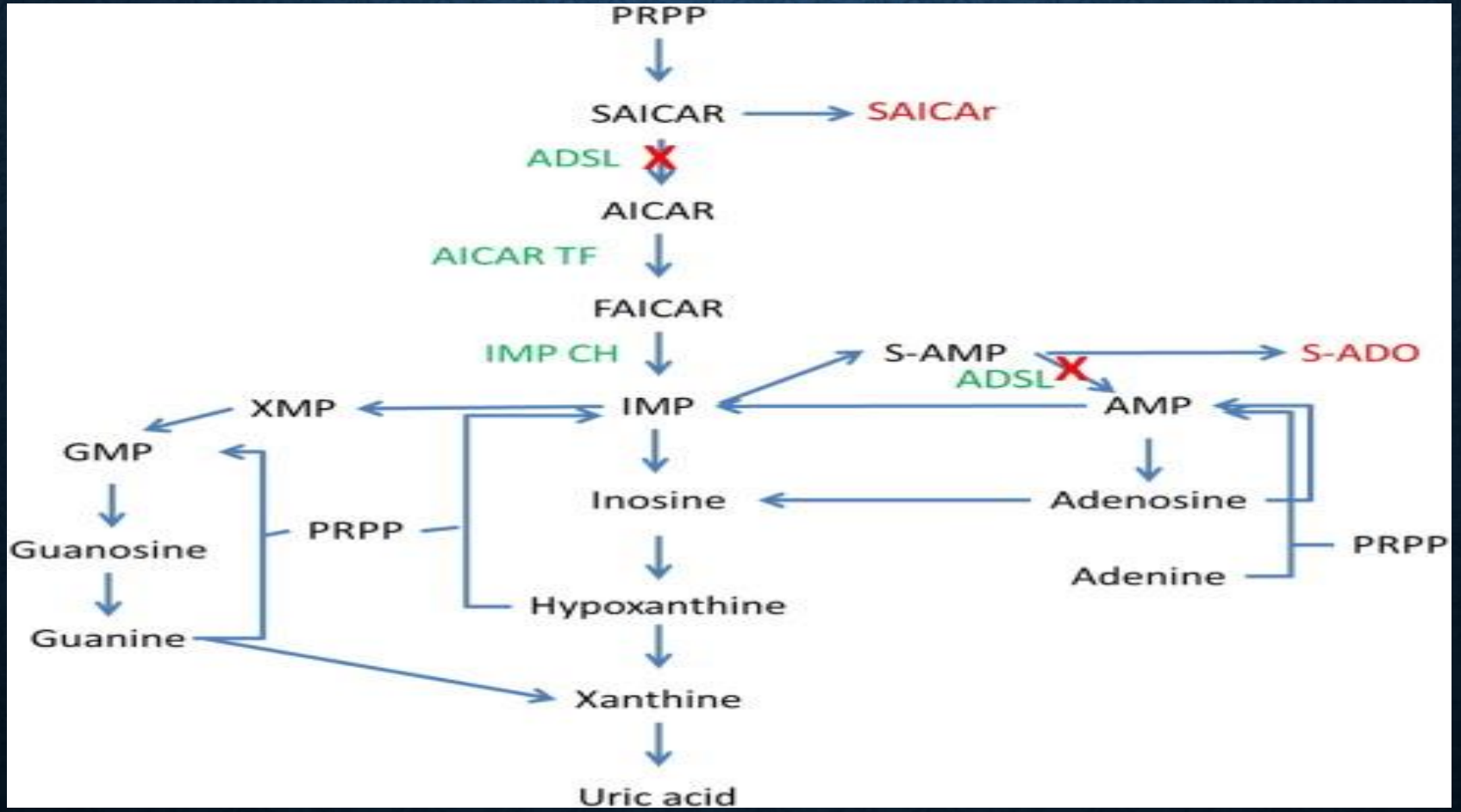
HISTORY AND EARLY SYMPTOMS:

- Patience: 34 years old female
- Born at term from unrelated parents, after a healthy pregnancy
- 1 elder sister, currently in good health
- Lack of eye contact after few months
- Muscular hypertonicity
- High acoustic sensitivity
- Delayed psycho-motor development
- Seizures from the age of 5
- Disharmonious walking
- Aphasia
- Normal skull size

On **february 28, 1992 she was diagnosed** with **Adenylosuccinate lyase deficiency (ASLD)**, a rare, inherited metabolic disorder due to a lack of the enzyme adenylosuccinate lyase (ASL). The defect is characterized by the appearance of succinylaminoimidazole carboxamide riboside (SAICAr) and succinyladenosine (S-Ado), in cerebrospinal fluid, in urine and, to a much smaller extent, in plasma.



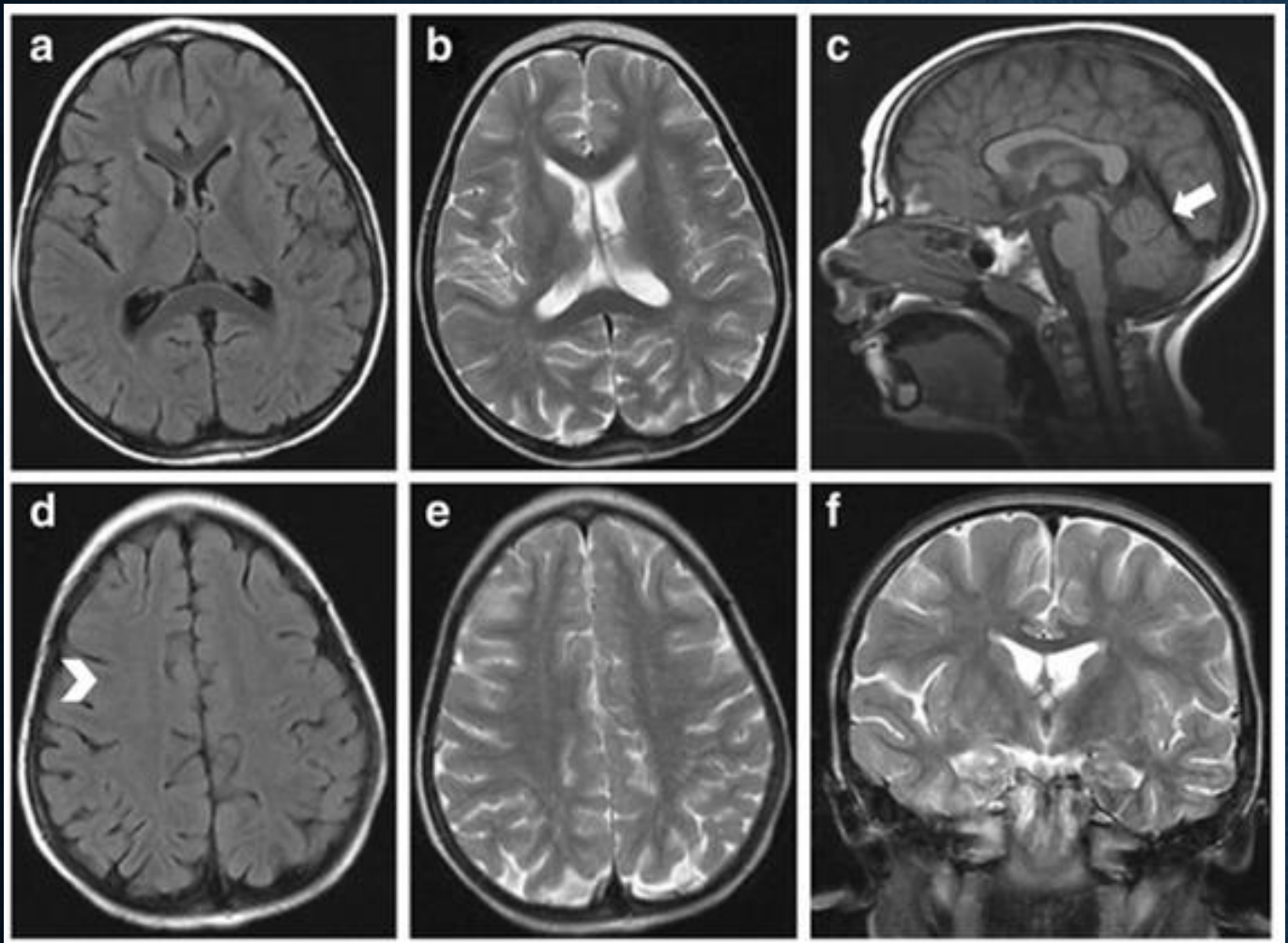
Adenylosuccinate lyase deficiency

ADSL disease was identified in 1984 as the first inborn defect of purine biosynthesis. It is a rare autosomal recessive disorder and over 50 different ADSL mutations have been identified. The phenotype severity is mainly determined by residual catalytic capacity of the enzyme. This is due to the role of the enzyme both in the synthesis of the purines and in the purine nucleotide cycle by which AMP and fumarate is obtained from the adenylosuccinate (fumarate is an oxidable substrates directly to the citric acid cycle). One of the biochemical disorder, for this, could be disorder of energy metabolism especially during energy consuming period, like stress. However the main pathogenic effects has been attributed to the toxic effects of accumulating succinyl purines (SAdo and SAICAR). All affected revealed a wide spectrum of symptoms from slowly to rapidly progressive forms: severe psychomotor retardation, hypotonia and spasticity but neurological symptoms are the most common, especially epilepsy. Epilepsy may be difficult to treat, due to the frequently drug resistence and there's no standardized therapy. Most current therapies have as goal symptom treatment, such as D ribose or uridine (to stimulate purine de novo synthesis), S adenosilmetionina or epilepsy treatment and on this last one we focus our attention, since the use of classical anticonvulsant or ketogenic diet has shown side effects.

1. Jurecka, A., Zikanova, M., Kmoch, S. & Tylki-Szymańska, A. Adenylosuccinate lyase deficiency. *J. Inherit. Metab. Dis.* **38,** 231–42 (2015).

SIGNS AND SYMPTOMS OF ADSL DEFICIENCY

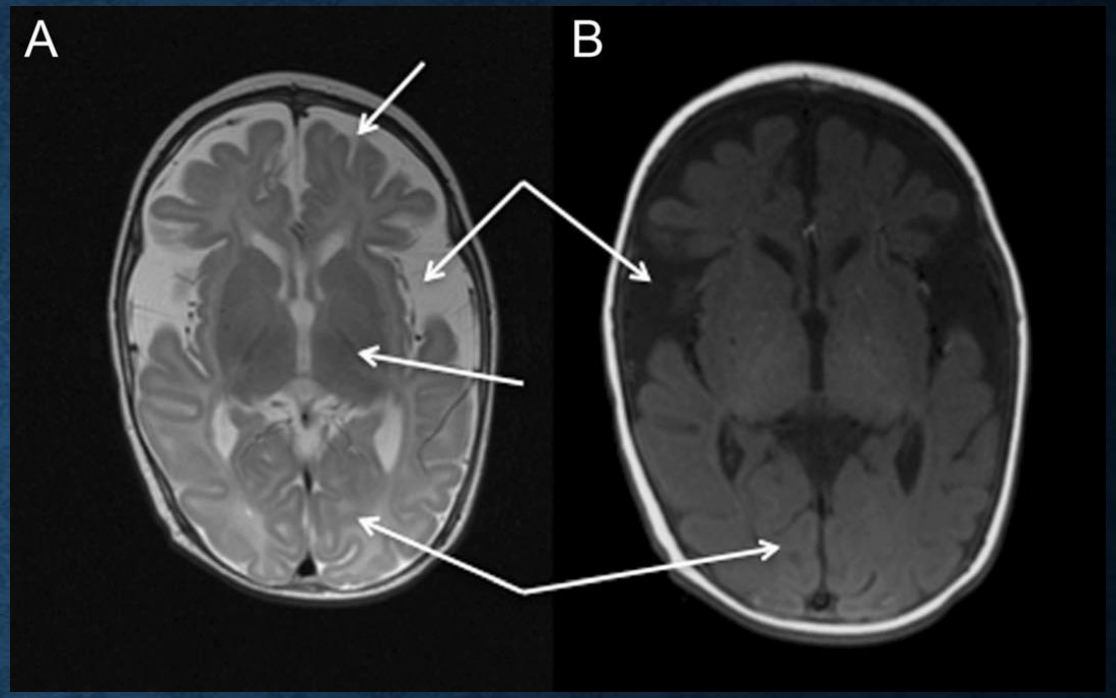
Head and Face	**Neurologic**
-Total body Growth retardation	Central Nervous System
- Brachycephaly	- Psychomotor delay
- Microcephaly	- Mental retardation
- Prominent metopic suture	- Hypotonia
- Thin upper lip	- Gait ataxia
- Long smooth philtrum	- Inability to walk
- Low-set ears	- Poor eye contact
- Strabismus	- Poor language and speech development
- Nystagmus	- Seizures
Behavioral Psychiatric Manifestations	- Spasticity
- Autistic features (?)	- Opisthotonus
- Hyperactivity	- Myoclonus
- Aggressive behavior	- Brisk reflexes
- Temper tantrums	- Cerebral atrophy
- Stereotypic movements	- Cerebellar atrophy
- Self-mutilation	- Hypomyelination
- Inappropriate laughter	- Atrophy of corpus callosum
- Happy demeanor	



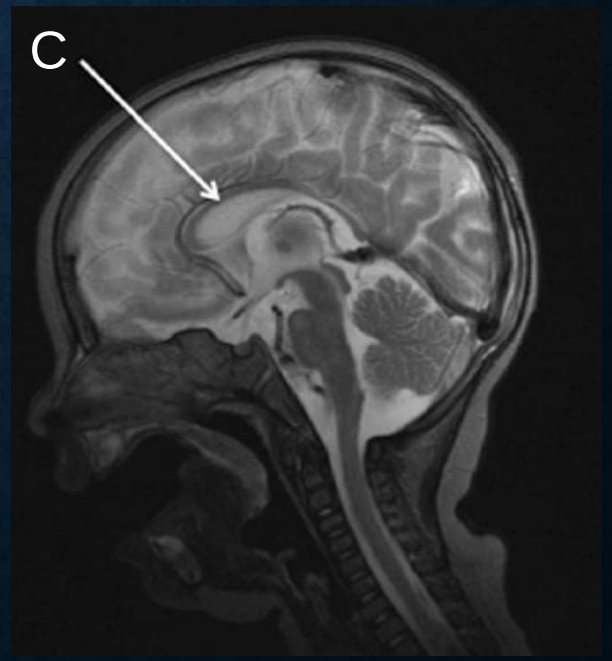
MRI of the patient at age 4 years. The images show very mild abnormalities of the white matter that are barely visible in FLAIR (**a, d**) (*arrow head*). Sagittal T1 weighted image shows a normal corpus callosum and a mild increased spacing of the cerebellar folia (*arrow*) (**c**). Coronal and axial T2 weighted images document peculiar fine stripes in T2 weighted image (**b, e, f**)

Magnetic resonance imaging (MRI) can be a useful tool for diagnosing and monitoring the pathological progression of the changes in the course of the disease.

12 reports of brain MRI associated with ADSL deficiency are correlated with atrophy of the cerebral cortex, corpus callosum, cerebellar vermis, lack of myelination, anomalies of the white matter.

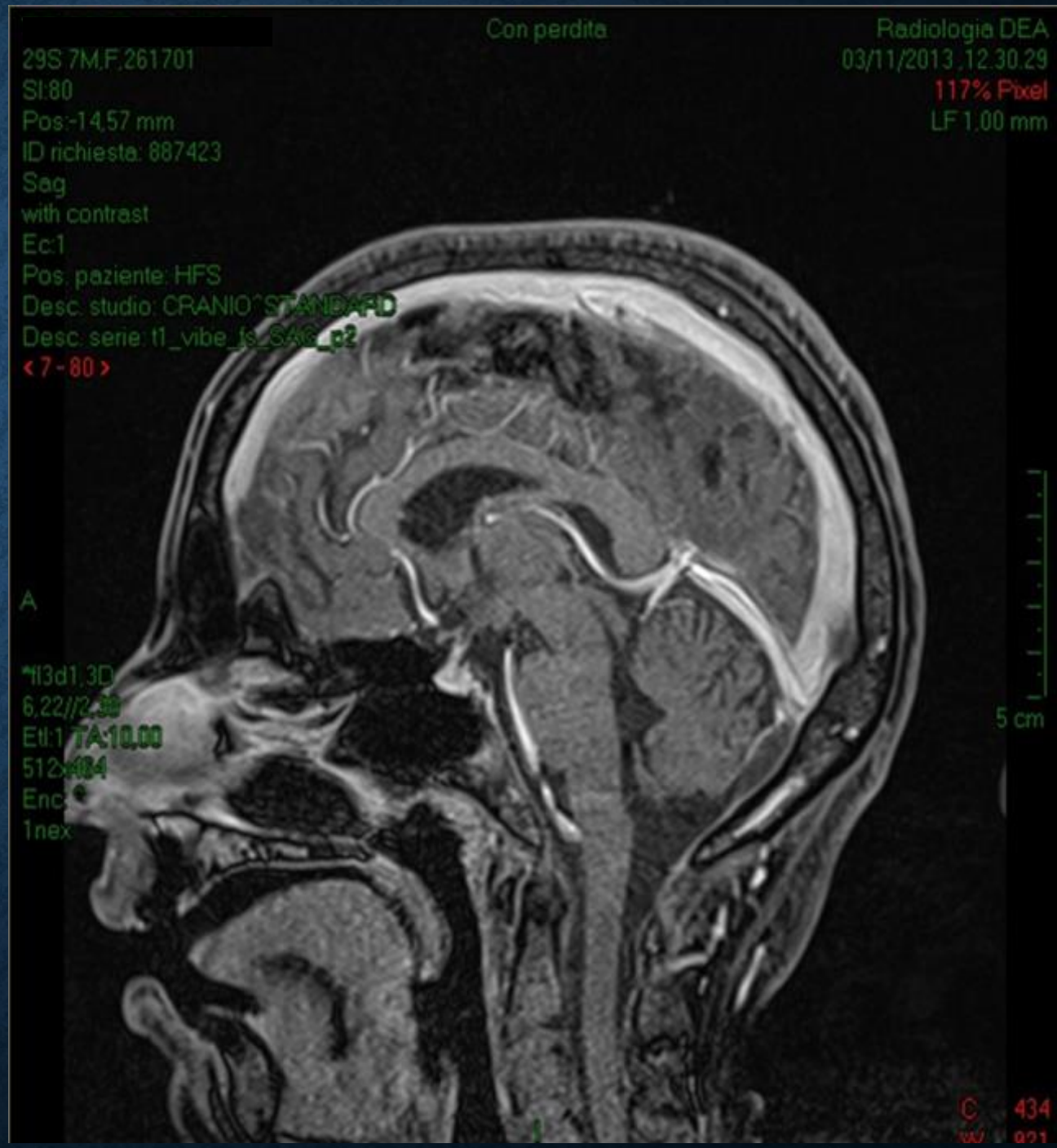


Enlarged subarachnoid space in the frontal regions (A), widened lateral ventricles (especially enlargement of the body and occipital horns), wide Sylvian fissures with hypoplasia operculum (B), thin corpus callosum and lack of myelination of the white matter volume (C).



OUR PATIENT DID NOT PRESENTED ANY
OF THE MRI ALTERATIONS OR SIGNS

03/11/2018
RM CRANIO GD e DIFFUSIONE
Non si rilevano aree di restrizione della diffusione, spia di lesioni ischemiche recenti.
Non sono state rilevate lesioni edemigene nei tessuti cerebrali nè aree di abnorme impregnazione dopo somministrazione di mdc.
Presenza di piccola lesione rotondeggiante (0,6 cm) ipointensa in GRE in sede cerebellare sinistra con altra lesione analoga (0,4 cm) in sede cerebellare destra, con aspetto compatibile con cavernomi, senza segni di sanguinamento recente.
Sistema ventricolare in asse, di regolari morfologia e dimensioni.
Regolare rappresentazione degli spazi subaracnoidei del mantello.
Strutture della linea mediana in asse.
Non colate trombotiche nei seni venosi endocranici.



EPILEPSY AND AMMONIA

Ammonia research: 03/05/2016

- 05/05 seizure: ammonia concentration of 104 microg/dl
- 07/05: menstrual phase and new seizure
- 08/05: eats an egg and takes branched-chain aminoacid
- 09/05: new seizure
- 10/05: ammonia concentration og 79.7 microg/dl

PICO QUESTION COMPONENT	KEYWORDS	MeSH WORDS
P POPULATION/PROBLEM	HYPERAMMONEMIA, ADSL DEFICIENCY, DRUG-RESISTANT EPILEPSY	''ADENYLOSUCCINATE LYASE'' OR ''ADSL DEFICIENCY'', ''HYPERAMMONEMIA'', ''EPILEPSY'' AND ''DRUG RESISTANT''
I INTERVENTION	BUMETANIDE OR OTHER NKCC GATE INHIBITOR	''BUMETANIDE'' AND ''HYPERAMMONEMIA'' OR ''NKCC'' AND ''EPILEPSY''
C COMPARISON	VALPROIC ACID, ANTICONVULSANT, NAGS N-CARBAMYLGLUTAMATE, CARBAMYLPHOSPATE	''VALPROATE'' AND ''SEIZURES'' OR ''NCG'', ''N-ACETYLGLUTAMATE SINTHASE''
O OUTCOMES	NORMAL AMMONEMIA, BETTER QUALITY OF LIFE, SEIZURES-FREE LIFE	''QUALITY OF LIFE'', ''SEIZURES-FREE''

PICO QUESTION

IN PATIENTS WITH METABOLISM DISORDERS, AFFECTED BY DRUG-RESISTANT EPILEPSY DURING PEAKS OF HYPERAMMONEMIA, IS IT BETTER TO TREAT HYPERAMMONEMIA WITH LOOP DIURETICS RATHER THAN OTHER DRUGS THAT ENHANCES THE UREA CYCLE?

SELECTION CRITERIA

INCLUSION

Patient characteristics:

- Metabolism enzymes deficiency
  - ADSL deficiency
  - Valproate-induced inhibition of urea cycle enzymes
  - N-acetil-glutamate deficiency
- Neurological symptoms as aphasia and altered neuro-motor developement
- Drug-resistant epilepsy

EXCLUSION

Patient characteristics:

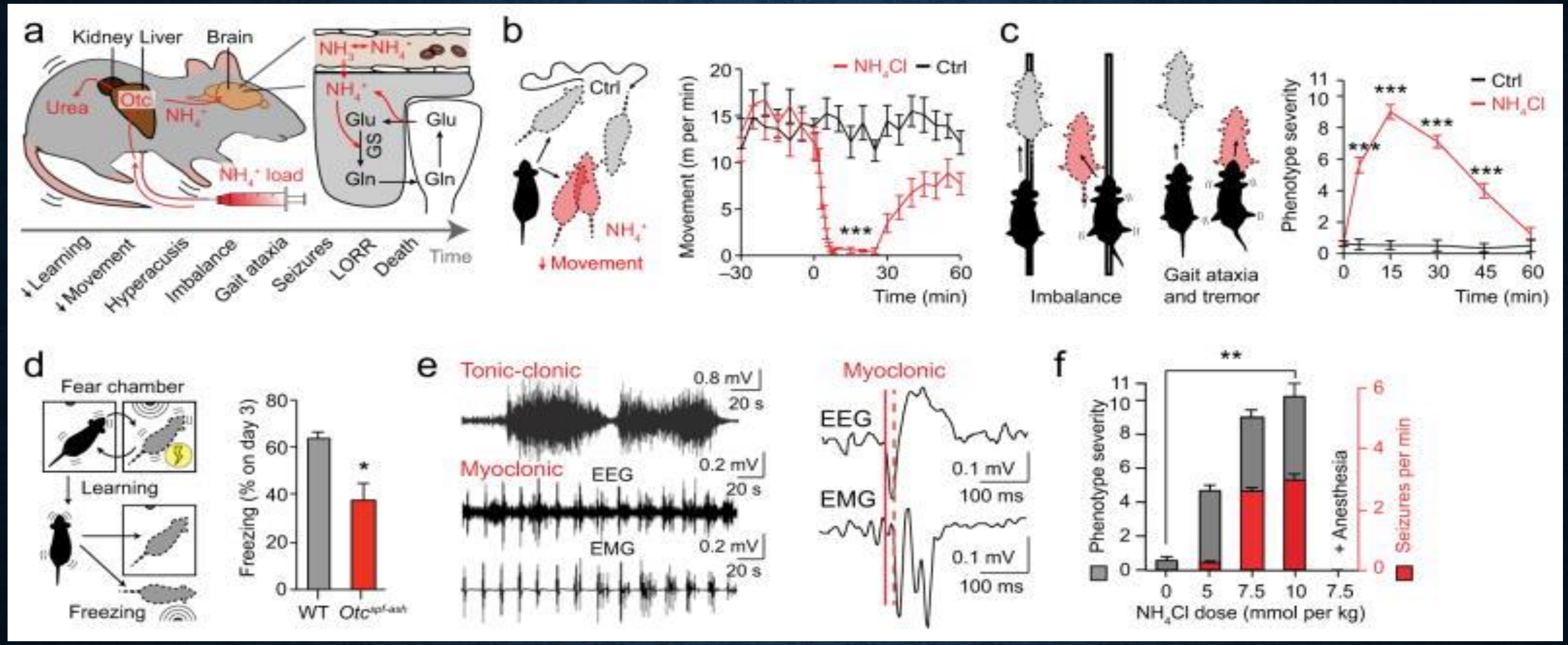
- Autism Spectrum disorders
- Epilepsy not associated to other systemic disorders.
- Responders to anti-epilectic drugs
- Estrogens-related hyperammonemia

ROLE OF AMMONIA IN DRUG RESISTANT SEIZURES

- **The brain is especially vulnerable to ammonia: «Ammonia triggers neuronal disinhibition and seizures by impairing astrocyte potassium buffering»**

- **Hyperammonemia causes lack of K+ re-uptake inside astrocytes and NKCC1 up-regulation, which leads to a deregulated GABA inhibitory activity.**

- **The neurotoxicity of hyperammonemia becomes evident with the recurrent seizures.**



We show that ammonia rapidly compromises astrocyte potassium buffering, increasing extracellular potassium concentration and overactivating the Na^+-K^+-$2Cl^-$cotransporter isoform 1 (NKCC1) in neurons. The consequent depolarization of the neuronal GABA reversal potential (E_{GABA}) selectively impairs cortical inhibitory networks.

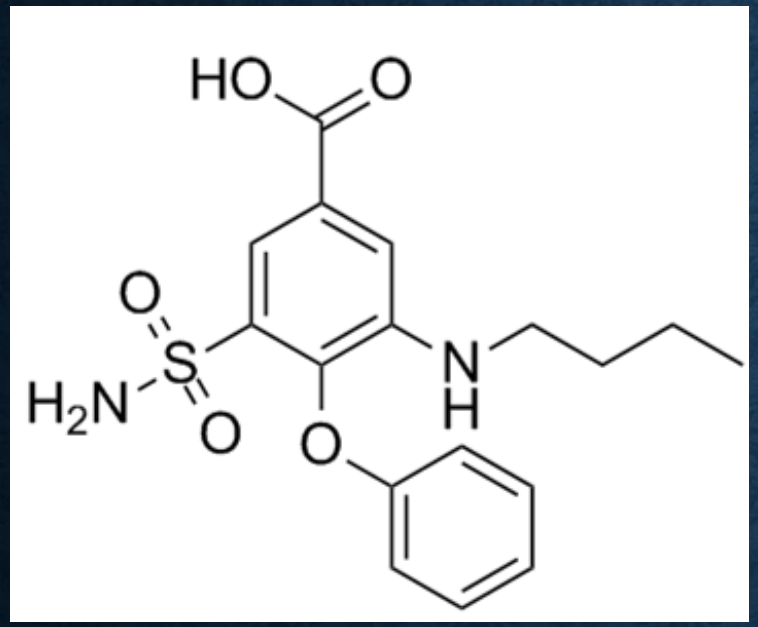
Ammonia triggers neuronal disinhibition and seizures by impairing astrocyte potassium buffering

- Hyperammonemia is characterized by stupor, seizures and coma.
- Astrocytes possess the primary enzyme necessary for ammonia detoxification
- Our observations indicate that elevated [NH4+]-out and [K+]-out drive an over-activation of neuronal NKCC1, leading to cortical disinhibition and seizures.

1. Rangroo Thrane, V. *et al.* Ammonia triggers neuronal disinhibition and seizures by impairing astrocyte potassium buffering. *Nat. Med.* **19,** 1643–8 (2013).

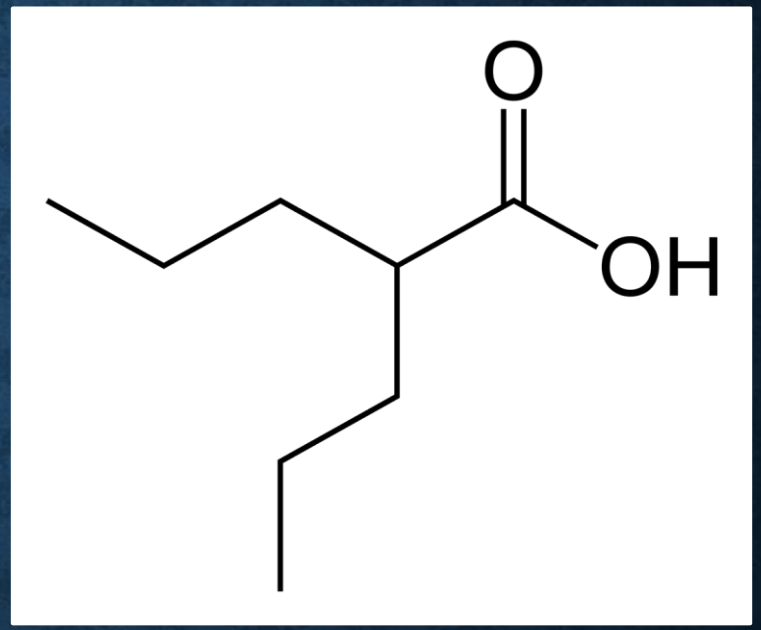
HOW TO TREAT THE SEIZURES?

BUMETANIDE



Bumetanide is a powerful sulfamoylanthranilic acid derivative, belonging to the class of loop diuretics. In the brain, bumetanide may prevent seizures in neonates by blocking the bumetanide-sensitive NKCC1, thereby inhibiting chloride uptake thus, decreasing the internal chloride concentration in neurons and may block the excitatory effect of GABA in neonates.

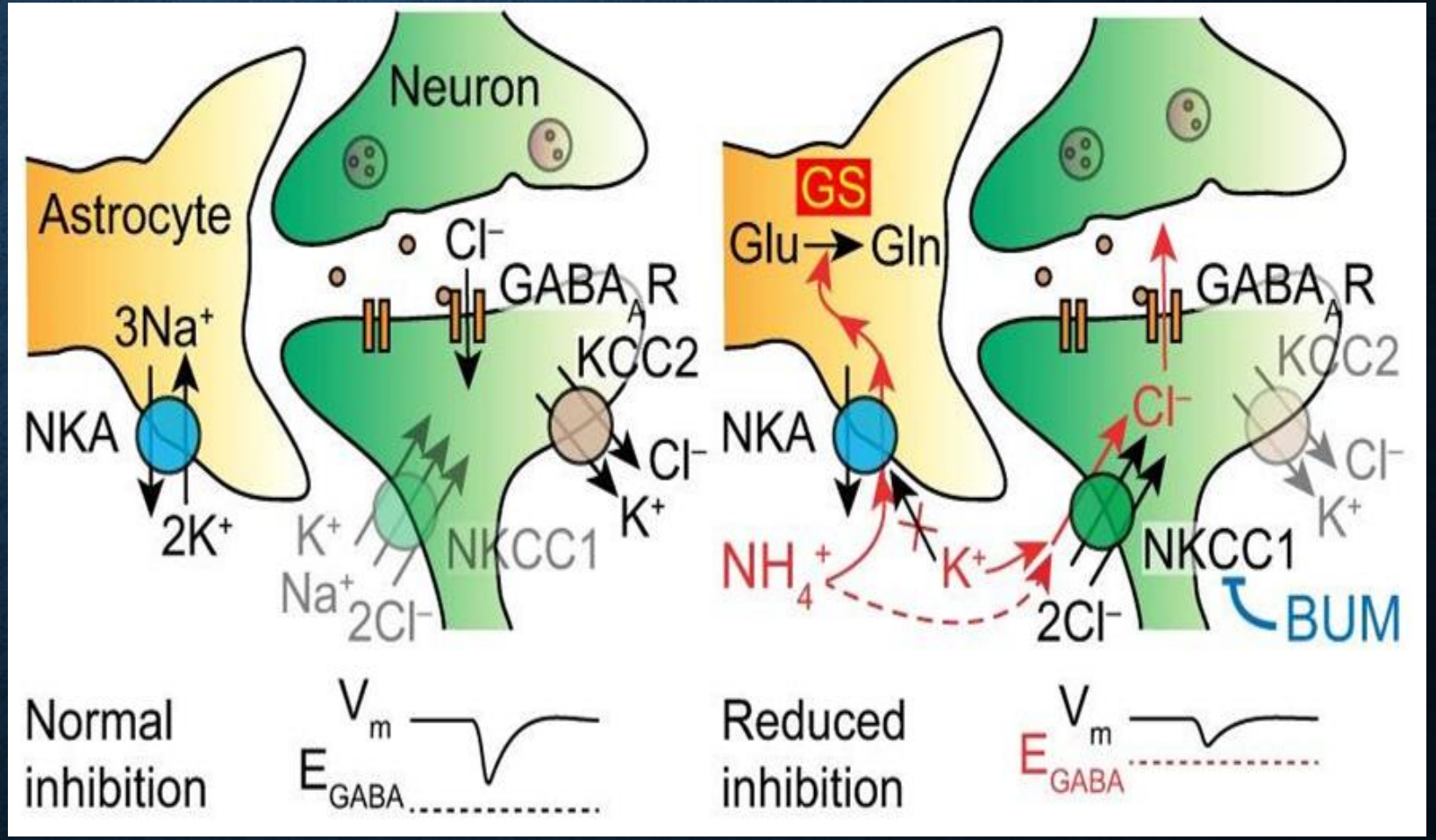
VALPROATO



Valproic acid, first manufactured as an anticonvulsant, is commonly used to treat both neurological and psychiatric conditions. A rare and deadly side effect of this medication is hyperammonemia, presenting as lethargy, confusion, seizure, and, ultimately, coma.

THE MECHANISM OF ACTION OF BUMETANIDE IS SELECTIVE BLOCKADE OF THE CATION-CHLORIDE COTRANSPORTER NKCC.

In the abnormally excitable neuron, known to harbor intracellular Cl^- levels comparable to immature neurons, loop diuretics such as bumetanide appear to return chloride balance toward "mature" levels. (Kahle & Staley, 2008).



Diuretics and epilepsy: Will the past and present meet?

- During **seizures**, the Cl– currents through the GABA A channel lead to **equilibration of the intra/extra-cellular [Cl-].** The end result is a more pronounced depolarizing shift in the GABA response, which reduces the efficacy of GABAergic inhibition.

- The intracellular level of chloride is established by **NKCC1 and KCC2.** If **NKCC1** activity predominates, it results in chloride accumulation and an efflux of chloride through the GABA A receptor upon GABA binding, eliciting **depolarizing excitation.**

- In adult neurons, **KCC2** activity predominates, resulting in a relatively low intracellular chloride concentration; therefore, chloride moves into the cell via the $GABA_A$ receptor and elicits **hyperpolarizing inhibition.**

- **Bumetanide** is a loop diuretic which blocks the cation-chloride cotransporter NKCC1. The diuretic effect of bumetanide is 40 times more powerful than furosemide, and maximum diuresis occurs within 15–30 minutes of intravenous administration

1. Maa, E. H., Kahle, K. T., Walcott, B. P., Spitz, M. C. & Staley, K. J. Diuretics and epilepsy: Will the past and present meet? *Epilepsia* **52,** 1559–1569 (2011).

Risk factors of hyperammonia in patience with epilepsy in treatment with VPA

- The study has proved that the **blood ammonia level was significantly related to the dosage of VPA.** Most of the patients with high blood levels of ammonia were asymptomatic.

- VPA seems to **cause hyperammonia** for its direct inhibition of the mitochondrial urea cycle enzyme, carbamoyl phosphate synthetase I (CPS I), through interference in the synthesis of N-acetylglutamate, and inhibition of the mitochondrial fatty acid beta-oxidation pathway.

- The study has also shown that the main risk factors for VPA-induced hyperammonemia is the concomitant use of VPA with liver enzyme-inducing anti-epilectic drugs (AEDs).

1. Tseng, Y.-L. *et al.* Risk factors of hyperammonemia in patients with epilepsy under valproic acid therapy. *Medicine (Baltimore).* **93,** e66 (2014).

- Carbamyl-phosphate synthetase I (CPSI), catalyzes the conversion of ammonia, bicarbonate and ATP to carbamyl-phosphate. Fig. 1

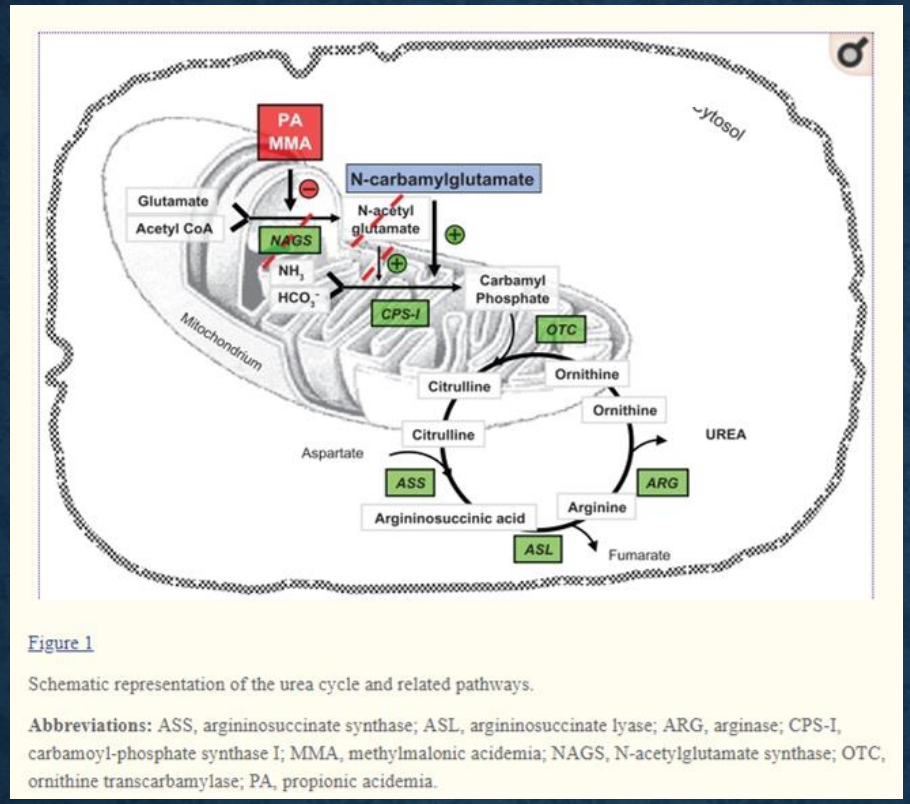


Figure 1

Schematic representation of the urea cycle and related pathways.

Abbreviations: ASS, argininosuccinate synthase; ASL, argininosuccinate lyase; ARG, arginase; CPS-I, carbamoyl-phosphate synthase I; MMA, methylmalonic acidemia; NAGS, N-acetylglutamate synthase; OTC, ornithine transcarbamylase; PA, propionic acidemia.

- However, CPSI is active only with N-acetylglutamate (NAG) which is catalytically produced from acetyl-CoA and glutamate by NAG synthase (NAGS) and so an NAGS deficiency
- (or the admnistration of valproate) can cause hyperammonemia.

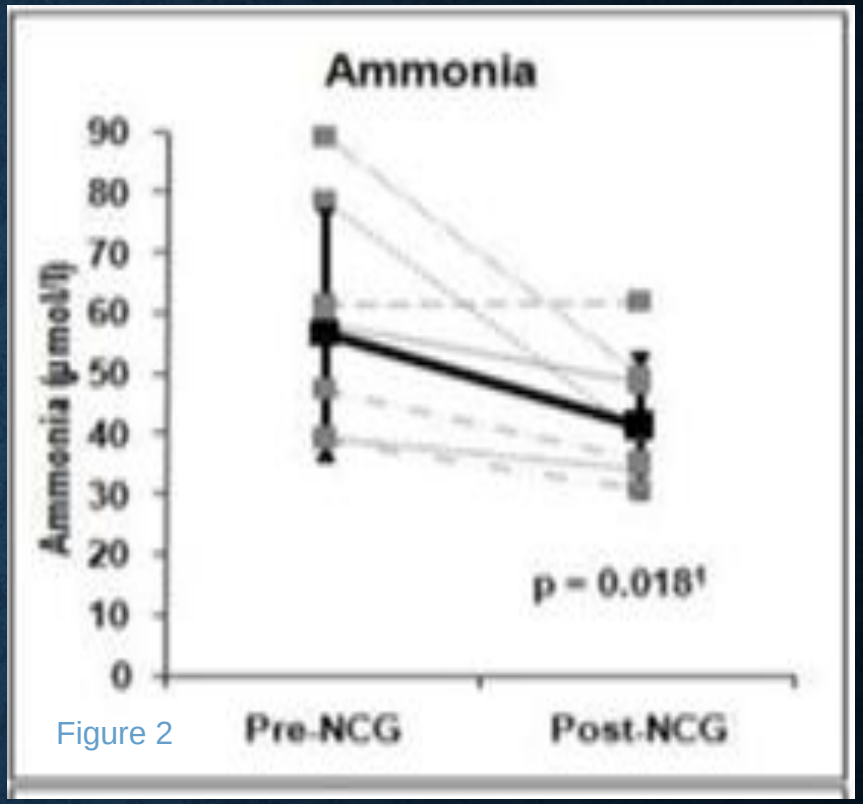


Figure 2

- Multiple blood samples of 7 patients aged 15 months-13 years showed us [NH4+] between 47µM and 171µM.
- N-carbamylglutamate (NCG), an analog of NAG, activates CPSI normalizing blood ammonia concentrations.
- After NCG, all patient [NH4+] has been lowered proportionally to their original values. Fig. 2

1. Ah Mew, N. *et al.* N-carbamylglutamate augments ureagenesis and reduces ammonia and glutamine in propionic acidemia. *Pediatrics* **126,** e208-14 (2010).

Survival after Treatment with Phenylacetate and Benzoate for Urea-Cycle Disorders[1]

This research is an open label study, uncontrolled, nonrandomized study, in which it is studied how to improve survival in small cohorts of patients with historically lethal urea-cycle enzyme defects and present hyperammonia. It is based from a combination of intravenous sodium phenylacetate and sodium benzoate has been shown to lower plasma ammonium levels. The primary end point was survival of the episode of hyperammonemia. The survival rate for hyperammonemic episodes was 96%.

It's important:

- **prompt recognition of a urea-cycle disorder**
- **treatment with both sodium phenylacetate and sodium benzoate, in conjunction with other therapies, such as intravenous arginine hydrochloride**
- **provision of adequate calories to prevent catabolism**, effectively lower plasma ammonium levels and result in survival in the majority of patients

1. Enns, G. M. *et al.* Survival after Treatment with Phenylacetate and Benzoate for Urea-Cycle Disorders. *N. Engl. J. Med.* **356,** 2282–2292 (2007).

PATIENT'S CONCLUSIONS

Studies have shown that metabolic diseases with drug-resistant epilepsy can only be treated by discovering the original cause. For the specific case of ADSL, we hypothesized that the seizure could be triggered by hyperammonia during the mestrual period. Starting from the analysis of the patient's blood sample, we explored the possible pathogenic mechanisms and effective drugs for the treatment.

We focused our research on the therapeutic effect of Bumetanide, in order to reduce ammonia blood levels. We also considered the side effects of this drug if used on patient with metabolic disorders, as it has been shown to cause gout, one of the mildest deseases in purine metabolism by increasing the riabsorption of uric acid.

There are several other drugs that can reduce ammonia levels, as L-arginine, which induces the transformation of ammonia into urea by the enzyme NAGS (N-acetil glutamate synthases). The intake on D-ribosio can also inhibit the synthesis of ammonia by increasing the energetic income.

In conclusion ADSL is a severe and rare metabolic disorder, with various clinical manifestation. In this specific case, the gene mutations of the patient, which is coded "McKusick608222.0003/0004", wasn't found in any other patient.

Drug resistant seizure can be treated using different drugs other than the standard anticonvulsivants, which are not effective on patients with metabolic disorder. In order not to produce further damage than those already caused by the desease, these patients should be under the medical care of experts in purines-related diseases and biochemistry.

We hope that these researches will be analyzed
in a more specific way by experts!!!

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