

# **BIOCHEMICAL ABNORMALITIES IN NEUROTRANSMITTER DISORDERS**

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*9<sup>th</sup> Annual CHILDx Fall Symposium*

*12 November 2009*

# OBJECTIVES

- Define neurotransmitter disorders identifiable with “routine” laboratory tests (PAA, UOA)
  - Glycine encephalopathy
  - Serine deficiency encephalopathies
  - Hyperprolinemia type 2
  - SSADH deficiency

## DISCLOSURES:

No relevant financial disclosures

# Case Presentation

**HPI: 5 do term female admitted for lethargy and severe generalized hypotonia. Stopped crying at 2 days of age, became sleepy on day 3 and stopped feeding. On day 4, she was lethargic, hypotonic with twitches.**

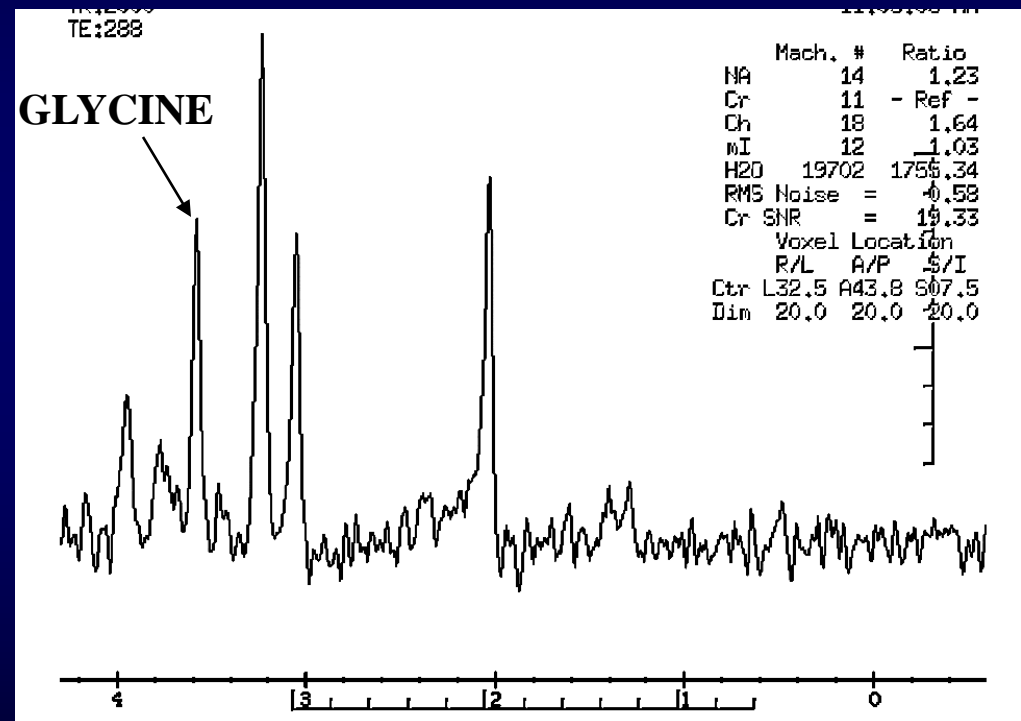
**There were normal fetal movements and history of hiccups before and after birth. Normal delivery.**

**Physical exam: shallow respirations; no gag or suck reflex; severe, generalized hypotonia; exaggerated Moro reflex; spontaneous, intermittent paroxysms of full body hiccups. No DTR or grasp reflexes, withdrawal from pain in all extremities.**

**Normal routine labs including CSF studies were performed.**

# NEURO EEG/MRI

- EEG: multifocal epileptiform discharges
- Brain MRI: diffusion restriction along the cortical spinal tracts with lack of normal myelination in posterior limb
- NMR: Elevated glycine



# GLYCINE ENCEPHALOPATHY (Nonketotic hyperglycinemia)

## CSF

ALA-SF	41.6	12.5-47.3	umol/L
ARG-SF	12.1	5.9-30.6	umol/L
ASN-SF	38.9	< 23.6	umol/L
ASP-SF	4.2	< 5.8	umol/L
CIT-SF	6.2	< 5.6	umol/L
CYS-SF	0.3	< 5.0	umol/L
GLN-SF	623.0	230.7-637.4	umol/L
GLU-SF	0.0	< 15.0	umol/L
<b>GLY-SF</b>	<b>179.5</b>	<b>3.1-21.0</b>	<b>umol/L</b>
HIS-SF	25.1	5.0-24.0	umol/L
HCY-SF	NONE DET		
OHP-SF	0.0	< 8.0	umol/L
ISO-SF	6.1	1.0-11.0	umol/L
LEU-SF	19.2	3.4-25.9	umol/L
LYS-SF	26.0	7.8-40.8	umol/L
METH-SF	4.3	0.4-9.4	umol/L
ORT-SF	4.0	1.6-12.0	umol/L
PHE-SF	12.6	6.9-25.1	umol/L
PRO-SF	0.0	< 8.0	umol/L
SER-SF	77.5	18.0-73.0	umol/L
TAU-SF	17.8	2.7-16.2	umol/L
THR-SF	56.2	10.8-74.9	umol/L
TYR-SF	11.3	5.4-23.7	umol/L
VAL-SF	23.6	7.0-37.1	umol/L

**Gly CSF/Gly PLASMA**

**179.5/2061=0.09**

**Normal < 0.02**

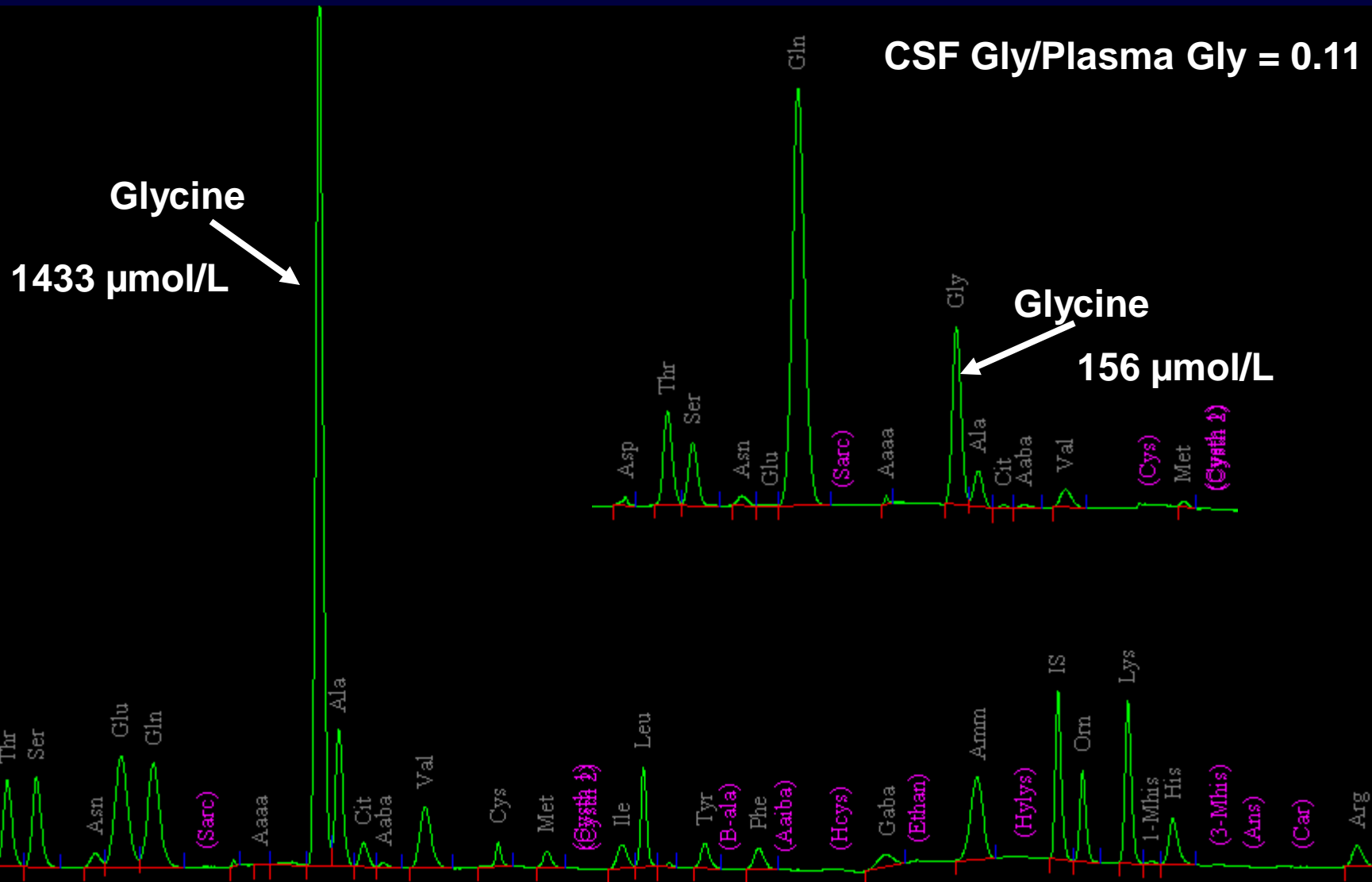
**Atypical 0.01-0.04**

**Classic > 0.08**

## PLASMA

ALANINE	382	200-600	umol/L
ARGININE	64	20-160	umol/L
ASPARTIC A	25	0-40	umol/L
CIT	24	6-60	umol/L
GLN	1187	410-960	umol/L
GLUACID	72	10-190	umol/L
<b>GLYCINE</b>	<b>2061</b>	<b>220-520</b>	<b>umol/L</b>
HISTIDINE	162	40-120	umol/L
HYDROXYPI	56	6-90	umol/L
ISOLEUCINE	57	20-130	umol/L
LEUCINE	89	40-230	umol/L
LYSINE	233	60-250	umol/L
METHION	45	10-60	umol/L
ORNITHINE	119	20-135	umol/L
PHENYLALA	65	30-100	umol/L
PROLINE	266	110-500	umol/L
SERINE	534	90-250	umol/L
TAURINE	169	25-160	umol/L
THREONINE	203	50-300	umol/L
TYR	50	30-140	umol/L
VALINE	156	110-300	umol/L
HCY	NOT DET	NOT DET	umol/L
A-ILE	NOT DET	NOT DET	umol/L
AA-INT	SEE NOTE		
CYSTINE P	59	7-70	umol/L

# GLYCINE ENCEPHALOPATHY (Nonketotic hyperglycinemia)



# GLYCINE ENCEPHALOPATHY (Nonketotic hyperglycinemia)

Recessive inborn error of glycine metabolism leading to the accumulation of large quantities of glycine in all body tissues, including the brain.

**Frequency:** 1:60,000 (second most frequent inborn error of amino acid metabolism)

**Cause:** Defect in glycine cleavage system (4 proteins – No mutations found yet in L-protein):

80%: P-protein (*GLDC* gene)

10-15%: T-protein (*AMT* gene)

<2%: H-protein (*GCSH* gene)

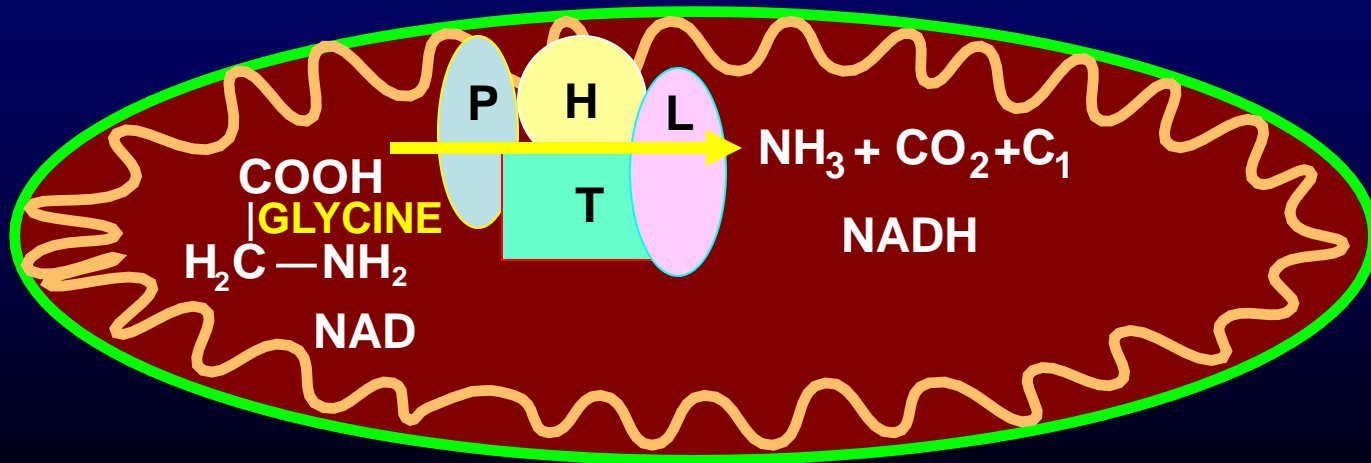
**Pathogenesis:** Accumulation of glycine in the brain affects neurotransmitter signaling and impairs brain development



- Glycine is the simplest amino acid
- It is present in the diet and can be synthesized from serine
- It can be converted to glucose and to a number of other compounds (creatine, oxalate, purines, porphyrins, etc.) in addition to participating in protein synthesis
- Glycine in the brain is removed by the glycine cleavage system

# GLYCINE CLEAVAGE SYSTEM

- The glycine cleavage system is composed of 4 proteins located in the inner mitochondrial membrane of liver, kidney, brain and placenta
- Glycine cleavage generates ammonia, carbon dioxide, a methyl group attached to tetrahydrofolate, and NADH



# GLYCINE CLEAVAGE SYSTEM

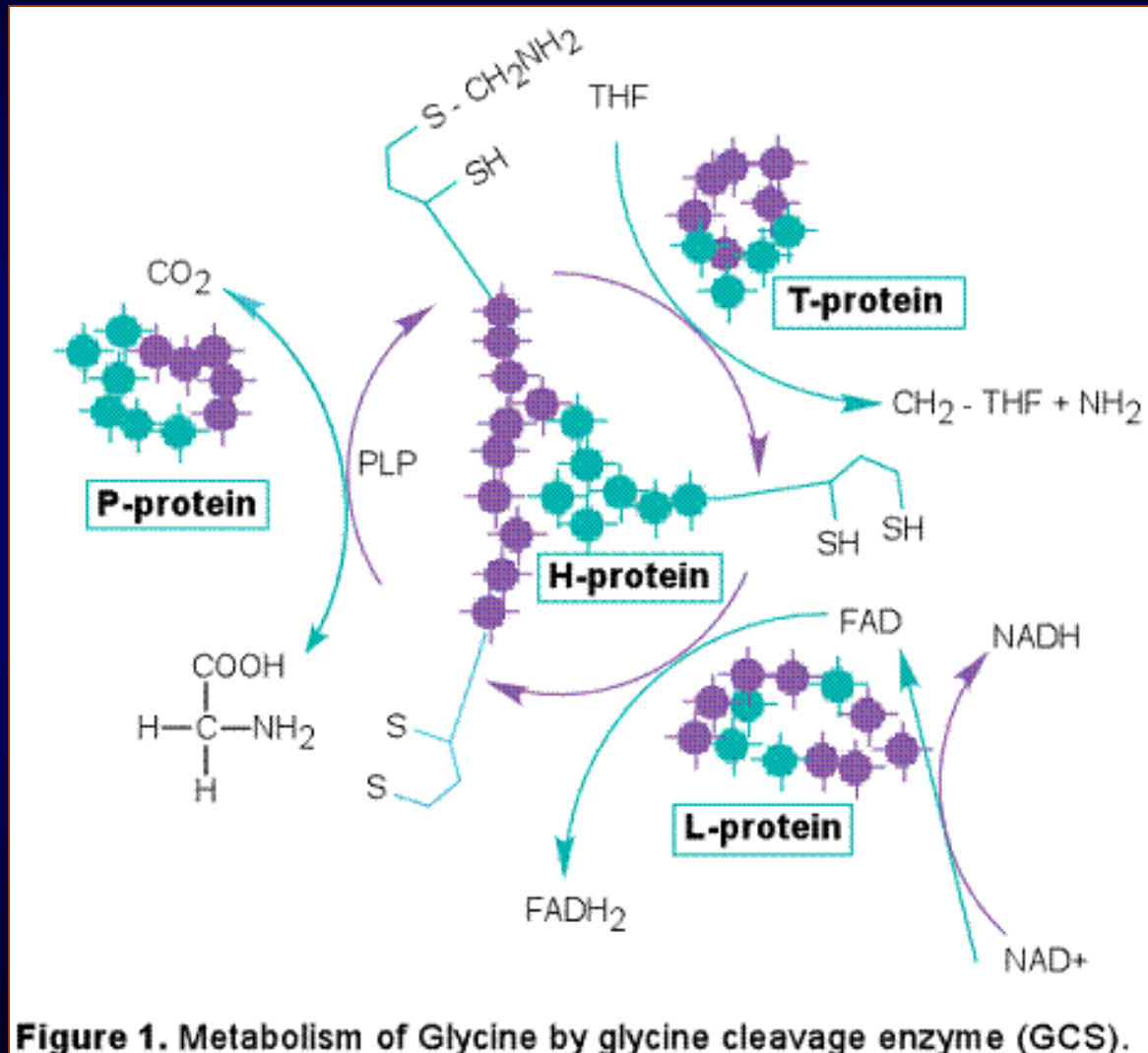


Figure 1. Metabolism of Glycine by glycine cleavage enzyme (GCS).

# GLYCINE ENCEPHALOPATHY (Nonketotic hyperglycinemia)

## Presentation:

1. **Neonatal (classic)** form (most frequent) manifests in the first hours to days of life with progressive lethargy, hypotonia, and myoclonic jerks leading to apnea and often death. Surviving infants develop profound mental retardation and intractable seizures. History of hiccups before and after birth.
2. **Atypical forms:**
  - A. **Infantile:** seizures beyond the neonatal period. Profound to moderate mental retardation.
  - B. **Mild-episodic:** mild mental retardation, chorea, agitated delirium, and vertical gaze palsy.
  - C. **Late-onset:** variable mild spastic paraparesis, optic atrophy, mild mental retardation, and choreoathetosis.
3. **Transient:** presents in the newborn period like the classic form, but improves spontaneously over time (2-8 weeks of age) with normalization of glycine levels.

# GLYCINE ENCEPHALOPATHY (Nonketotic hyperglycinemia)

- **Diagnosis:**
  - Plasma amino acids (high glycine),
  - Urine organic acids (normal),
  - Plasma acylcarnitine profile (Normal),
  - CSF amino acid (high glycine with elevated CSF/Plasma glycine  $> 0.08$  (normal  $<0.02$ )).
- **Newborn screening is NOT effective in identifying glycine encephalopathy.**

# **HYPERGLYCEMIA: DIFFERENTIAL DIAGNOSIS**

## **Nonketotic:**

**Nonketotic hyperglycemia**

**Transient neonatal hyperglycemia**

**Valproate therapy**

**D-glyceric acidemia**

## **Ketotic**

**Propionic acidemia**

**Methylmalonic acidemia**

**Isovaleric acidemia**

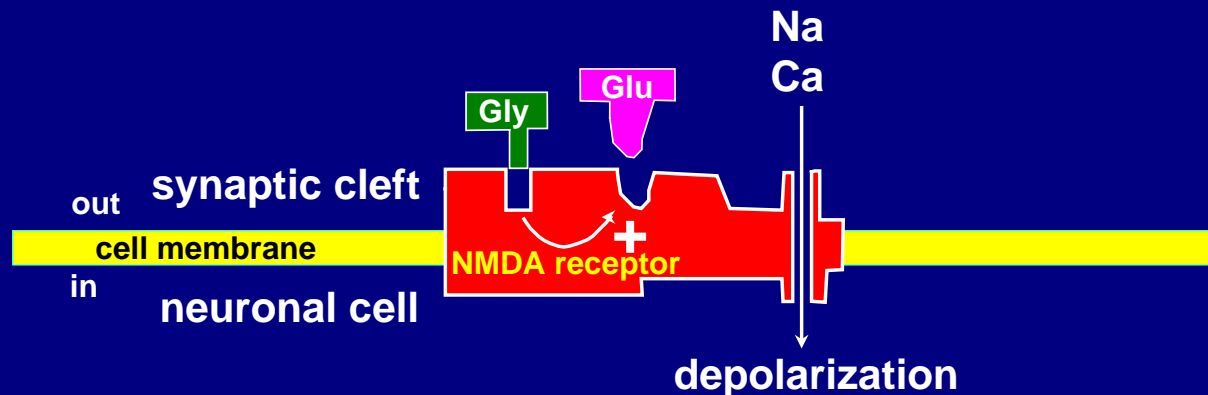
**$\beta$ -ketothiolase deficiency**

# **GLYCINE IS A NEUROTRANSMITTER**

- **Synaptic transmission in the brain is carried out by glutamate, gamma amino butyric acid (GABA) and glycine**
- **Glutamate is excitatory, GABA and glycine are inhibitory**
- **Glycine has specific inhibitory receptors (GlyR) in the brain and the ventral horns of the spinal cord. They increase chloride permeability and are inhibited by strychnine.**
- **Glycine facilitates excitation in the brain (by helping NMDA signaling) and inhibition in the spinal cord**

# GLYCINE FACILITATES THE NMDA RECEPTOR

- Glycine functions in the brain as a co-agonist of the N-methyl-D-aspartate (NMDA) receptor (a receptor for glutamate)
- This receptor activates prolonged electrical depolarization.
- This receptor plays a key role in brain development. Excessive stimulation causes seizures and excitotoxicity with neuronal death

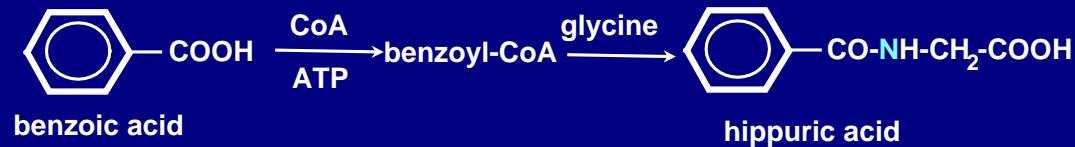


# **PATHOGENESIS**

- **Glycine impairs brain development even before birth. Some patients have agenesis of corpus callosum and delayed myelination**
- **After birth, children have seizures and apnea with severe hypotonia due to the excitatory/inhibitory actions of glycine on the brain and spine**

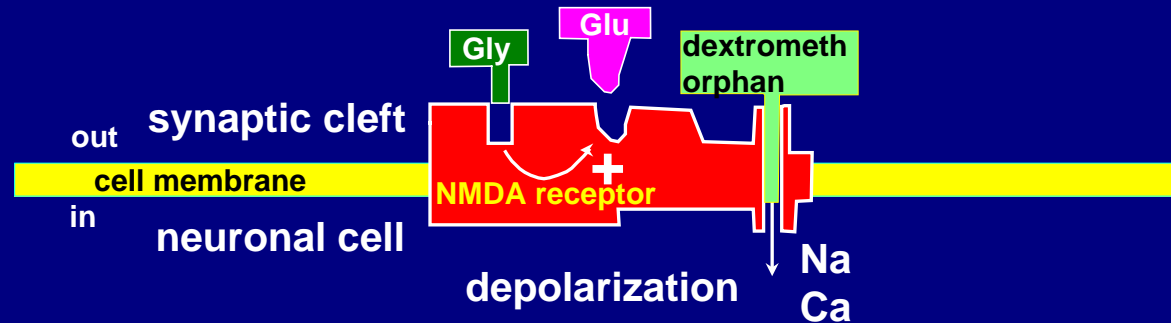
# THERAPY

- Glycine reduction: benzoate 250-750 mg/kg/day



- Receptor blockade:

Dextromethorphan, ketamine, felbamate, and lamictal : block NMDA channel

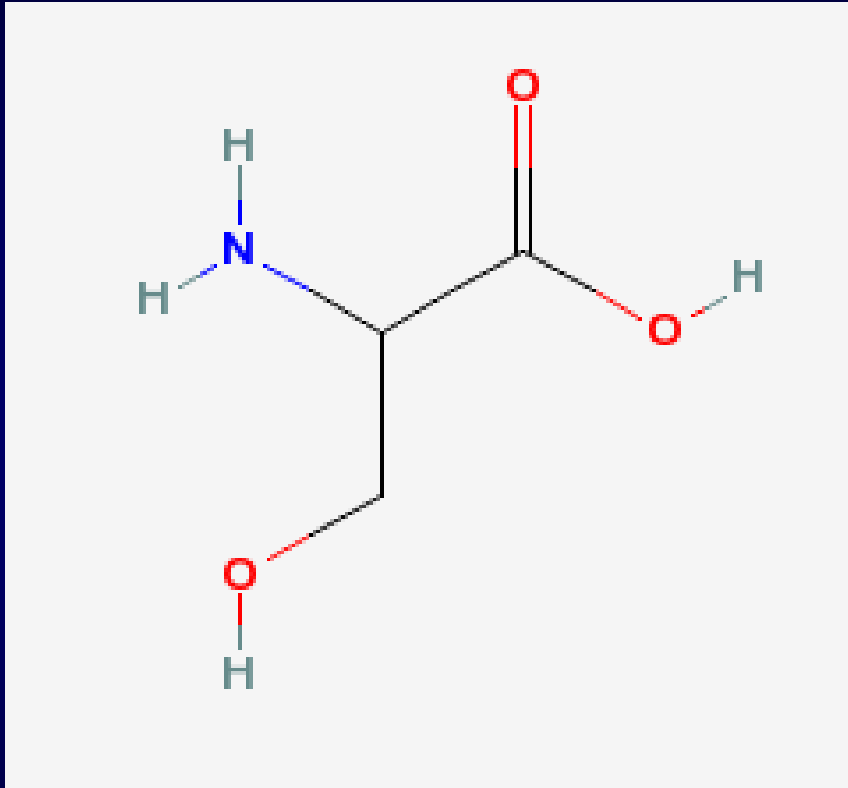


- Topiramate (AMPA receptor blocker)
- Diazepam (activates GABA-inhibitory receptor)
- Valproate is not used because it raises glycine levels

# PROGNOSIS

- **Poor for patients with neonatal presentation. Most die despite respiratory support. Survivors are profoundly delayed.**
- **Late-onset presentation allows some development (gross-motor, speech in some) in most. Rare patients have delays, but speak and walk relatively well.**
- **Prenatal diagnosis:** possible with chorionic villus sampling (not expressed in amniocytes) or DNA testing

# SERINE



- Serine derives from diet, protein degradation or it is synthesized from glycine or 3-phosphoglycerate.
- It is poorly transported into the brain that depends on local synthesis for adequate supply.
- Since glycine is synthesized from serine, defects in serine biosynthesis cause low levels of serine and glycine.

# SERINE METABOLISM

glucose → → 3-P-glycerate → → pyruvate

3-Phosphoglycerate dehydrogenase

3-P-hydroxypyruvate

3-Phosphopyruvate  
aminotransferase

3-P-serine

D-serine

3-Phosphoserine  
phosphatase

L-serine

pyruvate & ammonia

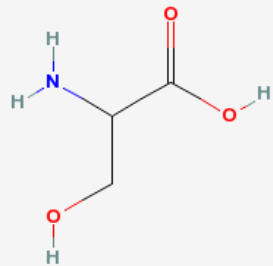
VII

phospholipids

III

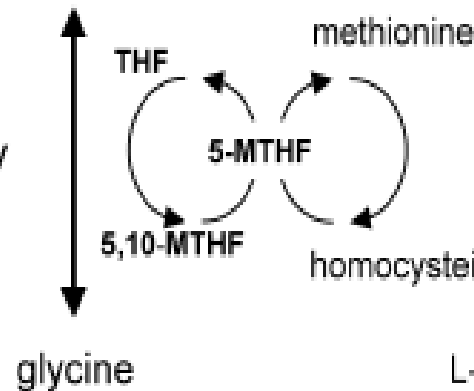
VIII

phosphoglycerides



Serine hydroxy  
methyltransferases

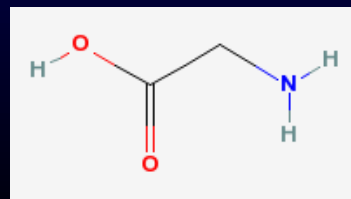
IV



glycine

L-serine

cystathionine → cysteine



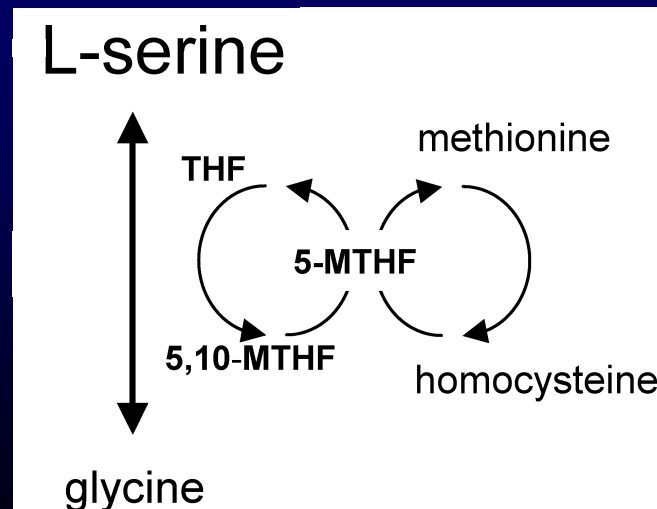
# SERINE DEFICIENCY ENCEPHALOPATHY

**Recessive disorders of serine synthesis of unknown frequency.**

**Cause: Defects in 3-Phosphoglycerate dehydrogenase or 3-Phosphopyruvate aminotransferase.**

**Pathogenesis: Decreased synthesis of D-serine, defective methylation**

**Serine hydroxy methyltransferases**



**1-carbon groups are essential for purine and pyrimidine metabolism and other methylation reactions.**

# SERINE

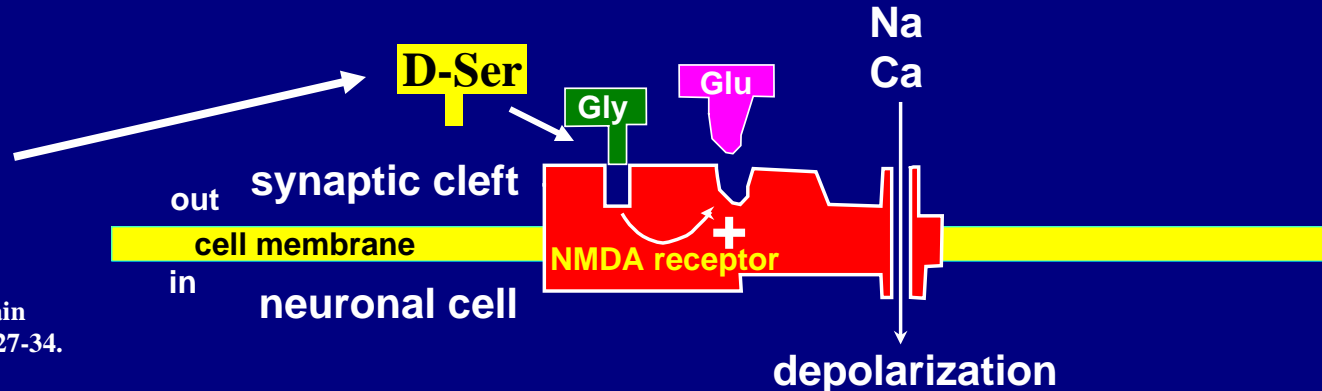
The mechanisms by which serine deficiency leads to pathological consequences are unknown, but they probably involve:

1. Decreased synthesis of serine-derived compounds (membrane lipids (phospholipids or ceramides));
2. The neuromodulators glycine or D-serine (also a co-agonist of the NMDA receptor), derived by isomerization of L-serine by serine racemase).

L-Serine → D-Serine



Yoshikawa et al (2007) The serine racemase mRNA is predominantly expressed in rat brain neurons. Arch Histol Cytol. 2007 Jul;70(2):127-34.



# SERINE DEFICIENCY ENCEPHALOPATHY

**Presentation:** Severe neurological symptoms such as congenital microcephaly and severe psychomotor retardation, intractable seizures.

**Diagnosis:** Plasma amino acids (low serine and glycine), CSF amino acid (low serine and glycine). Confirmation requires enzyme assay in fibroblasts (with residual enzyme activity ) or DNA testing.

**Therapy:** Effective if initiated early.

L-Serine 400-600 mg/kg per day

Glycine 200-300 mg/kg per day

Monitor plasma and CSF amino acids

Prenatal diagnosis: by DNA testing.

# PLASMA AND CSF SERINE AND GLYCINE IN PATIENTS WITH 3-PHOSPHOGLYCERATE DEHYDROGENASE DEFICIENCY

Table 1. Clinical and biochemical characteristics of reported patients with serine deficiency

Disorder	3-Phosphoglycerate dehydrogenase deficiency	3-Phosphoserine phosphatase deficiency	Control
Symptoms	Congenital microcephaly, <sup>a</sup> severe psychomotor retardation, seizures, spastic quadriplegia; in some patients, nystagmus, megaloblastic anemia, cataract, hypogonadism	Williams syndrome, IUGR, congenital microcephaly, psychomotor retardation, feeding problems	
CSF serine ( $\mu\text{mol/l}$ )	6–8	18	$38 \pm 2$
CSF glycine ( $\mu\text{mol/l}$ )	1–4	Normal	$7 \pm 2$
Plasma serine ( $\mu\text{mol/l}$ )	28–64	55–80	$130 \pm 30$
Plasma glycine ( $\mu\text{mol/l}$ )	128–190	Normal	$232 \pm 36$
Treatment	L-Serine at 400–600 mg/kg/day; when indicated, glycine at 200–300 mg/kg/day	L-Serine at 200–300 mg/kg/day	

<sup>a</sup>All patients presented with congenital microcephaly, except for one patient, reported by Hausler *et al.* [3], who had a normal head circumference at birth.

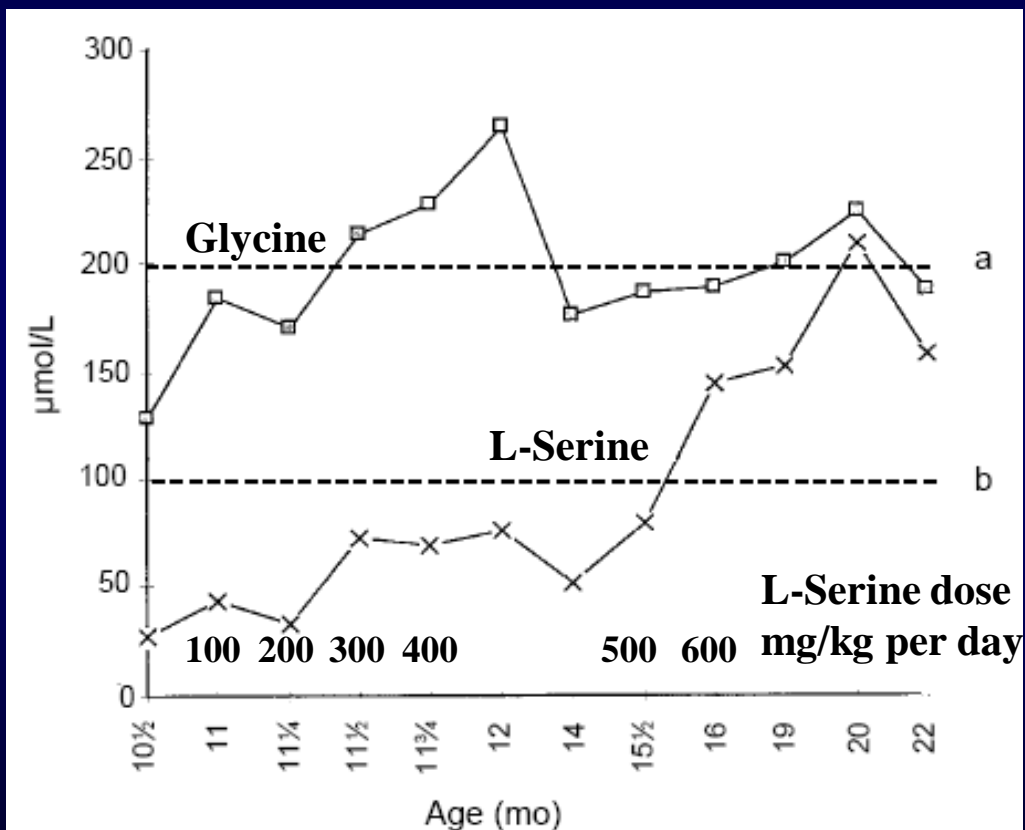
CSF, cerebrospinal fluid; IUGR, intrauterine growth retardation.

## Serine-deficiency syndromes

Tom J. de Koning and Leo W.J. Klomp

Current Opinion in Neurology 2004, 17:197–204

# EFFECT OF THERAPY ON PLASMA SERINE AND GLYCINE IN 3-PHOSPHOGLYCERATE DEHYDROGENASE DEFICIENCY



M Pineda MD PhD, M A Vilaseca PhD, R Artuch MD PhD, S Santos MD, MM García González MD, I Sau MD, A Aracil MD, E Van Schaftingen MD PhD, J Jaeken MD PhD (2000) 3-phosphoglycerate dehydrogenase deficiency in a patient with West syndrome.

# PLASMA AND CSF SERINE AND GLYCINE IN PATIENTS WITH PHOSPHOSERINE AMINOTRANSFERASE DEFICIENCY

**Table 1. Plasma and CSF Serine and Glycine Results for Patients 1 and 2 at Diagnosis**

Patient	Plasma <sup>2</sup> ( $\mu$ mol/liter)		CSF <sup>2</sup> ( $\mu$ mol/liter)	
	Serine	Glycine	Serine	Glycine
1	51 (60–300)	121 (140–420)	18 (35–80)	<1 (3–10)
2	30 (50–350)	110 (200–600)	5 (35–80)	<1 (3–10)

NOTE.—Plasma and CSF amino acids were measured by ion-exchange chromatography with ninhydrin detection.

<sup>2</sup> The normal range is shown in parentheses.

Phosphoserine Aminotransferase Deficiency: A Novel Disorder of the Serine Biosynthesis Pathway

Claire E. Hart, Valerie Race, Younes Achouri, Elsa Wiame, Mark Sharrard, Simon E. Olpin, Jennifer Watkinson, James R. Bonham, Jaak Jaeken, Gert Matthijs, and Emile Van Schaftingen

# CASE PRESENTATION

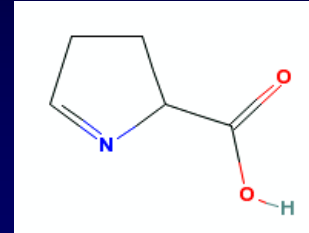
- **3 yo female referred for seizures and speech delays. Febrile seizures started at 11 months of age followed by seizures even without fever. She would “go limp, turn blue and stop breathing and her eyes would roll back in her head”. Delays were noted especially in speech.**

	Last Ref. Range	08/06/02 12:40	06/04/02 13:34
<b>Alanine</b>	240-600	<b>373</b>	<b>* 416</b>
<b>Arginine</b>	40-160	<b>70</b>	<b>* 64</b>
<b>Aspartic Acid</b>	0-20	<b>8</b>	<b>* 10</b>
<b>Citrulline</b>	10-60	<b>69 H</b>	<b>* 79 H</b>
<b>Cystine</b>	7-70	<b>18</b>	<b>* 7</b>
<b>Glutamate</b>	10-120	<b>45</b>	<b>* 34</b>
<b>Glutamine</b>	410-700	<b>442</b>	<b>* 666</b>
<b>Glycine</b>	140-490	<b>328</b>	<b>* 315</b>
<b>Histidine</b>	50-130	<b>114</b>	<b>* 113</b>
<b>Homocystine</b>	NDT	<b>* ND</b>	<b>* ND</b>
<b>Hydroxyproline</b>	6-50	<b>23</b>	<b>* 40</b>
<b>Isoleucine</b>	30-130	<b>27 L</b>	<b>* 34</b>
<b>All isoleucine</b>	NDT	<b>* ND</b>	<b>* ND</b>
<b>Leucine</b>	60-230	<b>68</b>	<b>* 62</b>
<b>Lysine</b>	80-250	<b>88</b>	<b>* 101</b>
<b>Methionine</b>	17-53	<b>28</b>	<b>* 19</b>
<b>Ornithine</b>	20-135	<b>119</b>	<b>* 140 H</b>
<b>Phenylalanine</b>	30-80	<b>58</b>	<b>* 48</b>
<b>Proline</b>	110-500	<b>2670 H</b>	<b>* 2958 H</b>
<b>Serine</b>	60-200	<b>93</b>	<b>* 94</b>
<b>Taurine</b>	25-80	<b>34</b>	<b>* 43</b>
<b>Threonine</b>	60-220	<b>127</b>	<b>* 134</b>
<b>Tyrosine</b>	30-120	<b>77</b>	<b>* 69</b>
<b>Valine</b>	140-350	<b>111 L</b>	<b>* 141</b>

# Plasma amino acids ( $\mu\text{M}$ ) Hyperprolinemia

# TWO TYPES OF HYPERPROLINEMIA

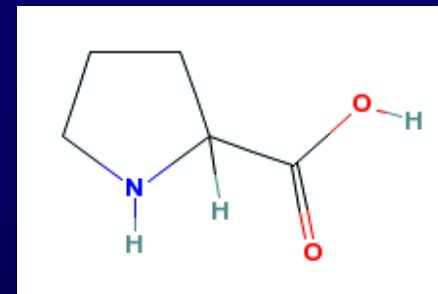
L-PROLINE



PROLINE OXIDASE  
*POX-PRODH*

HYPERPROLINEMIA TYPE 1

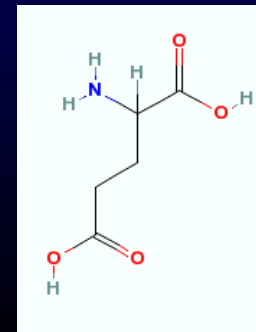
$\Delta$ 1-PYRROLINE-5-CARBOXYLIC ACID



P5C DEHYDROGENASE

HYPERPROLINEMIA TYPE 2

L-GLUTAMATE



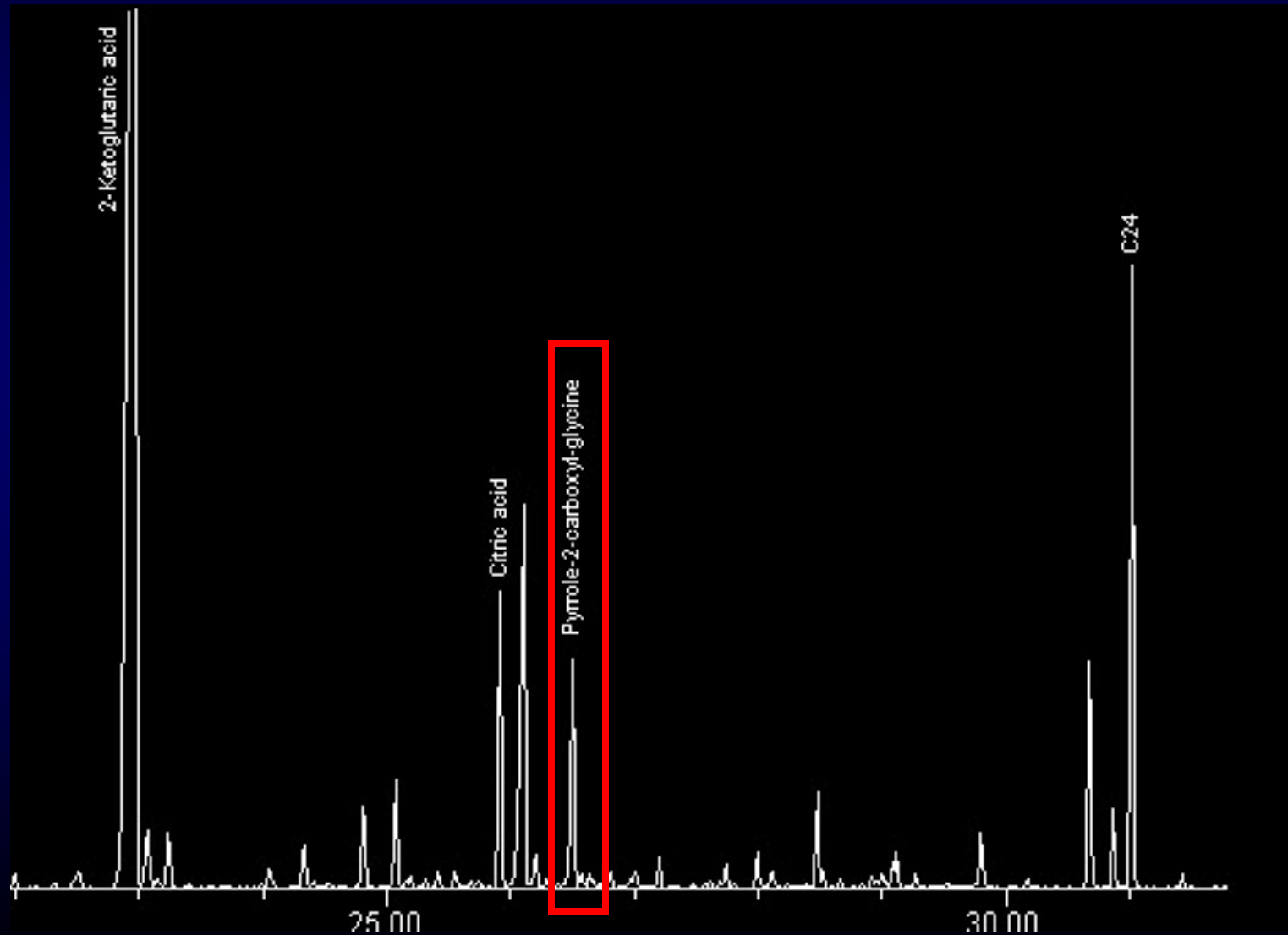
# **HYPERPROLINEMIA TYPE 1**

- Type I hyperprolinemia (HPI MIM 239500), an autosomal recessive inborn error, results from inherited deficiency of proline oxidase (POX), a mitochondrial enzyme expressed in kidney, liver and brain, which is encoded by the proline dehydrogenase (*PRODH*) gene.
- HPI is recognized by elevation of the plasma proline value above 550  $\mu\text{mol/L}$  without excretion of the degradation product of proline, 1-Pyrroline-5-carboxylate (P5C).

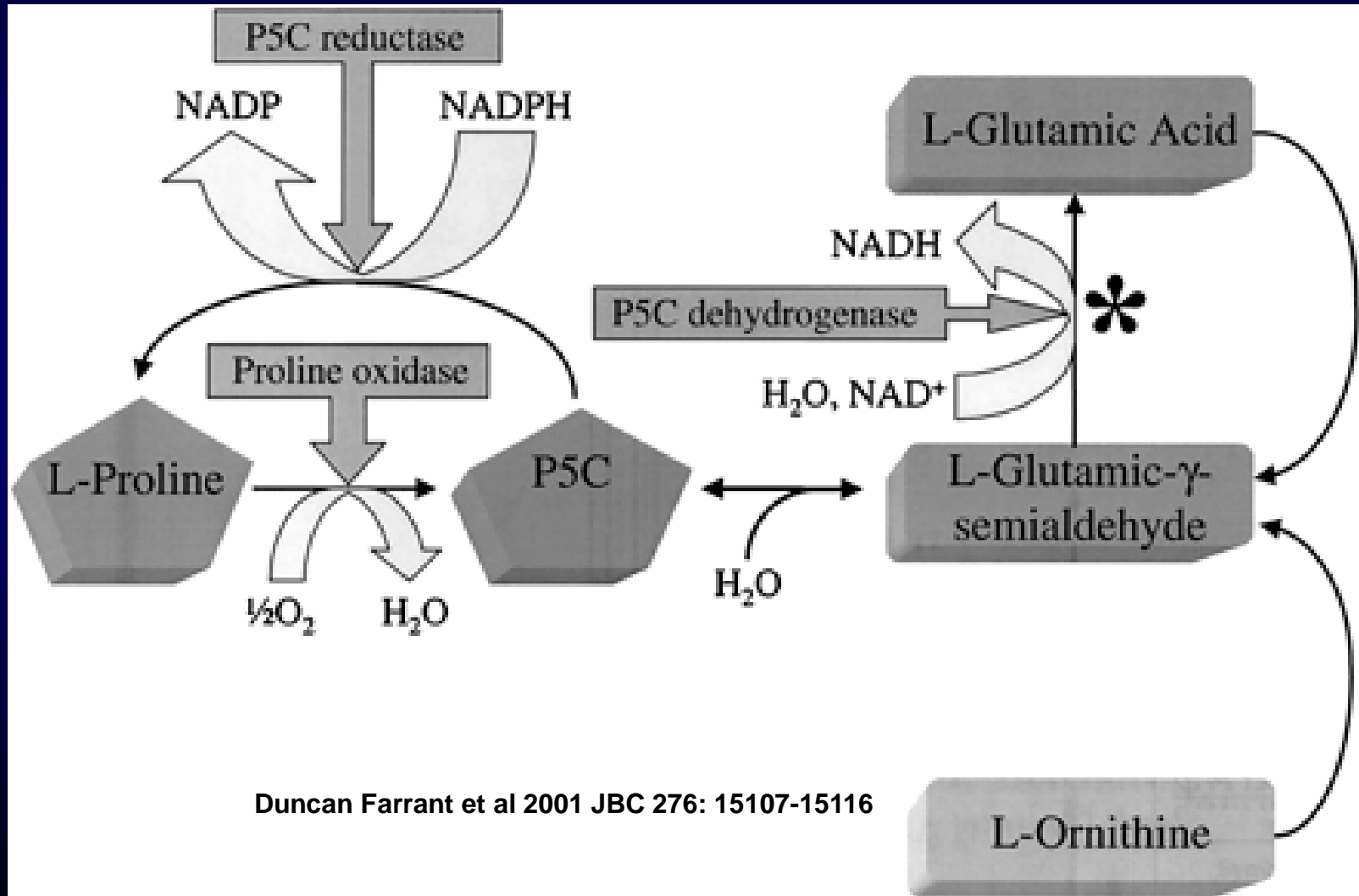
# **HYPERPROLINEMIA TYPE 2**

- **Type 2 hyperprolinemia (HPII MIM 239510) is a rare inherited metabolic disease due to mutations in the pyrroline-5-carboxylate dehydrogenase gene *P5CDH*.**
- **Some patients have neurological problems such as refractory convulsions and developmental delays. Seizures usually start as febrile seizures followed by seizures without fever.**
- **The gene maps to 1p36.**

# HYPERPROLINEMIA TYPE II



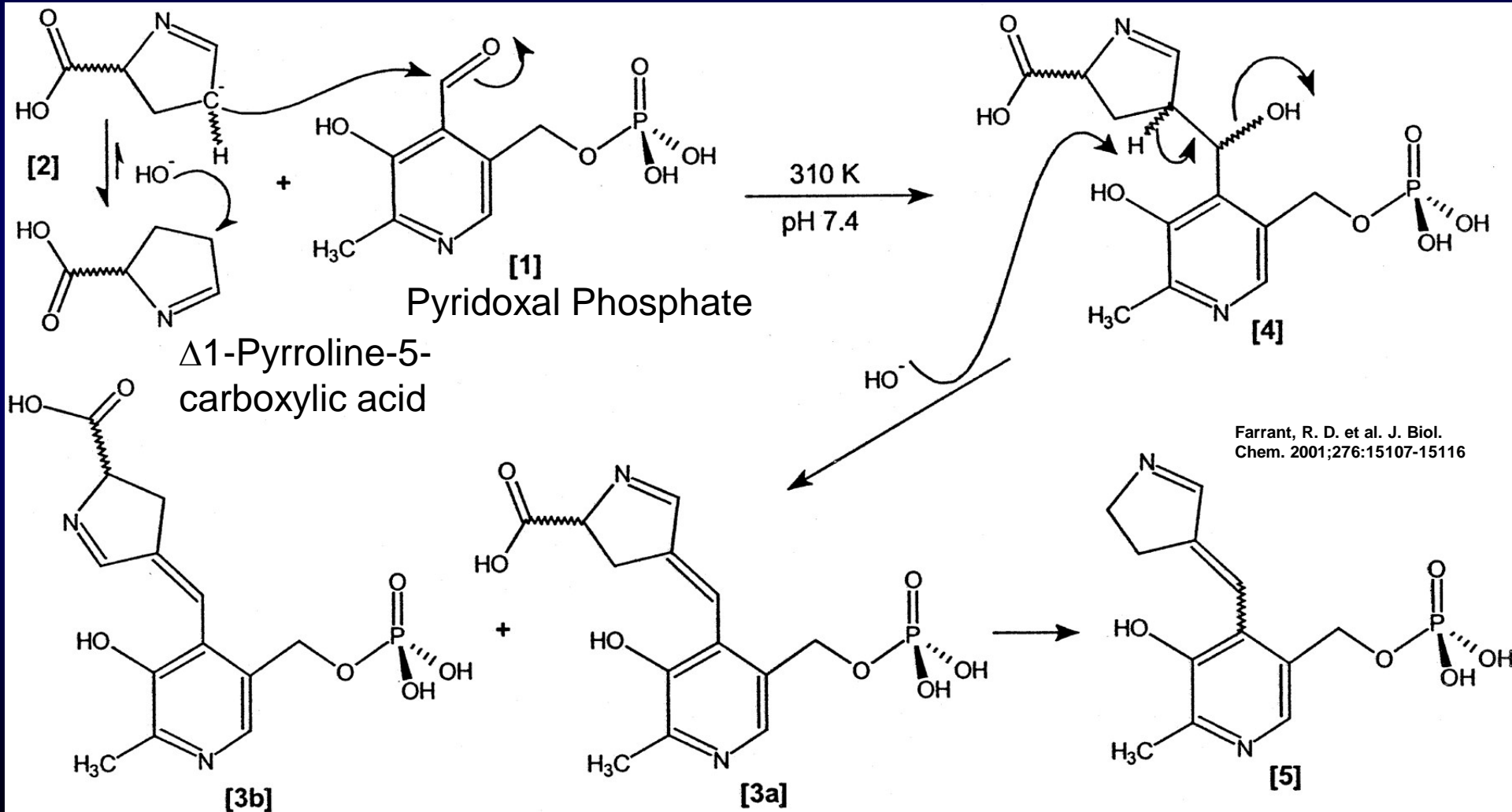
# HYPERPROLINEMIA TYPE 2

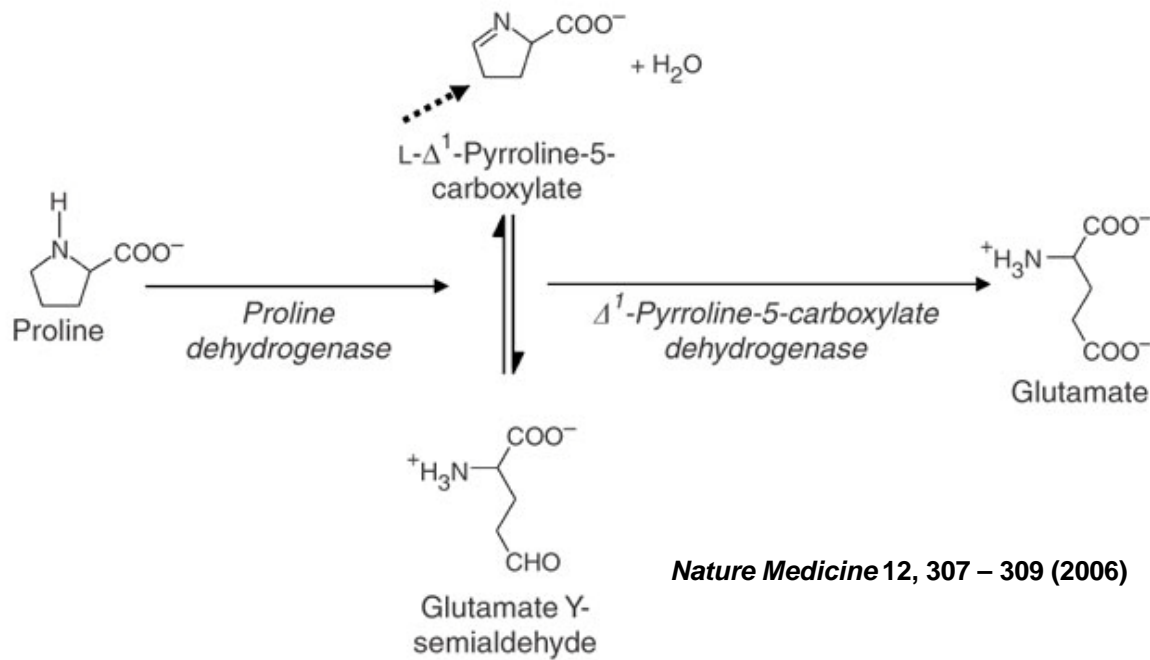
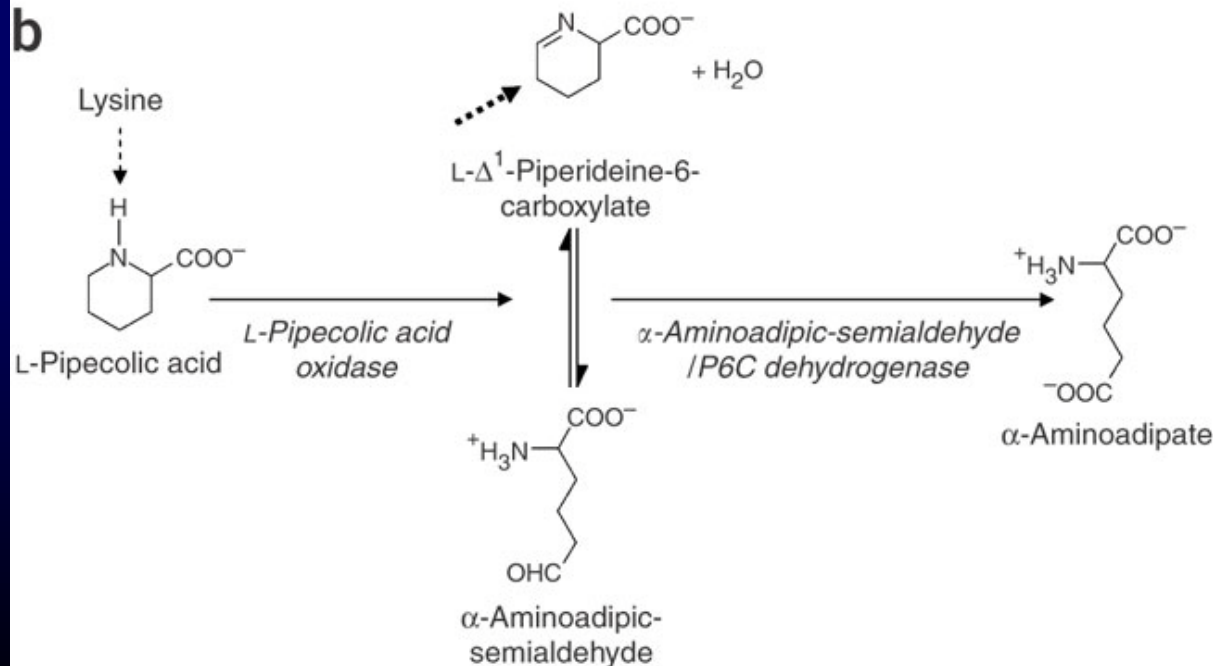


# **HYPERPROLINEMIA TYPE 2**

**The pathogenesis of this disorder seems related to pyridoxine deficiency. In fact, D1-pyrroline-5-carboxylic acid can combine with pyridoxal phosphate to sequester the active form of vitamin B6. Deficiency of this vitamin can lead to seizures.**

# HYPERPROLINEMIA TYPE 2



**a****b**

**SIMILAR  
MECHANISM OF  
PYRIDOXINE  
SEQUESTRATION  
IN HPA TYPE 2  
AND  
PYRIDOXINE-  
RESPONSIVE  
SEIZURES**

# TREATMENT

- Administration of vitamin B6 that should be continued for life.
- Unknown whether treatment before or at birth prevents delays.

# **4-HYDROXYBUTYRIC ACIDURIA**

## **Succinic Semialdehyde Dehydrogenase deficiency**

- **Autosomal recessive disorder in the second step of the conversion of GABA to succinic acid.**
- **The hallmark of the disease is the elevated concentration of 4-hydroxybutyric acid (GHB) due to enzymatic transformation of succinic semialdehyde as a consequence of the metabolic block.**

# 4-HYDROXYBUTYRIC ACIDURIA

- **CLINICAL PRESENTATION**

- Language delay, ataxia, hypotonia, mental retardation, seizures

- **DIAGNOSIS**

- Increased excretion of GHB in the urine

- Confirmation by enzyme assay in leukocytes

- **PRENATAL DIAGNOSIS**

- Analysis of GHB in amniotic fluid

- Enzyme activity in chorionic villi or amniocytes

# 4-HYDROXYBUTYRIC ACIDURIA

Glutamic acid



GABA

*GABA  
transaminase*

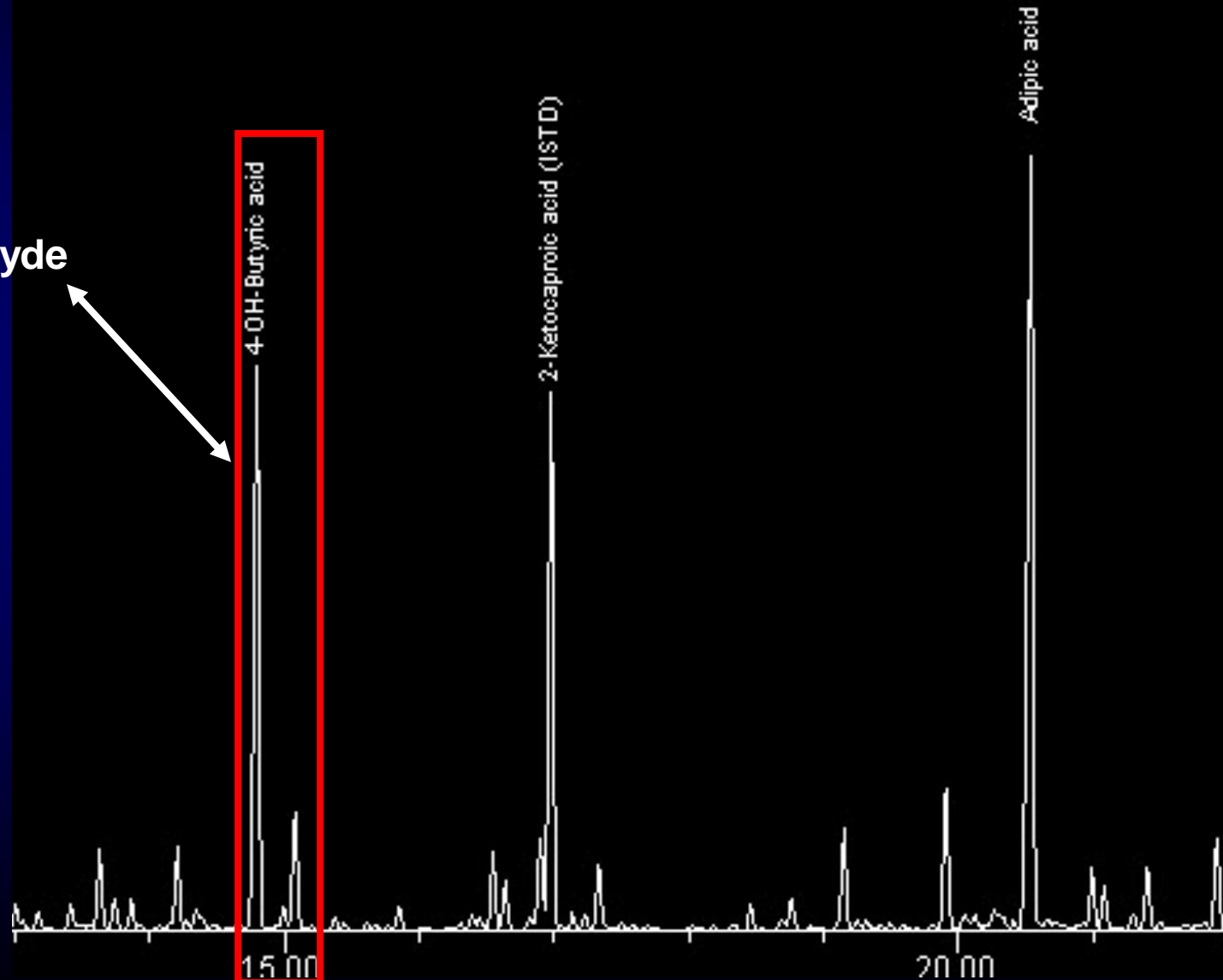


Succinic semialdehyde

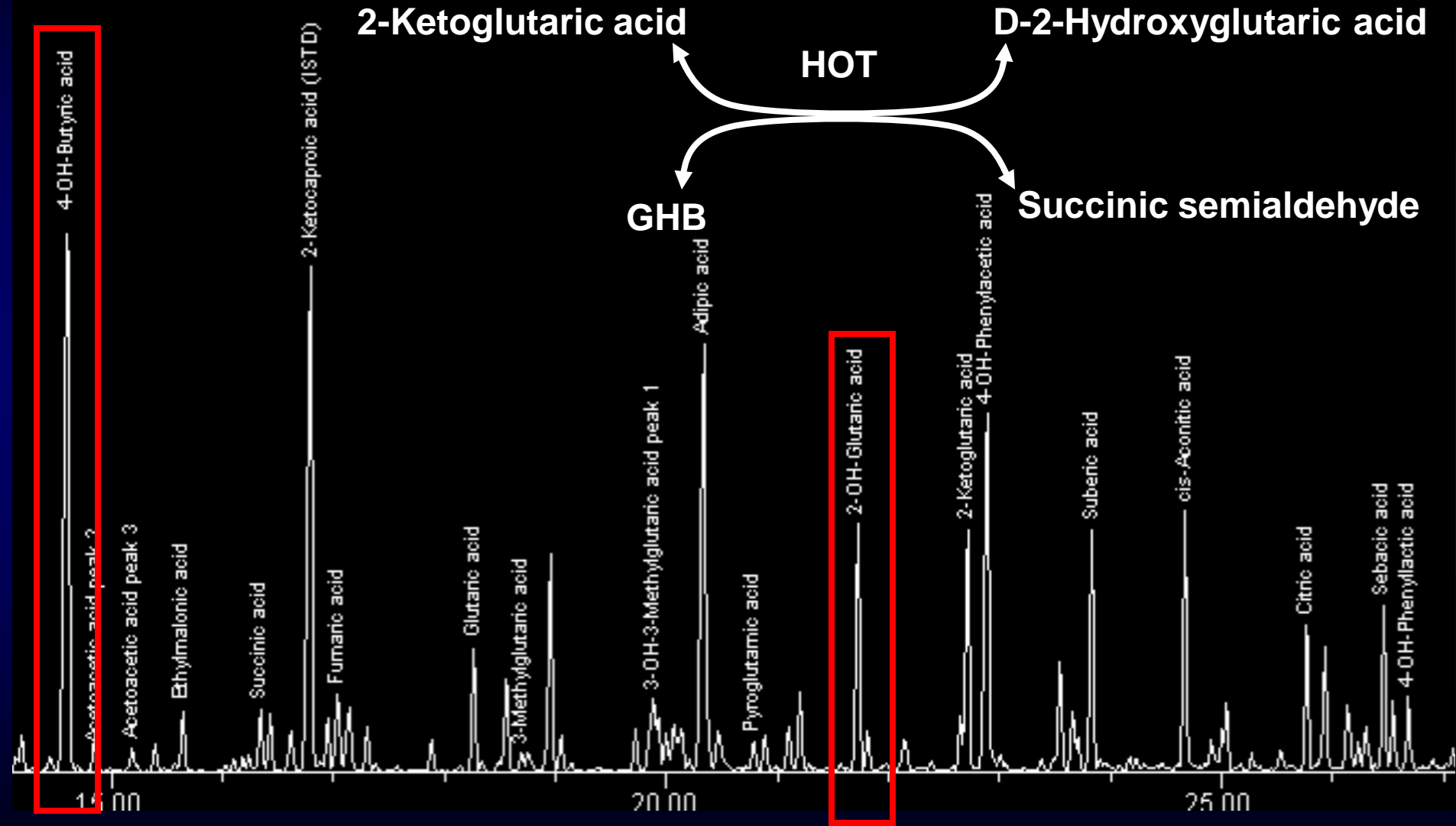
*SSADH*



Succinic acid



# 4-HYDROXYBUTYRIC ACIDURIA AND 2-HYDROXYGLUTARIC ACIDURIA



HOT = Hydroxyacid-Oxoacid Transhydrogenase

# SUMMARY

- **Biochemical abnormalities can be detected in patients with neurotransmitter disorders by “routine” biochemical genetics tests (plasma and CSF amino acids, urine organic acids).**
- **Plasma and CSF amino acids are helpful in the identification of disorders of serine and glycine metabolism.**
- **Plasma amino acids and urine organic acids are necessary to discriminate between hyperprolinemia type I and II.**
- **Urine organic acids are diagnostic for 4-hydroxybutyric aciduria.**