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Amino Acids Interpretation Scheme - What can we learn, how can we improve?

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Outline

1. Key points of the amino acid interpretation scheme
2. International classification of inherited metabolic disorders
3. Cases of 2024 scheme
4. Take to work message

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Key points of the amino acid interpretation scheme

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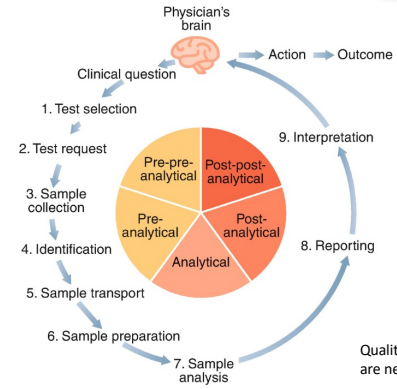

https://link.springer.com/chapter/10.1007/978-3-031-24958-7_6

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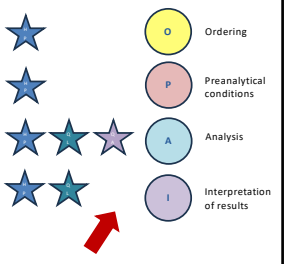
Various types of EQA are offered

- Quantitative EQA schemes **(QA)**
- Qualitative EQA schemes **(QL)**
- Hybrid proficiency tests **(HP)**

An interpretative EQA scheme, which does not require physical samples, represents a new approach.

Quality assurance measures aim at



EQA = external quality assessment

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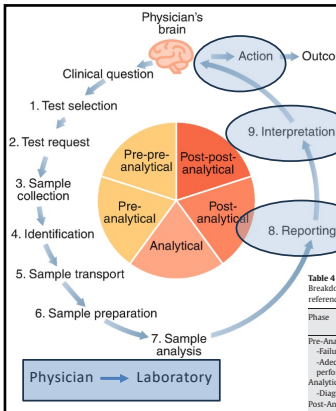

Laboratory → Physician

Amino acid interpretation scheme
1. Reporting
2. Interpretation
3. Recommendation

Table 4
Breakdown points in diagnostic processes leading to missed or delayed diagnoses (from reference [42], modified).

Phase	Percentage of all missed diagnosis
Pre-Analytic	
-Failure to order appropriate diagnostic or laboratory tests	55
-Adequate diagnostic or laboratory tests ordered but not performed	9
Analytic	
-Diagnostic or laboratory test performed incorrectly	8
Post-Analytic	
-Incorrect interpretation of diagnostic or laboratory tests	37
-Responsible provider did not receive diagnostic or laboratory test results.	12

https://link.springer.com/chapter/10.1007/978-3-031-24958-7_6 In Plebani 2009

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Interface 1: Optimal situation

Physician → Laboratory

- What symptoms does the patient have?
- Acute or chronic?
- Which ward is the patient in?
- Is there a clinical suspicion of a diagnosis?
- .....

Physician | Laboratory staff | No question – no answer!

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- Different languages
- Symptoms vs. pathways

The diagnosis of inborn disorders of metabolism is complex.

https://www.alamy.com

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Interface 2: Optimal situation

Laboratory → Physician

- KISS (keep it short and simple)
- Sometimes an explantation is needed
- .....

Physician | Laboratory staff

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Amino acid interpretation scheme
1. Reporting
2. Interpretation
3. Recommendation

Case 2025 - 4	
clinical information	noticeably tired and pale for several weeks, hypochromic
age at time of sample collection	1.5 years
sex	female
pregnancy	normal
birth	normal
family history	
initial symptoms	
treatment	none
miscellaneous	

amino acids in plasma, done with ion exchange chromatography with ninhydrin detection				
	µmol/L	reference range		
Taurine	27	19	-	130
Threonine	186	33	-	130
Serine	273	24	-	178
Asparagine	142	25	-	150
Glutamic Acid	23	6	-	80
Glutamine	666	200	-	600
Proline	517	51	-	185
Glycine	286	56	-	308
Alanine	698	99	-	350
Citrulline	28	5	-	24
Valine	92	57	-	270
Cystine	22	0	-	40
Methionine	631	3	-	29
Cystathionin/Alloisoleucin	3	0	-	0
Isoleucine	31	26	-	94
Leucine	36	45	-	155
Tyrosine	547	11	-	120
Phenylalanine	125	23	-	70
Homocystine	n.d.	0	-	0
Ornithine	57	10	-	107
Lysine	207	45	-	144
Histidine	69	24	-	112
Arginine	56	11	-	75
n.d. not detected				

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Amino acid interpretation scheme
1. Reporting
2. Interpretation
3. Recommendation

An example for the answer (anonymously for scorers, no ERN number):

Part. No.	Case 4 abnormalities	Case 4 diagnosis	Case 4 further testing recommendations
	Each 1 point: elevated tyr and met, maximum 2 points	Tyrosinaemia type I 2 points, liver failure (due to other IMD like galactosaemia, mitochondrial d., etc.) 1 point, tyrosinemia type II 0 points, maximum 2 points	each 1 point (maximum 2 points): alpha-fetoprotein, organic acids (urine, inclusive succinylacetone), 5-aminolaevulinic acid/ porphobilinogene synthase activity, molecular genetic analysis of FAH gene
x	High levels of Tyrosine, Methionine and slightly increased levels of Phe/Ala. High levels of Alanine, Proline and Lysine (Lactic Acidosis) and slight increase on Glutamine, threonine and serine. Leucine levels are low or borderline (Ile and Val) related with liver disease.	Tyrosinemia Type I (high levels of Tyr, Met, Phe/Ala). The anemia is a symptom between altered enzymes and coagulation test. Lactic acidosis (threonine and serine) could due to an hepatic failure and Glutamine by the secondary urea cycle failure. Other causes: mithocondrial diseases or liver failure	urine organic acids with succynilacetone, DBS succinylacetone, serum alpha-fetoprotein, transaminases, coagulation test. Urine aminoacids. Ammonia FAH gene or exome sequencing searching for another diseases like mithochondrial diseases.

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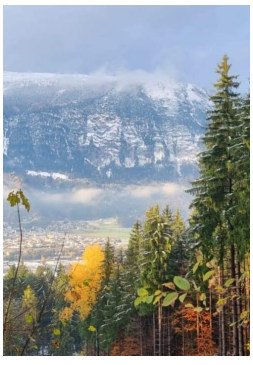

Amino Acid Interpretation scheme

- since 2023 full scheme
- 6 (2x3) cases per year
- 6 (3x2) points per case (findings, interpretation, recommendation)
- 56% cut off for poor performance
- concept of critical error

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Amino acid interpretation scheme

application → case details → submission → scoring → reports

application to the scheme via ERNDIM homepage
- there is a limit to the number of participants

publication of case details with
- a short clinical description and
- amino acid concentrations inclusive reference ranges
- there are two rounds of 3 cases per year

web based submission of results
- description of results
- diagnosis
- recomendation for further investigations
- each part scores a total of two points

scoring by two independent and blinded experts
- the scoring criteria and the criteria for critical error are predetermined
- in addition to sufficient participation, there are poor performers, partial submitters and non sbumitters

reports
- circulation reports
- meeting of the scientific advisors to validate the results and critical errors
- annual report together with the report on critical errors

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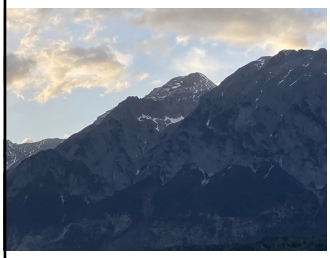

Nearly 2000 inherited metabolic disorders are known!

(a classification to get an overview is therefore necessary)

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International classification of inherited metabolic disorders

- **hierarchical, group-based collation** of (all) currently known inherited metabolic disorders
- includes any primary genetic condition in which **alteration of a biochemical pathway** is intrinsic to specific biochemical, clinical and/or pathophysiological features
- aims to **facilitate an improved understanding** of the interconnections between conditions that share functional, clinical and diagnostic features

http://www.icimd.org

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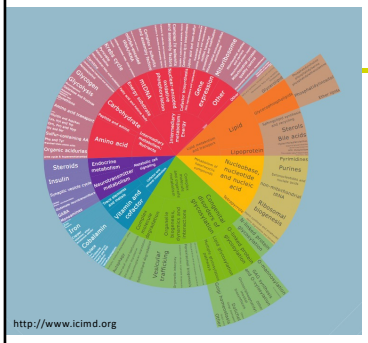

ICIMD

- > 1450 IMD known
- Classification in 6/23 groups
  - **Intermediary metabolism**
  - Lipid metabolism and transport
  - Metabolism of heterocyclic components
  - Complex molecule and organelle metabolism
  - Cofactor and mineral metabolism
  - Metabolic cell signaling
- Of these, approx. 17% have a treatment option.

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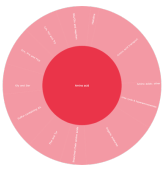

Amino acid profiles of

- Primary aminoacidopathies
  - Classical aminoacidopathies
  - Rare aminoacidopathies
- Secondary chances
- Healthy persons

Performance > 92%

Performance < 92%

Primary changes (examples)

primary amino acid changes (+ diagnostic)	urea cycle disorders	CPS deficiency
		OTC deficiency
		ASS deficiency
		ASL deficiency
		ARG deficiency
		HHH syndrome
		Citrin deficiency
	disorders of BCAA metabolism	MSUD
		E3 Dihydrolipoamid Dehydrogenase
		MADD deficiency
		BCAT 1 deficiency
	disorders of aromatic AA metabolism	PKU
		tyrosinemia type I
		tyrosinemia type II
		tyrosinemia type III
	disorders of sulfuric AA metabolism	MAT1A mutation
		MAT2 mutation
		homocystinuria
		MTHFR deficiency
		remethylation defects
		molybden cofactor deficiency
	other disorders of AA metabolism	histidinemia
		tryptophanemia
		serin deficiency
		NKG
		sarcatrophia
		hyperprolinemia
		LPI
		hyperlysinemia (AASS)
		cystinuria
		Hartnup disease
		prolidase deficiency
		P5C (pyrroline 5-carboxylate) synthase deficiency
		Asparaginase
		PNPO deficiency
		hypophosphatasia

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Some of the 2024 cases

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Case 2024-1

Case 2024 - 1	
clinical information	deterioration in general condition on the second day of life, suspected sepsis, antibiotic
age at time of sample col	three days old
sex	female
pregnancy	normal
birth	normal
family history	unremarkable
initial symptoms	
treatment	
miscellaneous	

amino acids in plasma, done with biochrome 30	µmol/L	reference range		
Taurine	130	10	-	95
Threonine	83	33	-	128
Serine	108	24	-	178
Glutamic acid	341	6	-	93
Glutamine ★	1711	200	-	550
Proline	506	52	-	277
Glycine	263	70	-	430
Alanine	555	99	-	380
Citrulline ★	589	0	-	29
Valine	68	57	-	262
Methionine ★	77	3	-	41
Isoleucine	3	26	-	53
Leucine	peak overlaid	46	-	109
Tyrosine	142	11	-	112
Phenylalanine	71	23	-	110
Ornithine	56	10	-	151
Histidine	156	25	-	114
Lysine	246	45	-	240
★ Arginine	11	10	-	85
★ Argininosuccinic acid	detectable			
n.d. not detected				

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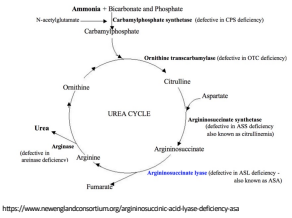
Case 2024-1: further details



https://www.newenglandconsortium.org/argininosuccinic-acid-lyase-deficiency-asa

	interpretation		scores (points)
findings, abnormalities, maximum 2 points (*has to be mentioned for 2 points)	elevated	gln, cit, met	1
	elevated*	argininosuccinic acid	1
	low	arg	1
diagnosis, maximum 2 points	argininosuccinic aciduria (ASL deficiency)		2
	citrullinemia type I (ASS deficiency)		0
	ASS and ASL deficiency		1
further tests (if molecular genetic recommended specify the gene), maximum 2 points	orotic acid in urine (organic acids in urine)		1
	genetic analysis of ASL gene		1
	enzyme studies in erythrocytes/fibroblasts		1
comments	As the detection of argininosuccinic acid is typical for the diagnosis of ASL (argininosuccinate lyase) deficiency, other urea cycle defects are not recognised as differential diagnoses and are therefore not awarded points. In addition, the mention of molecular genetic analysis (ASL gene) without additional metabolite diagnostics (such as determination of orotic acid) was only awarded one point.		
critical error	Overlooking an inborn disorder of metabolism is considered a critical error.		

	order	author	CE	level of difficulty (1-easy, 3-difficult)	estimated overall proficiency
ASL deficiency	2024_1	SSB, 93%	n=0	1	>90%

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Case 2024-2

Case 2024 - 2	
clinical information	Speech delay, mental retardation, eating difficulties, foot edema with lameness
age at time of sample collection	5 years
sex	F
pregnancy	No particularities. Born by ce-section due to medical antecedant of the mother (sevral ce-sections)
birth	Nothing
family history	Nothing
initial symptoms	
treatment	No treatment
miscellaneous	2 older siblings without particularities

amino acids in plasma, done with LC-MS/MS	µmol/L	reference range		
Glutamine	1032	334	-	666
Alanine	296	122	-	456
Glycine	354	135	-	274
Proline	196	57	-	266
Valine	129	158	-	310
Threonine	179	66	-	170
Lysine	59	94	-	224
Serine	238	75	-	179
Glutamic Acid	87	14	-	106
Leucine	54	68	-	168
Taurine	29	42	-	134
Histidine	62	48	-	112
Ornithine	12	27	-	93
Arginine	22	47	-	123
Tyrosine	31	38	-	94
Phenylalanine	35	33	-	74
Isoleucine	33	37	-	89
Cystine	41	48	-	120
Methionine	18	11	-	43
Citrulline	41	16	-	40

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Case 2024-2: further details

- during hospitalization: detection of anaemia due to a homozygous sickle cell disease, biological work-up also revealed a hyperferritinaemia and increased LDH
- after diagnosis treatment with protein restricted diet, ammonia scavenger and citrulline
- persistant feeding difficulties - gastrostomy
- **really hard diagnosis (but as often for LPI)** - probable malnutrition and unspecific decrease of some amino acids, but

→ **importance of asking for amino acids in urines if there is any doubt**

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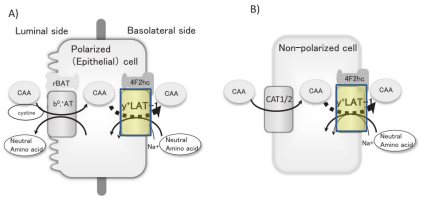
Lysinuric protein intolerance (LPI)

y^+LAT1 4F2hc	SLC7A7 SLC3A2	y^+L	LPI = déficit y^+LAT1 (SLC7A7)
$b^{0,+}$AT rBAT	SLC7A9 SLC3A1	$b^{0,+}$	Cystinurie type III = déficit $b^{0,+}$AT (SLC7A9) Cystinurie type I = déficit rBAT (SLC3A1)

Fig. 1 Membranous transport of cationic amino acids(CAA). y^+LAT and $b^{0,+}$AT transport both CAA and neutral amino acids. In addition, y^+LAT is Na dependent. **a** CAA transport at an polarized cell. **b** CAA transport at a non-polarized cell. CAT1/2 (cationic transporter-1/2) are Na^+ independent



Noguchi et al., J of Hum Genetics 2019

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Lysinuric protein intolerance (LPI)

- presentation at all ages (neonatal →adult)
- classic hepatobiliary presentation with spontaneous avoidance of protein in diet and secondary malnutrition
  - nausea, vomiting, and diarrhea
  - hepatosplenomegalia – hypotonia
  - altered consciousness (NH_3)
- **Complications (may lead to diagnosis)**
  - growth retardation, osteopenia
  - hematological and immunological abnormalities: cytopenia, macrophage activation syndrome, susceptibility to infections
  - renal involvement: tubulopathy, renal insufficiency, glomerulonephritis, nephrocalcinosis, proteinuria, hematuria
  - pulmonary involvement: interstitial alveolar pneumonia

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Lysinuric protein intolerance (LPI)

- plasma amino acids
  - ↓ of Lys, Orn, Arg
  - +/- non-specific signs of hyperNH3: ↑Gln + Glu +/- Ala
- urine amino acids
  - ↑ Lys, Orn, Arg, without associated cystinuria
- increased urinary orotic acid
- cytopenia: anemia, thrombocytopenia
- increased ferritin, LDH, TG
- hyperammonemia (variable)
- genetics (AR): mutations only in the *SLC7A7* gene, not in *SLC3A2*

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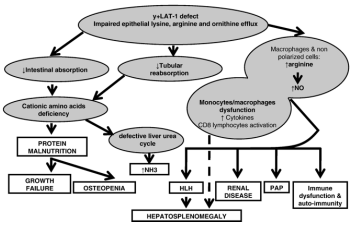
Lysinuric protein intolerance (LPI)

- complex pathophysiology that is still poorly understood
- intracellular accumulation of arginine in macrophages/ monocytes disrupts their activity → immune damage
- Arg = substrate of NOS →neurotransmission, vasodilation, inflammation
- role of malnutrition



Ogier de Baulny et al., MGM, 2012

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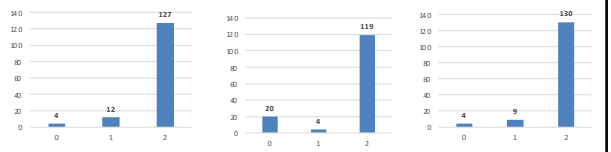
Case 2024-2: Results

Case 2 abnormalities	Case 2 diagnosis	Case 2 further testing recommendations
elevated Gln or Cit 1 point Reduced Lys, Arg, Orn 1 point Maximum 2 points	Lysinuric protein intolerance (LPI) 2 points, Malnutrition 1 point Maximum 2 points	each 1 point, maximum 2 points: orotic acid (organic acids) in urine; amino acids in urine; ammonia, ferritine, triglycerides; molecular genetic analysis *SLC7A7* gene



critical error: If elevated Gln concentration doesn't lead to further action or if the findings are quoted as unspecific.

N=1

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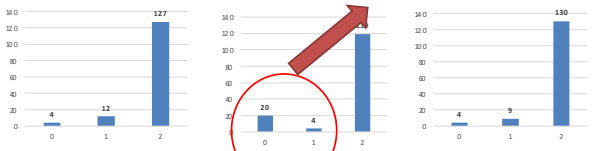
Case 2024-2: Results

Case 2 abnormalities

elevated Gln or Cit 1 point
Reduced Lys, Arg, Orn 1 point
Maximum 2 points

- Kwashiorkor or low protein intake
- glutamine synthetase deficiency
- glutaminase deficiency
- ornithine transcarbamylase deficiency
- carbamoyl phosphate synthetase I
- BCKD kinase deficiency



critical error: If elevated Gln concentration doesn't lead to further action or if the findings are quoted as unspecific.

N=1

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Case 2024-3

Case 2024 - 3	
clinical information	Cutis laxa, axial hypotonia, IUGR (height and weight <-3SD), microcephaly, joint hyperlaxity, inguinal hernia
age at time of sample collection	4 months
sex	male
pregnancy	
birth	IUGR (intrauterine growth retardation)
family history	
initial symptoms	
treatment	/
miscellaneous	

amino acids in plasma, done with LC-MS/MS	µmol/L	reference range		
Glutamine	873	515	-	651
Alanine	670	258	-	400
Glycine	337	190	-	268
Proline	78	144	-	220
Valine	140	174	-	246
Threonine	147	91	-	167
Lysine	116	129	-	191
Serine	189	110	-	168
Glutamic Acid	127	44	-	80
Leucine	92	117	-	142
Taurine	37	36	-	82
Histidine	73	63	-	91
Ornithine	19	43	-	83
Arginine	33	64	-	92
Tyrosine	69	51	-	85
Phenylalanine	53	46	-	82
Isoleucine	36	48	-	80
Cysteine	19	72	-	100
Methionine	25	21	-	33
Aspartic Acid	13	5	-	13
Citrulline	5	18	-	42

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Case 2024-3

- The results are from a 4 months-old boy from a non-consanguineous family.
- He presented an intrauterine growth retardation and joint hyperlaxity, inguinal hernia and cutis laxa at birth.
- At 2 months he presented a neurological deterioration with axial hypotonia, pyramidal syndrome and a cataract on the left eye was diagnosed.
- His growth was also severely impaired leading to naso-gastric feeding. He died at 7 month of age.
- Decreased Cit, Arg, Orn, Pro→ **De Barsy syndrome due to D1-Pyrroline-5-carboxylate (P5C) synthase deficiency** (*ALDH18A1* gene).
- This is an ultrarare metabolic disorder, but with the clinical symptoms (and pubmed search) and the description of the amino acid changes, the diagnosis could be made.

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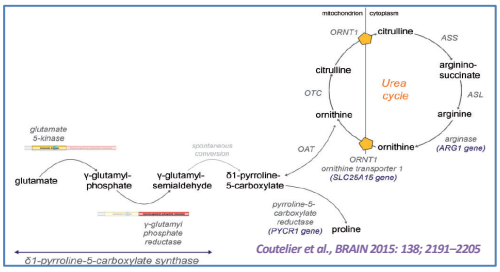
P5C synthase deficiency
1 gene = different diseases and transmission



1 gene: *ALDH18A1*
enzyme: P5C synthase

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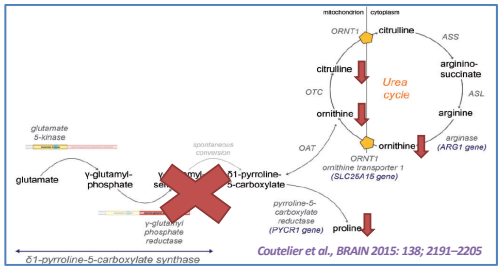
P5C synthase deficiency
1 gene = different diseases and transmission



1 gene: *ALDH18A1*
Enzyme: P5C synthase

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P5C synthase deficiency
1 gene = different diseases and transmission

- severe form: cutis laxa & hyperammonemia
- = de Barsy syndrome
  - AD or AR (mutation dependant)
  - symptoms
    - severe psychomotor retardation
    - cutis laxa
    - joint abnormalities
    - small size
    - cataract
    - microcephaly
    - rare (< 20 cases)
    - very severe (early death ++)

- adult form: spastic paraparesis
- autosomal dominant or autosomal recessive (mutation dependent)
- symptoms
  - Pyramidal syndrome with paraparesis and spasticity
- found by exome sequencing in large cohorts of families with spastic paraparesis

Fisher et al., Molecular Genetics and Metabolism 112 (2014)

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P5C synthase deficiency
1 gene = different diseases and transmission

- probably very «slight» pattern in the adult form of P5C synthase deficiency
- unmasked in fibroblast with loading test

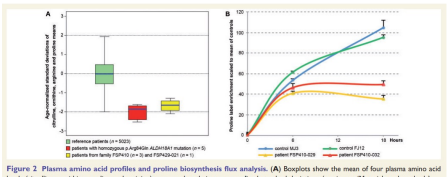

Figure 2 Plasma amino acid profiles and proline biosynthesis flux analysis. (**A**) Boxplots show the mean of four plasma amino acid levels (citrulline, ornithine, proline and arginine) expressed as their age-normalized standard deviations (y-axis; see 'Materials and methods' section) compared among three groups of patients: the full Necker hospital cohort of patients with no known metabolic disorders, aged > 15 years (n = 5023; green); patients from the first family in which *ALDH18A1* mutations were reported (n = 5; red); and the patients from Families FSP410 and FSP429 (this study) for which plasma amino acids were available (n = 4; yellow). The average of the four amino acid levels in *ALDH18A1* mutated patients mops at approximately −2 SD. (**B**) Time course of stable isotope ratio (labelled versus natural) enrichment of proline reflecting the rate of proline biosynthesis from $^{13}C_5$-labelled glutamine in fibroblasts. Two *ALDH18A1*-mutated patients (red and orange lines) are compared to two controls, i.e. members of the same family with no mutations (green and blue lines). Yaxis: label enrichment scaled to the mean of the controls at 18-h incubation. X-axis: hours of incubation in the presence of the tracer. Assuming a stationary phase at 18-h incubation time, proline biosynthesis in two patients harbouring *ALDH18A1* p.Val120Ala mutation was estimated to be 42% of controls.

Coutelier et al., BRAIN 2015: 138; 2191–2205

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Case 2024-3: Results

Case 3 abnormalities

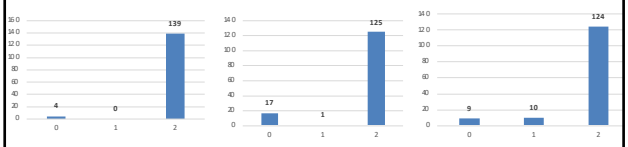
Elevated Glu, Gln 1 point
Decreased Cit, Arg, Orn, Pro: 2 points
Maximum 2 points

Case 3 diagnosis

P5C synthase deficiency (de Barsy syndrome) : 2 points
Proline metabolims abnormalities 1 point
Maximum 2 points

Case 3 further testing recommendations

Molecular genetic analysis of *ALDH18A1* gene: 2 points Organic acids, IEF transferrine; ammonia each 1 point
Maximum 2 points



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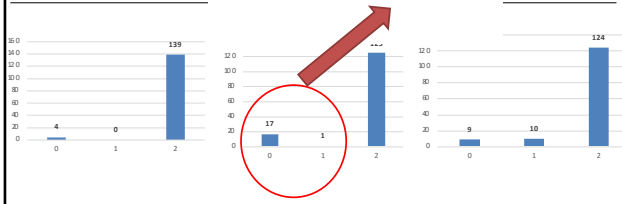
Case 2024-3: Results

Case 3 abnormalities

Elevated Glu, Gln 1 point
Decreased Cit, Arg, Orn, Pro: 2 points
Maximum 2 points

Cystathionine beta synthase deficiency ?
P5C = Pyrroline-5-carboxylate reductase
Urea cycle disorder
lysinuric proteine intolerance (LPI)
Clinical manifestation indicative of CDG.



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Case 2024-4

Case 2024 -4	
clinical information	anaemia, liver failure with disturbed clotting in the age of eight months
age at time of sample collection	8 months-old
sex	male
pregnancy	normal
birth	normal
family history	unremarkable, two healthy brothers
initial symptoms	icterus
treatment	
miscellaneous	

Laboratory results	
ammonia	79 µmol/L (15-70 µmol/L)

amino acids in plasma, done with biochrome 30				
	µmol/L	reference range		
Glutamine	576	200	-	550
Alanine	279	99	-	320
Glycine	188	56	-	308
Proline	174	51	-	185
Valine	97	57	-	262
Threonine	195	33	-	128
Lysine	196	45	-	150
Serine	109	24	-	178
Glutamic Acid	24	6	-	70
Leucine	58	45	-	155
Taurine	17	10	-	130
Histidine	61	24	-	112
Ornithine	82	10	-	107
Arginine	75	11	-	75
Tyrosine	222	11	-	112
Phenylalanine	55	23	-	70
Isoleucine	29	26	-	94
Cystine	49	10	-	40
Methionine	174	3	-	29
Aspartic acid	n.d.		-	
Citrulline	48	5	-	24
n.d. not detected				

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Case 2024-4: further details

	interpretation		scores (points)
findings, abnormalities, maximum 2 points	elevated	met, tyr	1
	elevated	cit	1
	elevated	thr	1
diagnosis, maximum 2 points	liver failure		1
	tyrosinaemia type I		1
	citrin deficiency		2
	galactosaemia		1
further tests (if molecular genetic recommended specify the gene), maximum 2 points	organic acids (urine) inclusive succinylacetone		1
	galactose-1-phosphate, GALT activity		1
	molecular genetic analyses (*SLC25A13*)		2
comments	This is a rather difficult case, as the changes in the plasma aminogram are not very pronounced (therefore the concentrations of threonine and citrulline have been slightly adjusted). Nevertheless, it is important to consider this metabolic disorder in the case of liver failure in infancy, as the therapy consists of a fat/protein-defined diet and not a carbohydrate-defined diet as in the case of other congenital metabolic disorders with liver failure.		
critical error	If the participant does not recognise that the liver failure is due to a metabolic disorder, this is considered a critical error.		

	order	author	CE	level of difficulty (1-easy, 3-difficult)	estimated overall proficiency	ful
	2024_4	SSB_87%	n=0	3	<80%	

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Case 2024-5

clinical information	microcephaly, psychomotor retardation, failure to thrive
age at time of sample collection	5 years
sex	male
pregnancy	
birth	born preterm (gestational week 35+6)
family history	consanguineous parents
initial symptoms	
treatment	
miscellaneous	MRI: enlargement of the peri-cerebral spaces of the lateral ventricles and delayed myelinisation

amino acids in plasma, done with LC MS/MS				
	µmol/L	reference range		
Glutamine	541	334	-	666
Alanine	313	122	-	456
Glycine	190	135	-	274
Proline	126	57	-	266
Valine	49	158	-	310
Threonine	127	66	-	170
Lysine	174	94	-	224
Serine	131	75	-	179
Glutamic Acid	66	14	-	106
Leucine	16	68	-	168
Taurine	69	42	-	134
Histidine	59	48	-	112
Ornithine	61	27	-	93
Arginine	54	47	-	123
Tyrosine	79	38	-	94
Phenylalanine	57	33	-	74
Isoleucine	7	37	-	89
Cystine	44	48	-	120
Methionine	19	11	-	43
Citrulline	18	16	-	40

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Case 2024-5

- The sample was taken from a five-year-old boy who had microcephaly, mental retardation and a failure to thrive.
- In the extended work-up, cerebral MRI showed enlargement of the peri-cerebral spaces of the lateral ventricles and delayed myelinisation.
- The analysis of amino acids showed isolated reduced concentrations of branched-chain amino acids.
- → BCKDK deficiency

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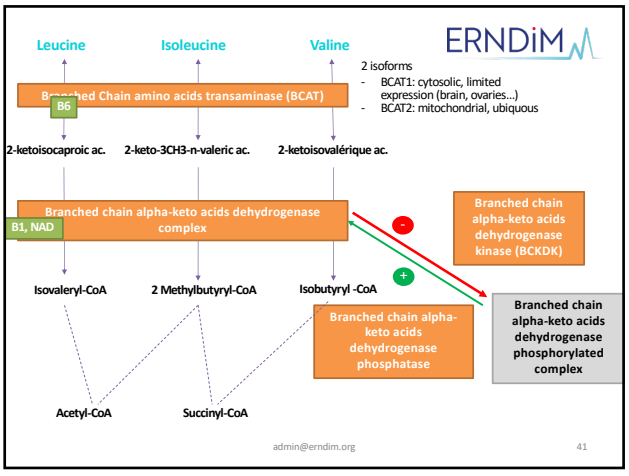

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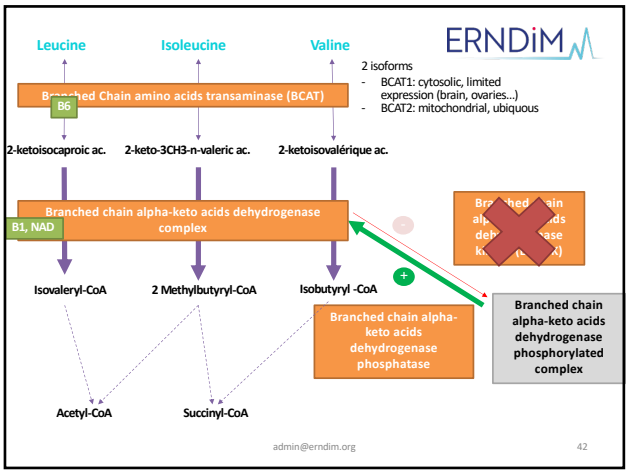

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Case 2024-5

- First description of BCKDK deficiency in 2012

Mutations in *BCKD-kinase* Lead to a Potentially Treatable Form of Autism with Epilepsy

Gaia Novarino[1,7,†], Paul El-Fishawy[2,*], Hulya Kayserili[3], Nagwa A. Meguid[4], Eric M. Scott[1], Jana Schroth[1], Jennifer L. Silhavy[1], Majdi Kara[5], Rehab O. Khalil[4], Tawfeg Ben-Omran[6], A. Gulhan Ercan-Sencicek[7], Adel F. Hashish[4], Stephan J. Sanders[7], Abha R. Gupta[8], Hebatalla S. Hashem[4], Dietrich Matern[9], Stacey Gabriel[10], Larry Sweetman[11], Yasmeen Rahimi[12], Robert A. Harris[12], Matthew W. State[7], and Joseph G. Gleeson[1,†]

- Large cohort (17 patients) described in 2023

BCKDK deficiency: a treatable neurodevelopmental disease amenable to newborn screening

Trine Tangeraas,[1,2,†] Juliana R. Constante,[2,3,4,†] Paul Hoff Backe,[2,5,6] Alfonso Oyarzábal,[2,3,4] Julia Neugebauer,[2,7,8] Natalie Welnhold,[2,7,8] Francois Boemer,[2,9] François G. Debray,[2,10] Burcu Ozturk-Hism,[11] Gumus Evren,[12] Eminoglu F. Tuba,[13] Oncul Umrnuhan,[13] Emma Footitt,[2,14,15] James Davison,[2,14,15] Caroline Martinez,[16] Clarissa Bueno,[17] Irene Machado,[18] Pilar Rodríguez-Pombo,[19] Nouriya Al-Sannaa,[20] Mariela De Los Santos,[2,4,21] Jordi Muchart López,[22] Hatice Ozturkmen-Akay,[23] Meryem Karaca,[24] Mustafa Tekin,[25] Sonia Pajares,[4,26] Aida Ormazabal,[2,4,27] Stephanie D. Stoway,[28,29] Rafael Artuch,[2,4,27] Marjorie Dixon,[2,30] Lars Mørkrid[2,5,31] and Angeles García-Cazorla[2,3,4]

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Table 1 BCKDK phenotype at diagnosis

Phenotype	HPO code	Frequency n (%)
Progressive microcephaly	HP:0000253	17/20 (85)
Global developmental delay	HP:0001263	21 (100)
Motor delay	HP:0001270	21 (100)
Language impairment	HP:0002463	17 (100)
Intellectual disability	HP:0001249	16 (100)
Intellectual disability, severe	HP:0010864	15/16 (93.8)
Developmental regression	HP:0002376	5/8 (62.5)
Behavioural abnormality	HP:0000708	20/21 (95.2)
Autistic behaviour	HP:0000729	14/19 (73.7)
Autism	HP:0000717	11/19 (58)
Self-injurious behaviour	HP:0100716	5/17 (28)
Aggressive behaviour	HP:0000718	8/18 (44.4)
Hyperactivity	HP:0000752	5/21 (23.8)
Restlessness	HP:0000711	6/21 (26.8)
Attention deficit hyperactivity disorder	HP:0007018	4/18 (22.2)
Seizure	HP:0001250	9/21 (42.9)
Bilateral tonic–clonic seizure	HP:0002069	3/18 (16.7)
Generalized myoclonic seizure	HP:0002123	1/18 (5.6)
Typical absence seizure	HP:0011147	2/18 (11.1)
Generalized-onset seizure	HP:0002197	9/21 (42.9)
Focal-onset seizure	HP:0007359	1/21 (4.8)
Interictal epileptiform activity	HP:0011182	12/18 (66.7)
Hypotonia	HP:0001252	5/17 (29.4)
Abnormality of movement	HP:0100022	3/20 (15)
Dystonia	HP:0001332	2/10 (10)
Ataxia	HP:0001251	1/20 (5)
Hyperkinetic movements	HP:0002487	1/20 (5)
Clumsiness	HP:0002312	12/15 (80)
Feeding difficulties	HP:0011968	5/20 (25)
Sensorineural hearing impairment	HP:0000407	3/21 (14.3)
Hyperreflexia	HP:0001347	5/17 (29.4)
Polyneuropathy	HP:0001271	2/17 (11.8)
Abnormal facial shape	HP:0001999	10/21 (47.6)
Full cheeks	HP:0000293	4/21 (19)
Thin upper lip vermillion	HP:0000219	4/21 (19)
Abnormal nasal bridge morphology	HP:0000422	2/21 (9.5)
Hypoplastic philtrum	HP:0003326	2/21 (9.5)
Small forehead	HP:0000350	3/21 (14.3)
Coarse hair	HP:0002208	1/17 (5.9)
Dry skin	HP:0000958	2/17 (11.8)
Inflammatory abnormality of the skin	HP:0011123	3/17 (17.6)
Hydronephrosis	HP:0000126	1/17 (5.9)

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Case 2024-5

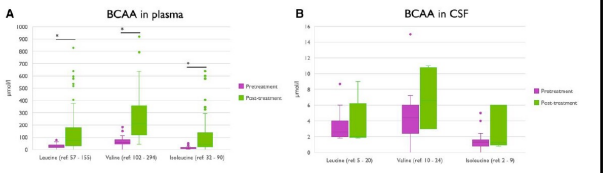

Figure 3 Branched chain amino acids (BCAA) before BCAA supplementation and a follow-up. (A) Pretreatment: $n = 12/21$; post-treatment: $n = 5/21$, 1/5 patients did not have CSF leucine levels pretreatment. No significant differences between the samples from before and after treatment. Mean of 1.2 samples per patient pretreatment and 1 sample post-treatment. (B) Pretreatment: $n = 19/21$; post-treatment: $n = 14/21$; $P < 0.001$ for leucine, valine and isoleucine independently. Mean of 1.8 samples per patient pretreatment and 6.4 samples post-treatment.

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- !!! Sample must be taken while fasting

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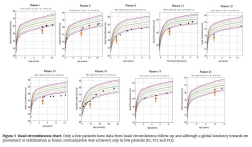
Case 2024-5

- Possibly treatable disease if treatment is started early



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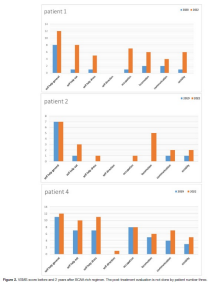
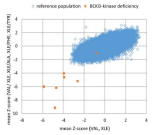


- Neonatal screening ?



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Case 2024-5: Results

Case 5 abnormalities

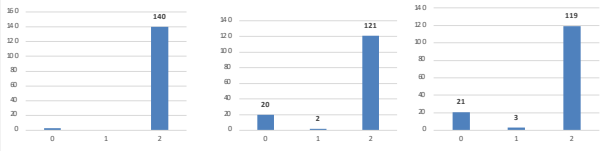
Decreased BCAA (Ile, Val, Leu): 2 points

Case 5 diagnosis

BCKDK (branched-chain 2-ketoacid dehydrogenase kinase) deficiency 2 points,
Glucose infusion/anabolism (hyperinsulism) 1 point
Maximum 2 points

Case 5 further testing recommendations

Repetition of the determination of amino acids in plasma;: 1 point
Molecular genetic analysis of BCKDK : 2 points
Maximum 2 points



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Case 2024-5: Results

Case 5 abnormalities

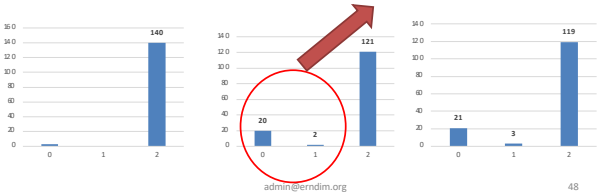
Decreased BCAA (Ile, Val, Leu): 2 points

MSUD in treatment
Congenital hyperinsulinism
Denutrition
lysosomal or peroxysomal diseases
treated organic aciduria
Autosomal recessive microcephaly
Hartnup disease
Leigh syndrome

nmendations

ition of amino acids

of BCKDK : 2 points



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Case 2024-6

Case 2024 - 6	
clinical information	seizures (beginn 6 days old), responsive to pyridoxine
age at time of sample collection	7 days
sex	female
pregnancy	uneventful
birth	normal
family history	unremarkable
initial symptoms	
treatment	
miscellaneous	

Laboratory results in plasma	
ASAT	24 U/L (R: 10 - 50)
ALAT	7 U/L (R: 3 - 38)
g-GT	96 U/L (R: 0 - 265)
alkaline phosphatase ★	< 20 U/L (R: 48 - 406)
Ammonia	14 µmol/L (R: 31 - 104)
Lactate	18 mg/dL (R: 1 - 26)

amino acids in urine, done with Biochrome 30				
	mmol/mol Crea	reference range		
Glutamine	**157**	52	-	205
Alanine	**205**	75	-	244
Glycine	**825**	283	-	1097
Proline	**194**	21	-	213
Valine	**20**	3	-	26
Threonine	**52**	20	-	138
Lysine	**44**	22	-	171
Serine	**132**	80	-	282
Glutamic Acid	**15**	0	-	30
Leucine	**20**	3	-	25
Taurine	**442**	8	-	226
Histidine	**199**	80	-	295
Ornithine	**6**	0	-	19
Arginine	**5**	0	-	14
Tyrosine	**27**	6	-	55
Phenylalanine	**16**	4	-	32
Isoleucine	**4**	0	-	6
Cystine	**15**	24	-	78
Methionine	**4**	7	-	27
Aspartic acid	**7**	2	-	12
Citrulline	**5**	0	-	11
Phosphoethanolamine ★	**705**	n.s.	-	n.s.

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Case 2024-6: further details

	interpretation		scores (points)
findings, abnormalities, maximum 2 points	elevated	PEA	2
	decreased	AP	2
diagnosis, maximum 2 points	Hypophosphatasia		2
	Pyridoxine-responsive seizures		1
further tests (if molecular genetic recommended specify the gene), **maximum 2 points**	Phosphoethanolamine (P, CSF, U)		1
	alkaline phosphatase (AP) in plasma		1
	determination of vitamin B6 metabolites		1
	molecular genetic analysis of *ALPL* gene		2
	molecular genetic analysis of *ALDH7A1* ge		1
comments	The participant should recognise that it is a case of vitamin B6-dependent epilepsy and begin further clarification of the cause.		
critical error	If the participant does not recognise that an inborn metabolic disorder is present.		

	order	author	CE	level of difficulty (1-easy, 3-difficult)	estimated overall proficiency
hypophosphatasia	2024_6	SSB, 98%	no CE	1	>90%

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Take to work message

- The aai aims to improve the interpretation of complex laboratory results.
- Real-life cases are used.
- The performance of the participating laboratories is generally good.
- Errors arise from overlooking a relevant laboratory result, misinterpretation, or a lack of recommendations for further investigation (the term ‘molecular genetic analysis’ as the only recommendation is insufficient).
- We hope that the cases have been instructive for you so far.

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