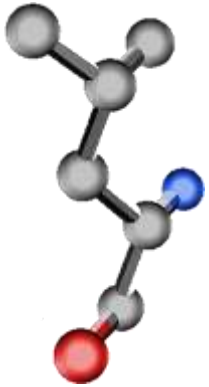
ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

SSIEM ANNUAL SYMPOSIUM 2019
2-6 SEPTEMBER | ROTTERDAM, THE NETHERLANDS



Defects of BCAA metabolism

Clinical manifestations and Biomarkers

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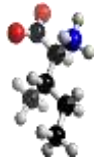
SJD Sant Joan de Déu Barcelona · Hospital

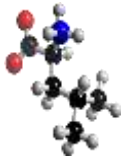
ciberer isciii

European Reference Network

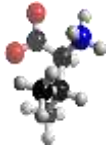
BCAAs NITROGEN DONORS involved in ANABOLIC PATHWAYS (protein and lipid synthesis, inhibiton of autophagy), and in METABOLIC HOMEOSTHASIS



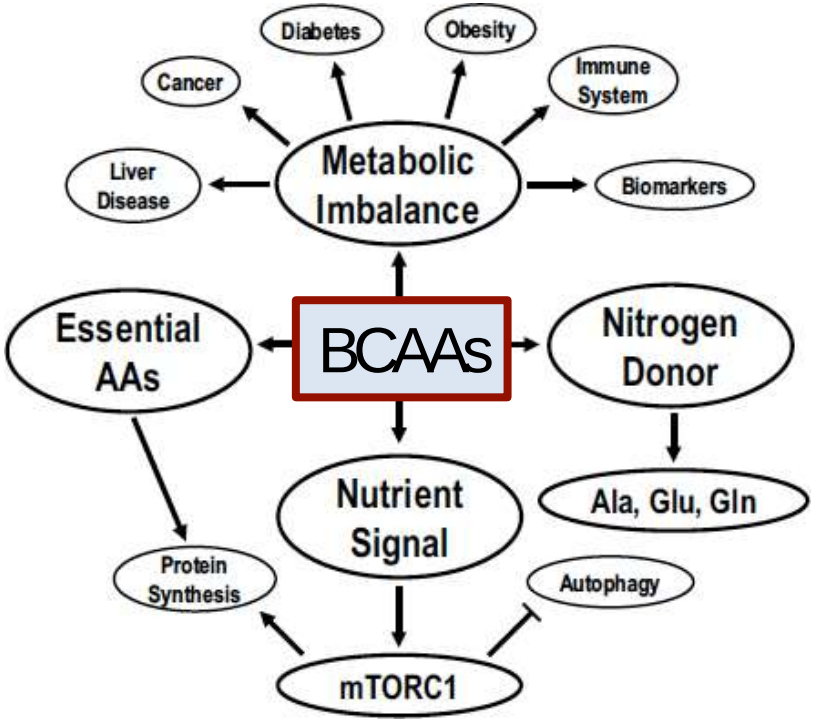
Leucine



Isoleucine

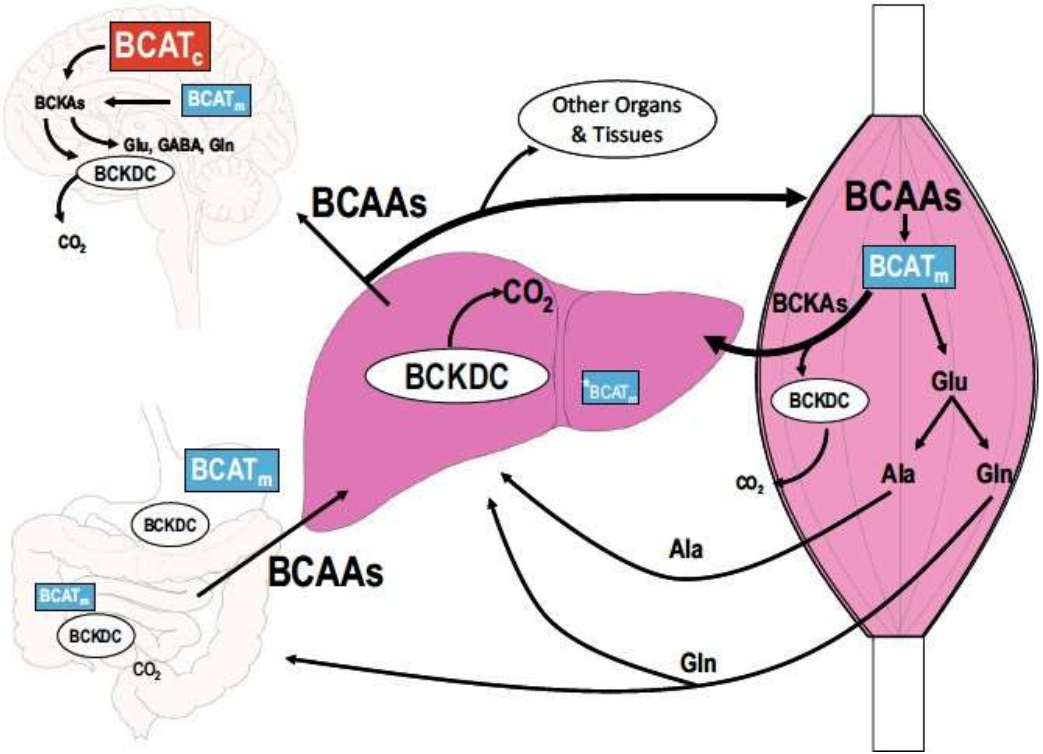


Valine

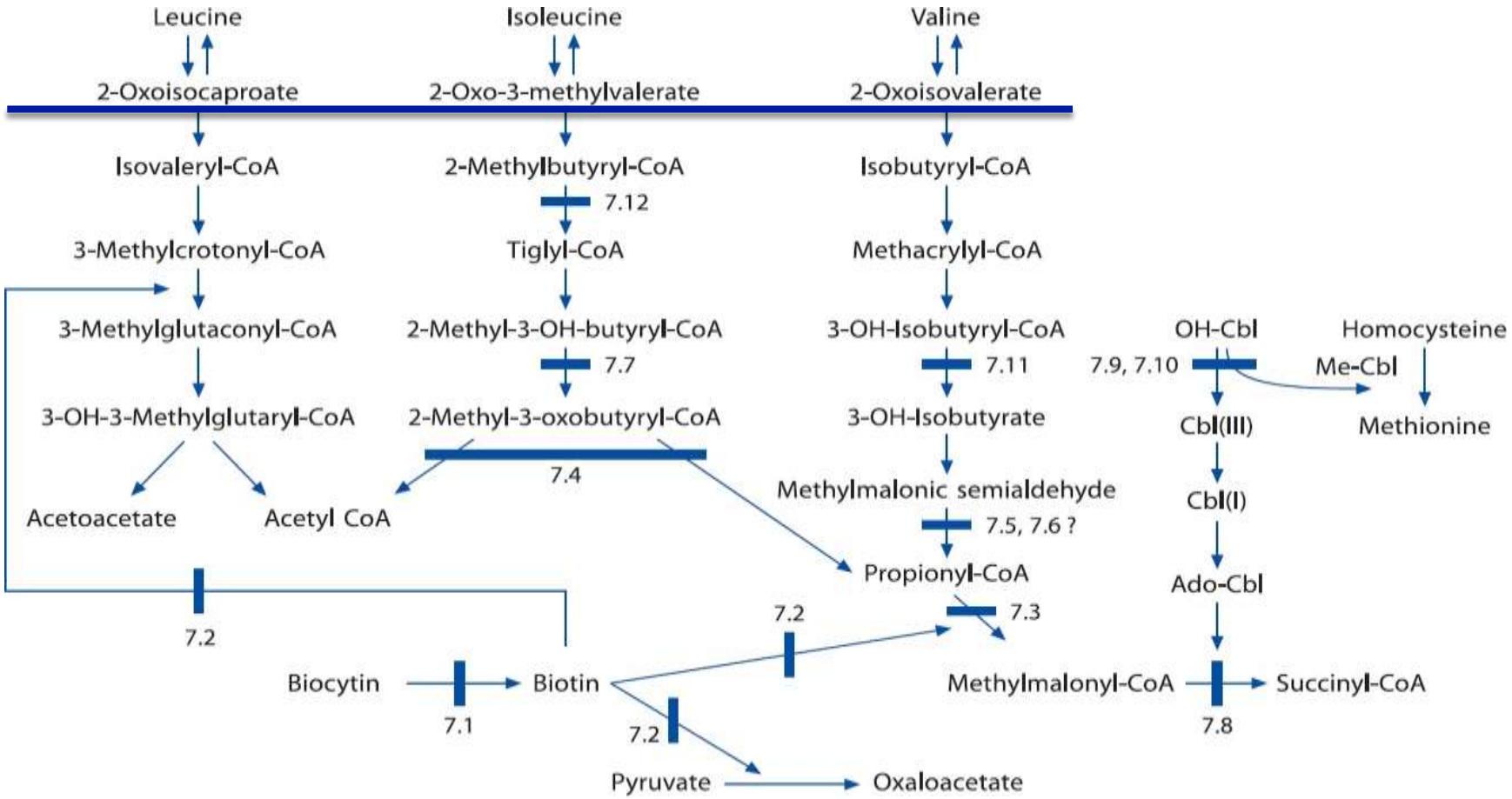


Sperringer et al, 2017

Liver and Skeletal Muscle play a major role in INTERORGAN SHUTTLING of BCAA nitrogen whereas in brain, INTERCELLULAR SHUTTLING predominates



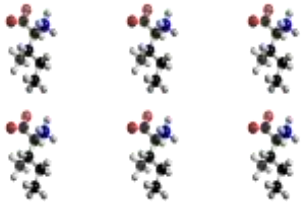
Sperringer et al, 2017



Blau et al, 2007

IEMs of BCAA that involve the 3 of them: Leu, Isol, Val

HIGH

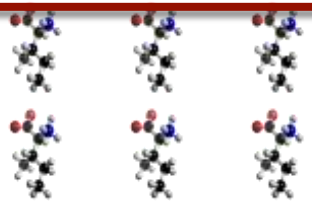


MSUD/MSUD like

BCAA Catabolism and transport

LOW

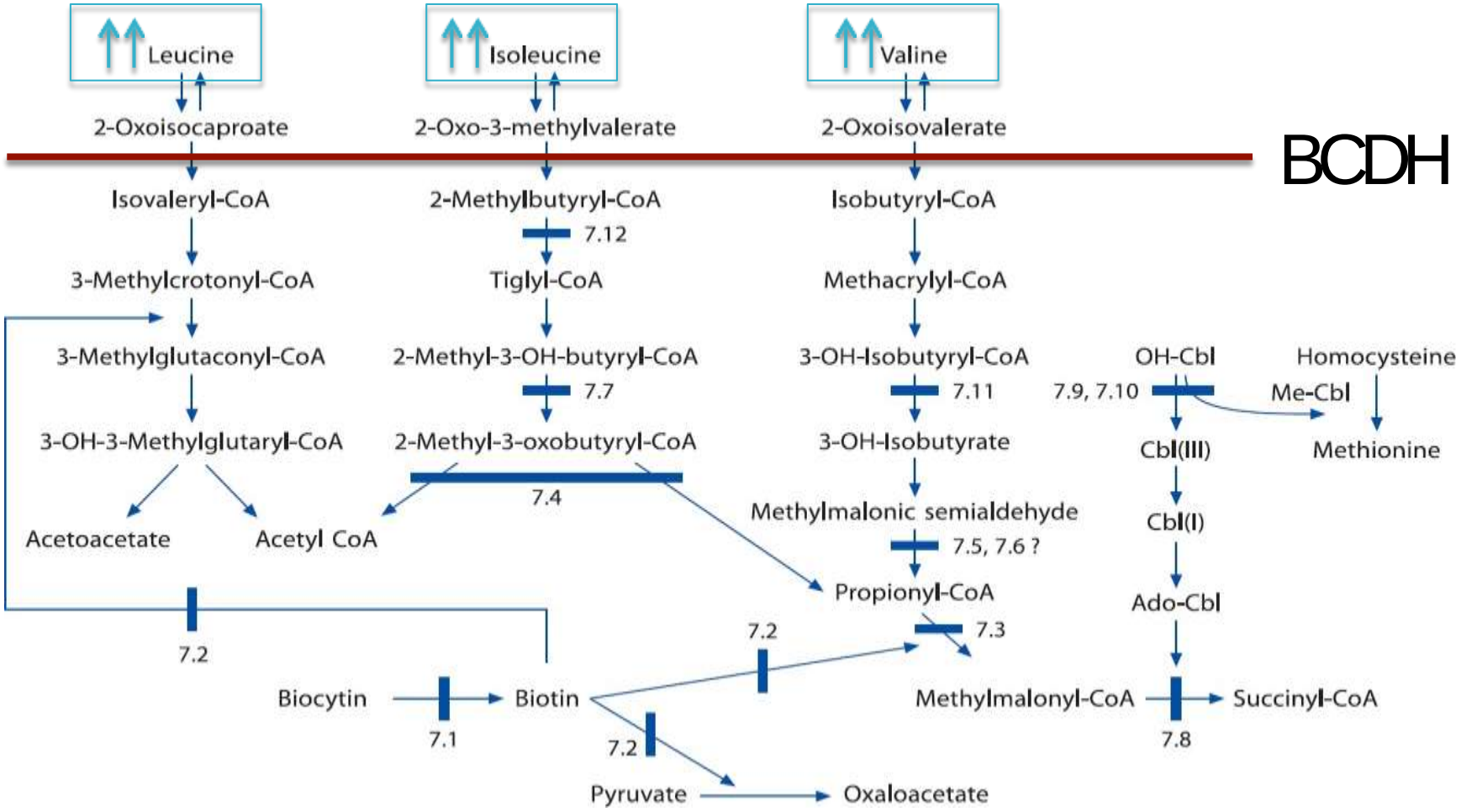
HIGH



MSUD/MSUD like

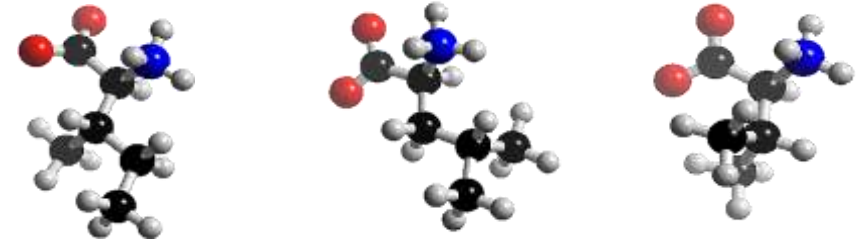
BCAA Catabolism and transport

LOW



Blau et al, 2007

MSUD: the most studied IEM of BCAA



ISOLEUCINE LEUCINE VALINE

BRANCHED CHAIN DEHYDROGENASE

Branched-chain α-ketoacid dehydrogenase complex

BCAA catabolism

Clinical presentation

MSUD type	Age of onset	Genes	BCKAD subunit	Clinical features	Biochemical features
Classic	Neonatal	*BCKDHA*; *BCKDHB*; *DBT*	E1α; E1β; E2	Neonatal period: maple syrup odor in cerumen and urine, irritability, poor feeding, lethargy, intermittent apnea, opisthotonus,"bicycling" movements. Infant and toddler: nausea, anorexia, dystonia, ataxia. Older: cognitive impairment, hyperactivity, sleep disturbances, hallucinations, focal dystonia, choreoathetosis, ataxia	Elevated BCAAs and alloisoleucine in plasma; elevated branched-chain ketoacids in urine
Intermediate	Variable	*BCKDHA*; *BCKDHB*; *DBT*	E1α; E1β; E2	Neonatal period: maple syrup odor in cerumen and urine. Older: feeding problems, poor growth, developmental delay	Similar but less severe than the classic form
Intermittent	Variable	*BCKDHA*; *BCKDHB*; *DBT*	E1α; E1β; E2	Normal growth and neurological development. In stress situations, may present with encephalopathy	Normal BCAAs when well; similar to the classical form during illness
Thiamine-responsive	Variable	*DBT*	E2	Similar to the intermediate form	Improvement of leucine tolerance and levels of BCAAs when on thiamine supplementation
E3-deficient	Variable	*DLD*	E3	Early-onset neurologic phenotype: hypotonia, developmental delay, emesis, hepatomegaly, lethargy, seizures, spasticity, Leigh syndrome, failure to thrive. Hepatic phenotype: nausea, emesis, hepatomegaly, hepatic encephalopathy	Elevated BCAAs, alloisoleucine, lactate, pyruvate, and alanine in plasma; elevated branched-chain ketoacids and α-ketoglutarate in urine

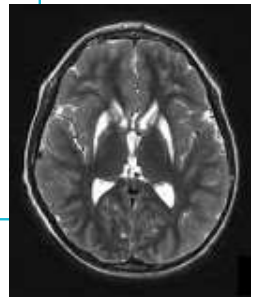
Blackburn et al, 2017

NEONATAL

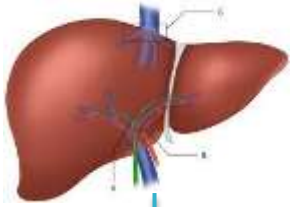
Maple syrup odor
Encephalopathy:
Hypotonia, Lethargy,
“Cycling, Boxing”,
Opisthotonus

NEUROLOGIC

Cognitive impairment
Neuropsychiatric symptoms
Focal dystonia
Ataxia
Leigh syndrome

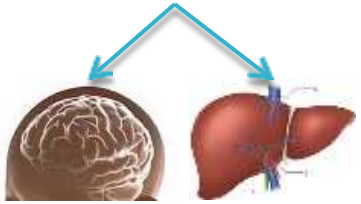


GASTRO-HEPATIC



Hepatomegaly
Nausea, emesis
Failure to thrive

INTERMITTENT

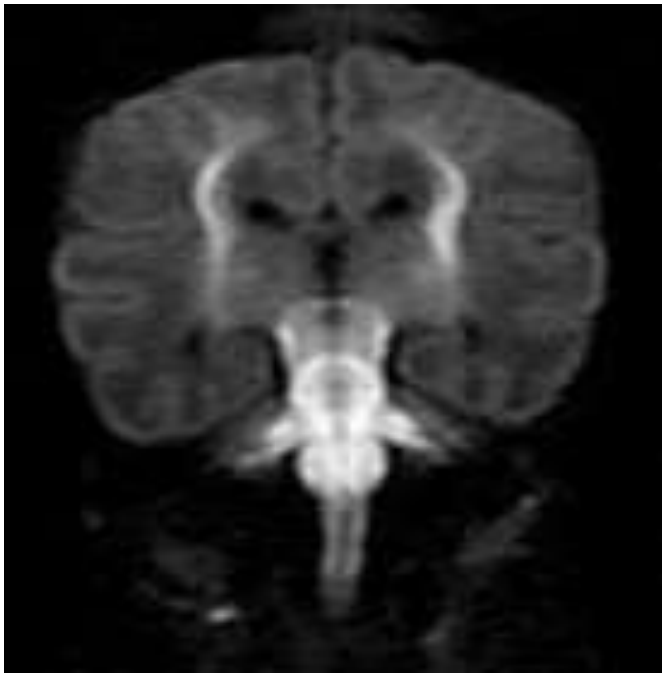


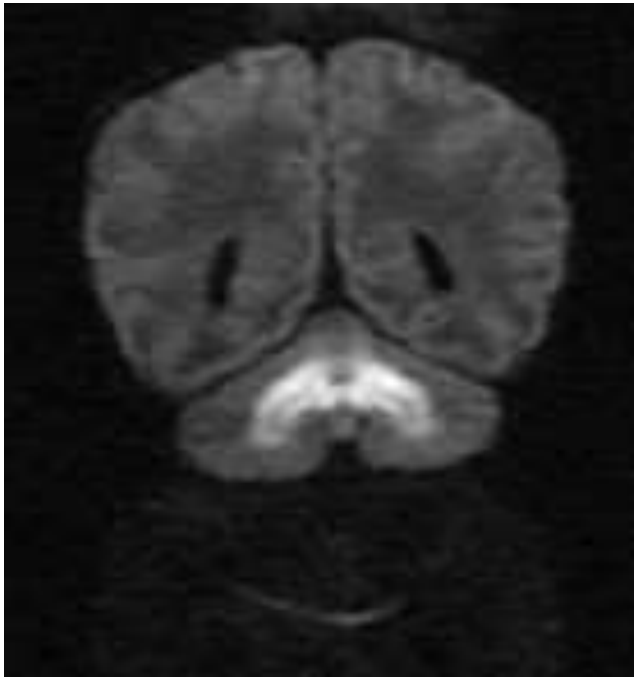
CLINICAL CASE: CS, NEONATAL PRESENTATION

Thursday 4th Sept , parallel session 11-12:30 h. Dr. Alejandra Darling (O-076)

VIDEO

Thursday 4th Sept , parallel session 11-12:30 h. Dr. Alejandra Darling (O-076)





Diffuse cerebral edema and symmetric restricted diffusion in bilateral cerebellar white matter, dorsal brainstem (in all of them), basal ganglia, posterior limbs of internal capsules, and corona radiata

CLINICAL CASE. CS, OUTCOME

[redacted]

Acute encephalopathic crisis at 16 days of life

4 years

6 years

Learning difficulties (IQ 62)

Much better at school. No decompensations

- First 2 years: Optimal metabolic control

- From 2-4 years: Several decompensations (ataxia, somnolence)

Liver transplantation

Long-term follow-up 35 patients

(1964-2013; Age: 2-49 years; m:16)

Decompensations (leucine >380 μ mol/L)

-More frequent during the 1st year of life and after 15 y (infection and dietary noncompliance)

-Leucine levels increased significantly in adulthood

Mental health, personal autonomy, quality of life

-56% needed occasional or sustained psychological or psychiatric care (mood, emotional, and anxiety disorders being the most common)

-Patients needing psychiatric care were significantly older [mean 22.6 y] than patients needing only psychological follow-up [mean 14.3 y]
-Patients with psychological follow-up experienced the highest lifetime number of decompensations

Abi-Wardé et al, 2017

How to improve clinical outcome?

Newborn Screening

New Therapies

New Biomarkers

Other than low-protein diet + supplements

Newborn Screening

NS: Better psychomotor development and IQ, less decompensations

Irritability/ lethargy	Stereotyped movements	Coma	Sweet odor smelling	Cerebral edema	Dialysis	Psychomotor development index/intellectual quotient (age at testing)	Hospital admissions (number)	Days admitted to hospital	Leu >1000 μM (days)
+					–	117 (9 y)	5	19	1
+		+	+	+	PD	89 (8 y)	5	38	0
+			+		PD	104 (6 y)	1	11	1
					–	108 (4 y)	0	0	0
					–	92 (2 y)	0	0	0
+		+	+	+	H	88	2	2	4
+					H	90 (1 y)	0	0	0
					–	93	0	0	0
+	+	+	+	+	H	80	7	90	13
+	+	+	+	+	H	83	5	62	10
+	+	+		+	H	≥85	6	46	6
+				+	H	≥85	5	34	1
+					H	≥85	4	32	2
+				+	H	≥85	5	54	8

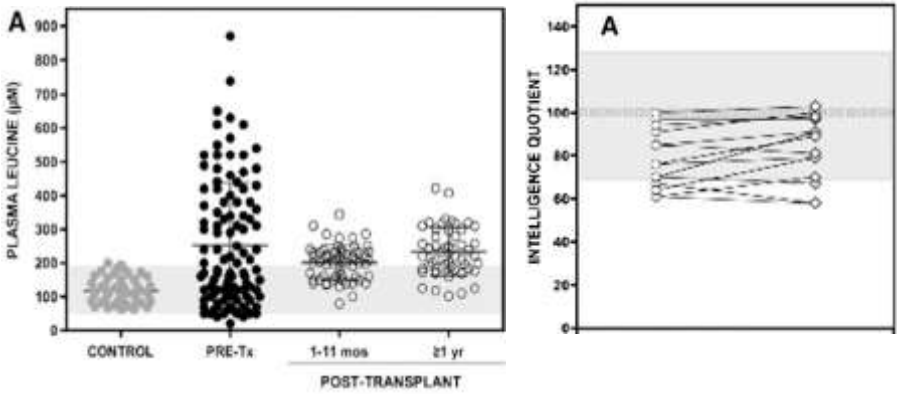
NS

Couce et al, 2015

Liver Transplantation

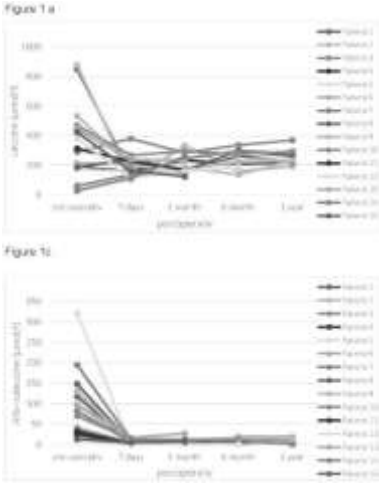
Long-Term Follow-up in 37 patients

After LT: Lower Leucine Levels, Higher IQ scores



Mazariegos et al, 2012

Domino Liver Transplant in 15 patients



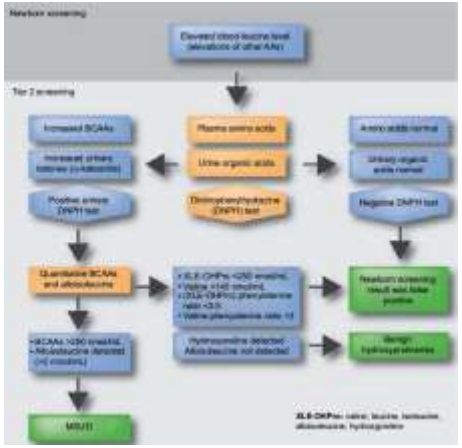
Uta et al, 2019

Biomarkers

CLASSIC

Elevations of the branched-chain amino acids (BCAAs) in plasma, α-ketoacids in urine, and alloisoleucine

NEONATAL SCREENING



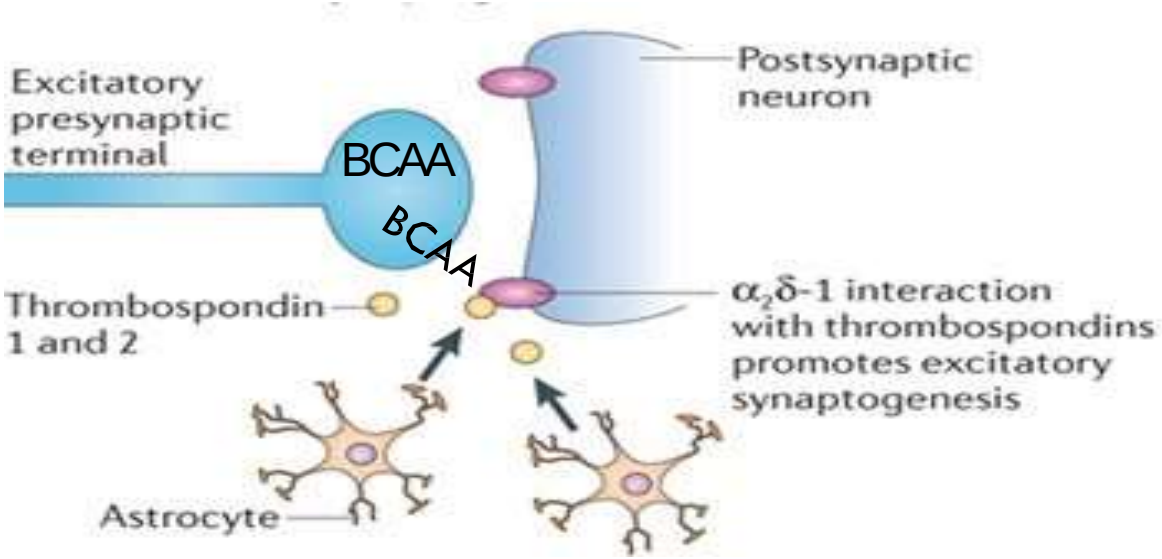
NEW

Inflammatory
(cytokines)
Scaini et al, 2018

Neurodegenerative
(BDNF, NCAM, Cathepsin D)
Scaini et al, 2016

Synaptic/ Neuronal
(Cacna2d2)
Castells et al, 2019

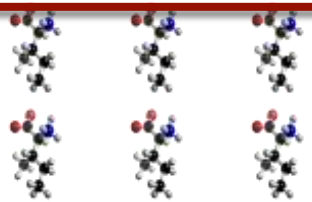
Downregulation of Cacna2d2 (mRNA) inversely correlated with chronically elevated BCAAs levels



Castells et al, 2019

Panel of genes related to neuronal function in peripheral blood at the mRNA level

HIGH



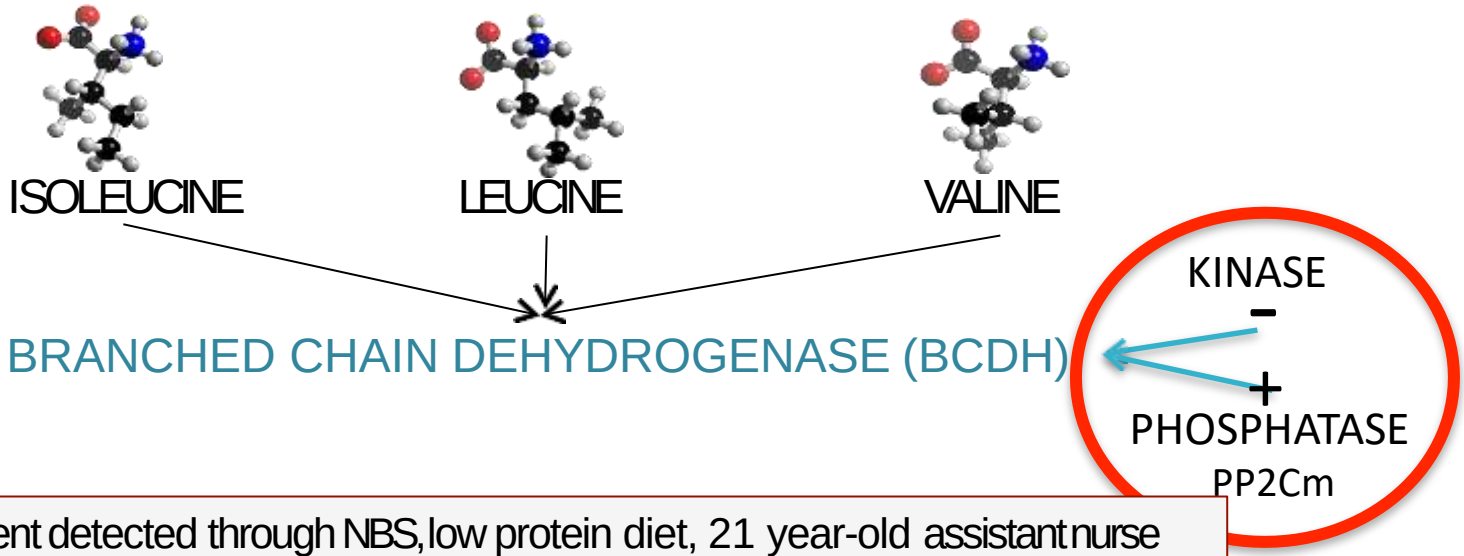
MSUD/MSUD like

BCAA Catabolism and transport

LOW

A Novel Regulatory Defect in the BCDH complex due to a Mutation in the PPM1K Gene (that encodes PP2Cm) Causes a Mild Variant of MSUD

Oyarzábal et al, 2012

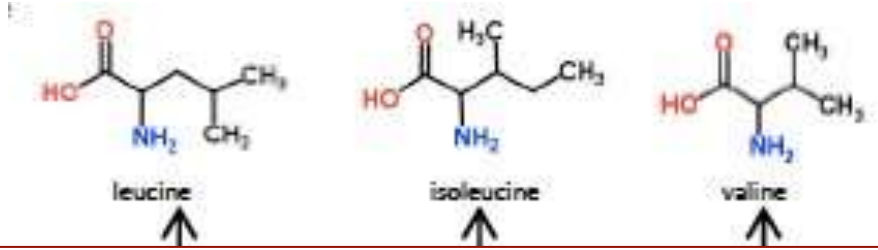


Patient detected through NBS, low protein diet, 21 year-old assistant nurse

	Leu	Ileu	Val	Aleu	Aleu/Ileu	α-KIC	α-KMV	α-KIV
Diagnosis	471	218	448	33.7	0.15	143	43	38
*Controls	98 ± 38	50 ± 21	149 ± 48	ND		123 ± 23	38 ± 15	8 ± 6
Follow-up	210–470	9–200	240–500	22–45	0.10–0.32	220–769	117–237	41–89
**Controls	115 ± 26	58 ± 15	219 ± 47	ND				

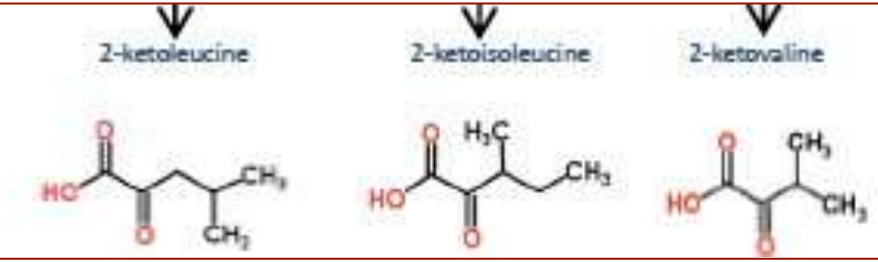
BCAT2 deficiency

BCAA: high



Branched chain amino acid aminotransferase (BCAT)

In Cytosol	In Mit
BCAT1	BCAT2



alpha-ketoacids:
low-normal

undetectable
L-allo-isoleucine

Branched chain amino acid dehydrogenase complex (BCDH)

BCAA
catabolism

Knerr et al, 2019

Subject #	1	2	3	4		5		6	
	ID+Autism	DD+Ataxia +SP	No symptoms	ID+Autism		No symptoms		Mild memory impairment	
Age (years)	29	11 (daughter of subject 3)	37 (mother of subject 2)	17		2		25	
Source	This study	This study	This study	This study		This study		Wang et al	
BCAT2 genotype	c.545T>G p.(Val182Gly); c.1021G>A p.(Ala341Thr)	BCAT2 c.600C>A; p.(Tyr200Ter)	BCAT2 c.600C>A; p.(Tyr200Ter)	Hom c.136_147 del; p.(His46_Pro		_1160delinsTGGATGCCCTCT p.(Ala385Valfs*35)		c.509G > A p.(Arg170Gln); c.790G > A p.(Glu264Lys)	
GnomAD frequency	NA; $2e^{-5}$	$4.95e^{-5}$	$4.95e^{-5}$			NA		$4e^{-5}$;$1.2e^{-5}$	
Stage at measurement	Without specific treatment	Without specific treatment	W	Pre-treatment	Post dietary restriction (monitoring after 3 months on diet with 1.5 g natural protein/kg/ day plus BCAA-free mixture)	Pre-treatment	Post dietary restriction (monitoring after 3 months on diet with 1.8 g natural protein/kg/day plus BCAA-free mixture)	Pre-treatment	Post pyridoxine treatment (100-200 mg/day for 3 months)
Leucine (59-180)	687	328	538	3446	215	325	86	Leu +Ile combined: 646	Leu +Ile combined: 464
Valine (64-320)	1528	1106	1108	3935	421	606	173	1755	452
Isoleucine (30-105)	505	474	370	2774	123	248	61	NA	NA
Allo-isoleucine (<5)	<5	<5	<5	<5	<5	<5	NA	Not detected	Not detected

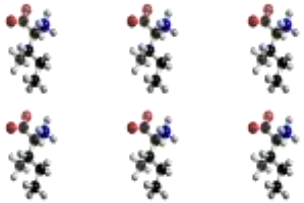
No acute encephalopathic crisis

Knerr et al, 2019

Biomarkers of diseases leading to raised BCAA

	BCAA	Alloisoleucine	Alpha-ketoacids	Others
MSUD BCKDHA, B, DBT DLD	Increased	Increased	Increased	 Lactate, pyruvate, alanine, alpha-ketoglutarate in urine
PHOSPHATASE PP1MK (1case)	Increased	Increased	Increased	Milder elevation
BCAT2 (6 cases)	Increased	Undectactable	Low-Normal	

HIGH

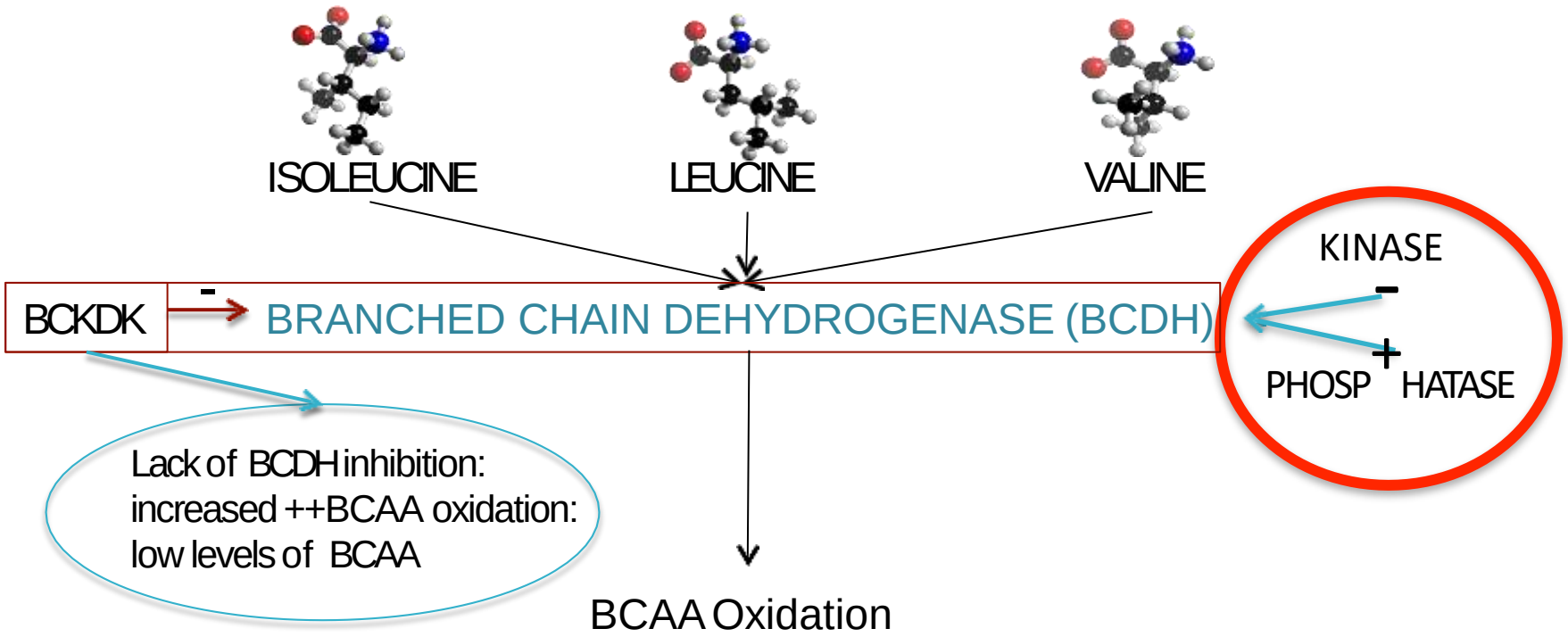


MSUD/MSUD like

BCAA Catabolism and transport

LOW

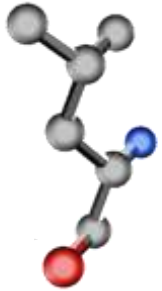
Novarino et al, 2012; García-Cazorla et al, 2014



VIDEO

PATIENT 1

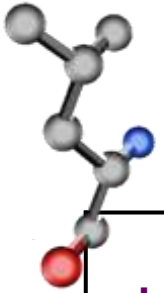
NRL DEVELOPMENT	++ **Hypotonia,** hold his head steady at 9 m . **Microcephaly** Sit without support at 16 m. Walks with support at 4 years Smiles at faces at 15 m **Babbles, no communication** **DQ of 12 m at 4 y**
BEHAVIOUR	Hyperactivity, rocking and hand flapping, hands and toy sucking, poor eye contact
GROWTH	W -1 SD; H-2 SD; **HC -2.5 SD**
EEG	Multiple spikes (> temporal)
BRAIN MRI	Delayed myelinisation at 4 y (> temporal)



	Plasma (umol/l)	CSF (umol/l)
LEUCINE	12-17 (109+/-31)	2-4 (10.9 ± 2.9)
ISOLEUCINE	4-14 (54+/-16)	0-0 (4.0+/-1.2)
VALINE	43-85 (212+/-53)	0-2 (13.9+/-2.9)

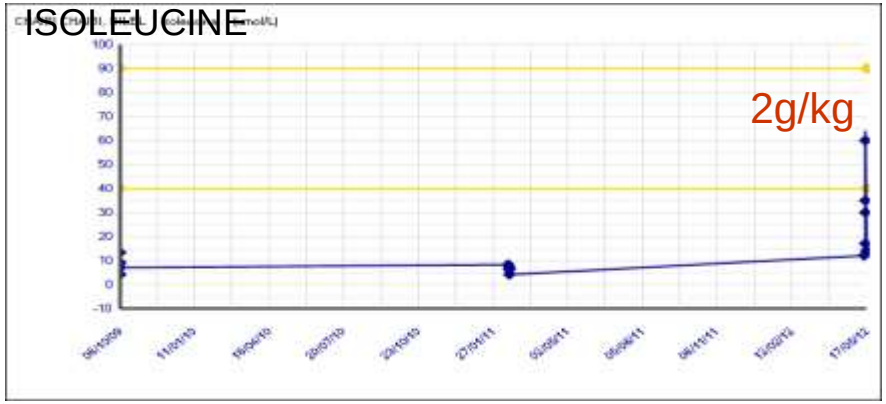
García-Cazorla et al, 2014

	PATIENT 1	PATIENT 2
NRL DEVELOPMENT	**++ Hypotonia,** hold his head steady at 9 m . **Microcephaly** Sit without support at 16 m. Walks with support at 4 years Smiles at faces at 15 m **Babbles, no communication** **DQ of 12 m at 4 y**	**Moderate Hypotonia. Microcephaly** Walks at 22 m Says his name and few other words at 5 years **DQ of 19 m at 5 y**
BEHAVIOUR	Hyperactivity, rocking and hand flapping, hands and toy sucking, poor eye contact	Hyperactivity, rocking and hand flapping, hands and toy sucking. At 5 y self-aggressive
GROWTH	W -1 SD; H -2 SD; **HC -2.5 SD**	W 0 SD; H 0.5 SD; **HC -2.5 SD**
EEG	Multiple spikes (> temporal)	Multiple spikes
BRAIN MRI	Delayed myelinisation at 4 y (> temporal)	Reduced WM volume at 4 y



	PLASMA (µmol/L)			CSF (µmol/L)		
	P1	**P2**	Control	**P 1**	**P 2**	Control
LEUCINE	12-17	28 – 48	109 ± 31	2-4	2.2-3.2	10.9 ± 2.9
ISOLEUCINE	4-14	10 – 24	54 ± 16	0-0	0.8 -1.3	4.0 ± 1.2
VALINE	43-85	70 - 151	212 ± 53	0-2	4.7-7.2	13.9 ± 2.9
α-KIC		5 - 8	11 – 57			
α-KMVal		4 - 5	10 - 32			
α-KIV		3 - 5	9 - 22			

PROTEIN OVERLOAD AND NORMALIZATION OF BCAA



MAIN OBJECTIVE

Normalize plasma BCAA levels at any time during the day and night

Patient with anorexia +++

2 g/kg/day of natural proteins + BCAA supplements

Normal BCCA post-intake but low preprandial BCAA

3,5 g/kg/day natural proteins +

100 mg/kg/d BCAA every 5 hours

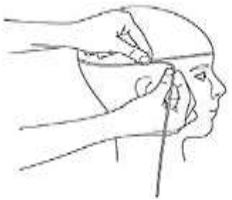
Continuous feeding during night

NORMALIZATION OF PLASMA BCAA AT ANY MOMENT

PATIENT 1. IMPROVEMENT AFTER 6 months OF TREATMENT

Longer attention spans, less hyperactivity and stereotypies, better communication strategies, autonomous walk, happier in general

VINELAND	BEFORE TREATMENT	AFTER TREATMENT
COMMUNICATION Receptive-Expressive	6-10	8-12
DAILY LIVING SKILLS Personal	0	5
SOCIALIZATION Interpers relationship Play and leisure time	17 4	19 5
MOTOR SKILLS Gross-Fine	16-10	26-10
GROWTH		
WEIGHT, HEIGHT, HEAD CIRCUMFERENCE	W: 14,5 kg(-2 SD) H:101 cm (-1.8 SD) HC: 48 cm (-3 SD)	W: 16 kg (-1.4 SD) H: 106 cm (-1.7 SD) HC: 49 cm (-2.17 SD)



García-Cazorla et al, 2014

LONG TERMOUTCOME

SEVEREID, BEHAVIOURALPROBLEMS++

VIDEOS

PATIENT 3

Abnormal NN examination !!
Peripheral hypertonia,
HC: -1.2DS
W: -1.5DS

Sits up only with hand support, does not explore objects, poor spontaneous movements, brisk reflexes

Babbles, no words, good social interaction, walks with help, imitates simple actions, Can't point the finger

TREATMENT

NEWBORN SCREENING TYROSINEMIA?

TYR 546
(41-206 umol/l)

LEU 14, 52, (57-155)
VAL 52, 100 (102-294)
ISO 17, 32 (32-90)

BCKDK Diagnosis

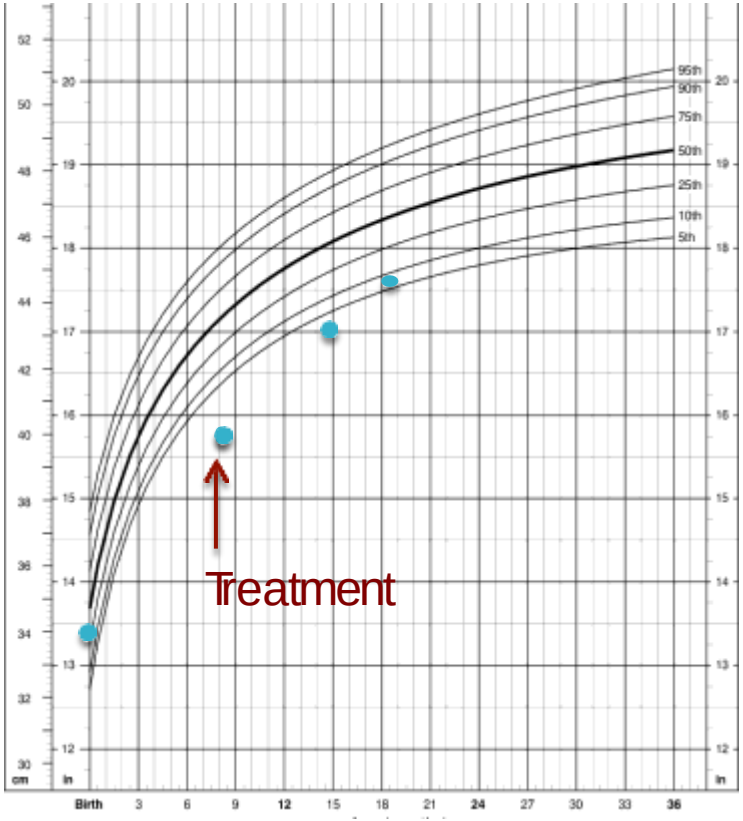
Progressive HC deceleration (-3.5 SD)
Developmental delay

6 m
WES negative
AA re-reading

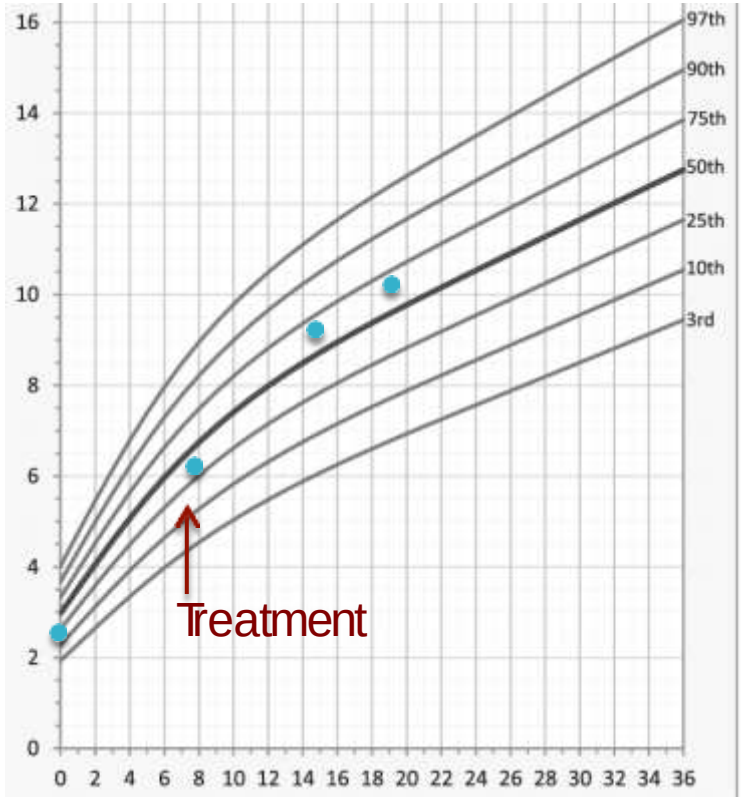
15 m
HC -2.2 SD
Quick ND improvement

19 m
HC -1.8 SD
Current situation

Head Circumference



Weight



LEUCINE

ISOLEUCINE

VALINE

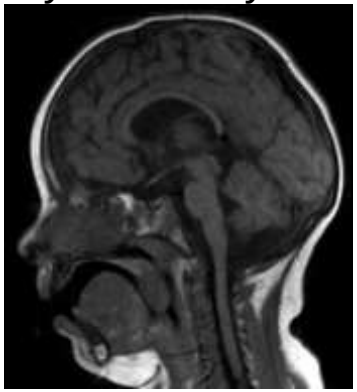
0.5g of every BCAA,
7 times/day.
Baseline high protein diet
(3 g/kg/d)

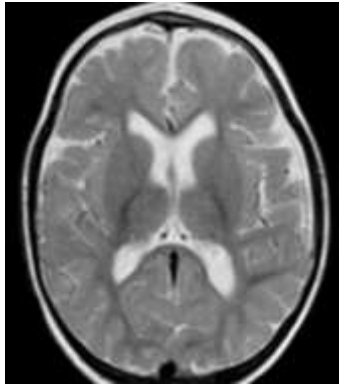
PATIENT 1

Reduced white matter volume, thin corpus callosum
hypomyelination

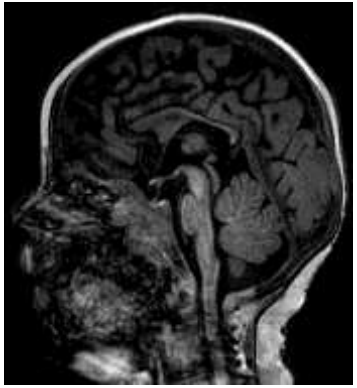
PATIENT 3

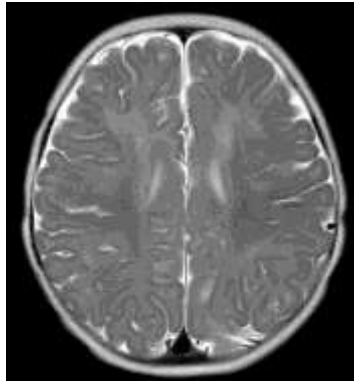
4 year-old boy



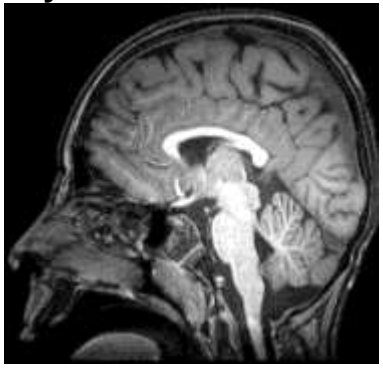


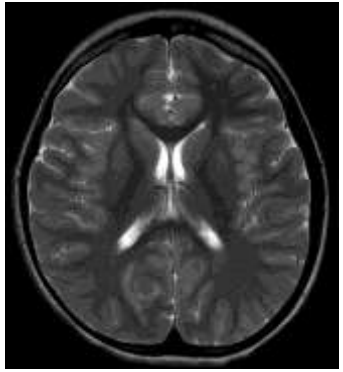
6 month-old girl





6 years of treatment: NORMAL BRAIN MRI





BCKDK: Biomarkers

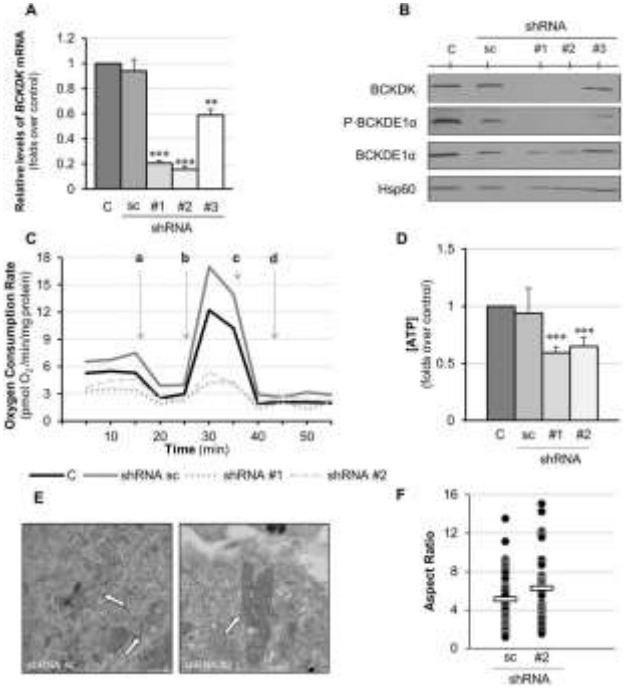
Low concentration of BCAA in plasma and CSF

Other AA may be abnormal but not constantly, LNAA?...specific pattern?

Abnormal mitochondrial function

O2 comsumption, ATP production, mit shape

Oyarzábal, 2016



Low concentration of BCAA in plasma and CSF

Other AA may be abnormal but not constantly, LNAA?...specific pattern?

Treonina	Serina	Asparagina	Ac glutámico	Glutamina	Prolina	Glicina	Alfa alanina
219	155	76	28	882	239	340	295
394	220	68	43	826	436	416	597
162	163	53	121	645	181	244	323
149	185	68	78	703	217	233	462
							315
58-292	103-197	50-120	5-80	326-674	90-270	103-293	167-439
137	116	52	12	639	223	310	530
68	67	35	11	435	127	180	181
93	98	45	21	539	128	167	259
210	139	64	34	492	229	299	698
152	133	48	42	539	145	273	313
133	127	49	42	531	134	272	227
108	109	46	15	520	131	238	250
78	88	35	20	411	126	205	226
243	192	74	20	651	236	392	760
164	133	55	13	612	166	279	393
191	144	50	35	540	180	305	496
171	129	51	13	541	160	280	448
169	130	60	10	594	160	852	432
203	147	71	20	410	143	282	328
							659
239	136	93	15	656	376	239	666
130	106	66	8	589	230	178	494
153	130	89	16	703	331	201	622
164	141	100	17	709	371	215	724
175	140	97	17	680	445	215	697
193	124	81	9	690	313	232	609
403	101	64	12	713	179	293	481
402	107	77	17	692	257	294	745
320	185	118	8	756	505	357	937
153	103	57	6	582	146	298	316
238	200	136	59	664	348	316	610
249	145	92	9	626	295	283	656
143	115	74	10	624	186	248	751
165	127	56	16	610	285	375	730
179	144	55	15	508	276	323	826
169	99	65	26	560	244	194	404

BCKDK Natural History Study

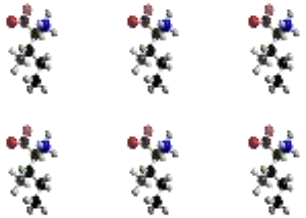
MetabERN project, co-directed by AOA and NOMPS subnetworks (T. Tangeraas and A. García-Cazorla)

About 20 patients in Europe

Courtesy of Dr. Trine Tangeraas

- Clinical and biochemical presentation
- Growth and development
- Genetics
- Neuroimaging, neurophysiology
- Neurodevelopmental tests (when performed)
- Describe clinical response to BCAA treatment (when available)
- *Collect NBS profile (BCKDK tool active in CLIR, Mayo Clinic)**
- *In case of CSF availability we'll perform a detailed multi-omics study.**

HIGH

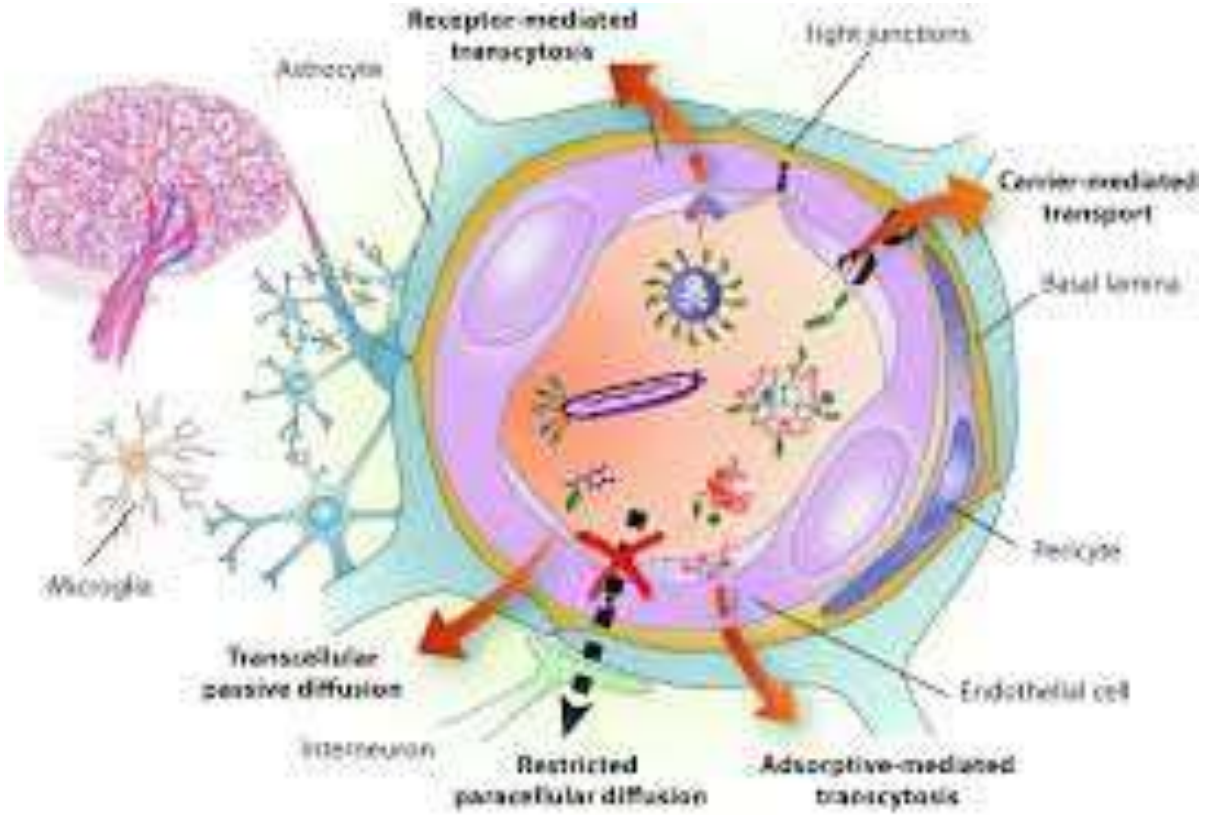


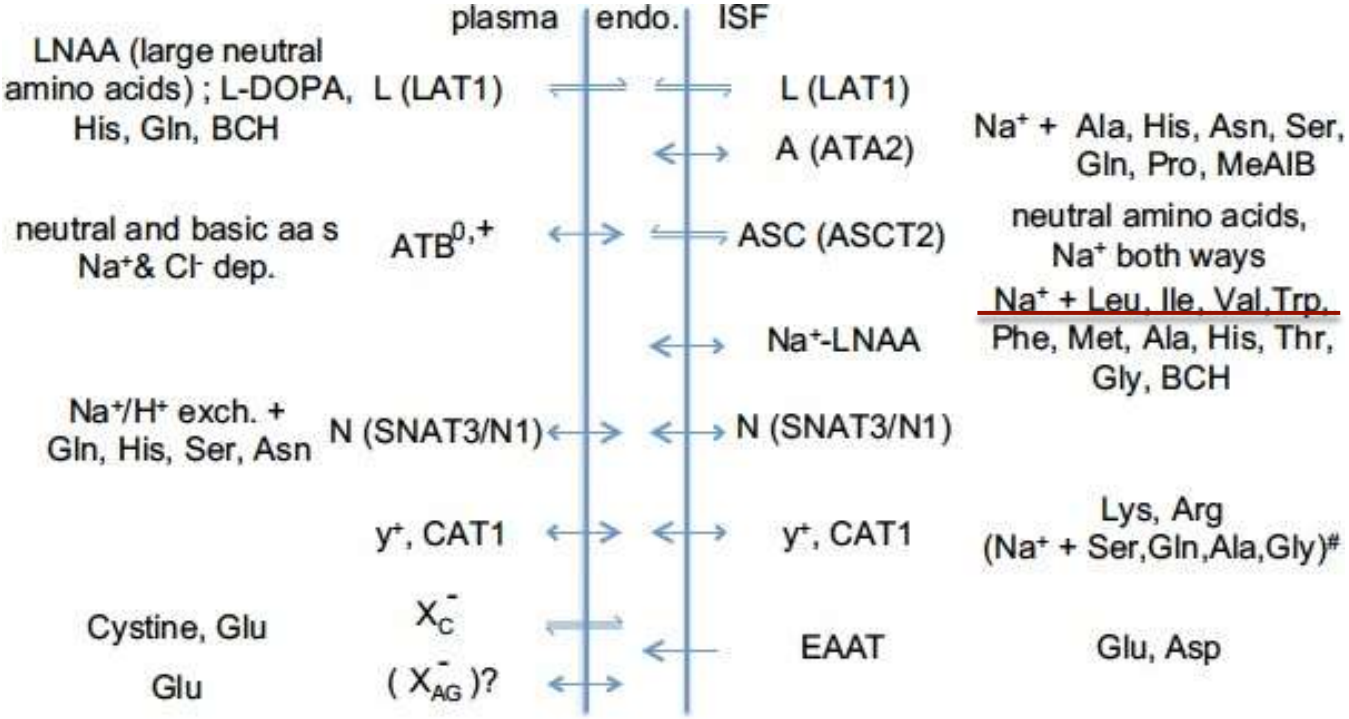
MSUD/MSUD-like

BCAA Catabolism and transport

LOW

BCCA transport across the blood-brain barriers



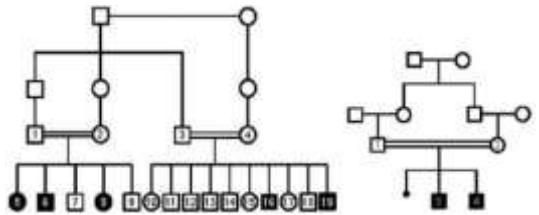

plasma
endo.
ISF
LNAA (large neutral amino acids) ; L-DOPA, His, Gln, BCH
L (LAT1)
L (LAT1)
A (ATA2)
Na+ + Ala, His, Asn, Ser, Gln, Pro, MeAIB
neutral and basic aa s Na+& Cl- dep.
ATB0,+
ASC (ASCT2)
neutral amino acids, Na+ both ways
Na+ + Leu, Ile, Val,Trp, Phe, Met, Ala, His, Thr, Gly, BCH
Na+-LNAA
Na+/H+ exch. + Gln, His, Ser, Asn
N (SNAT3/N1)
N (SNAT3/N1)
y+, CAT1
y+, CAT1
Lys, Arg
(Na+ + Ser,Gln,Ala,Gly)#
Cystine, Glu
XC-
EAAT
Glu, Asp
Glu
(XAG-)?
obligatory exchanger
net flux, rev.
net flux, little backflux
SNAT3/N1 SLC38A3;
ATA2 SLC38A2;
CAT1 SLC7A1;
XC- SLC7A11+SLC3A2;
Na+-LNAA unknown;
ATB0,+ SLC6A14;
L SLC7A5+SLC3A2;
EAAT SLC1A3-1;
ASC SLC1A5


Slc7a5

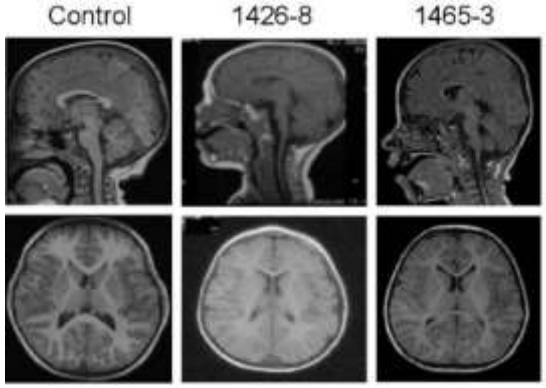
Hladky, 2018

SLA7A5 mutations identified in individuals with autism, microcephaly and motor deficits

Patient	1426-5	1426-6	1426-8	1426-19	1465-3	1465-4
Gender	F	M	F	M	M	M
Origin	Libya	Libya	Libya	Libya	Turkish	Turkish
Age at diagnosis	N/A	N/A	5 months	N/A	2 weeks	5-6 months
HC at birth (SD)	N/A	-2/-3	N/A	N/A	- 3	- 3
HC at latest examination (SD)	-5	-2,3	-5,5	-5	N/A	N/A
Developmental milestones						
Gross motor (normal/delayed/absent)	Delayed	Delayed	Delayed	Delayed	Delayed	Delayed
Fine motor (normal/delayed/absent)	Delayed	Delayed	Delayed	Delayed	Delayed	Delayed
Language (normal/delayed/absent)	Delayed	Delayed	Delayed	Delayed	Absent	Absent
Social (normal/delayed/absent)	Delayed	Delayed	Delayed	Delayed	Delayed	Delayed
Seizure						
Type	-	-	-	N/A	GTC	GTC
Onset	-	-	-	N/A	1year	6 months
Autism associated disorder						
Impaired social interactions	+	+	+	N/A	+	+
Impaired eye-to-eye gaze, facial expression	+	+	+	N/A	+	+
Impaired ability to form peer relationships	+	+	+	N/A	+	+
Lack of spontaneous play	+	+	+	N/A	+	+
Restrictive behavior, interests and activities	+	+	+	N/A	+	+
Stereotyped, repetitive behavior	-	-	-	N/A	+	+
Inflexible adherence to routines or rituals	+	+	+	N/A	+	+
Additional CNS investigations						
MRI	N/A	Cortical atrophy	Normal	N/A	Thin CC	Thin CC



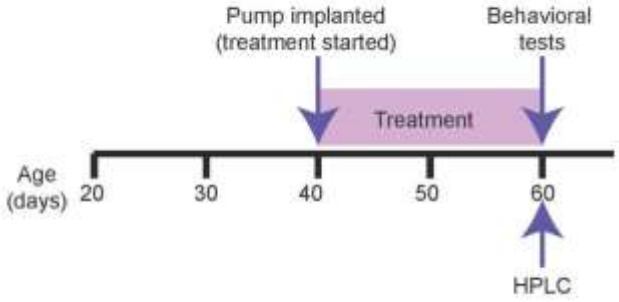
Tarlungeanu et al, 2016

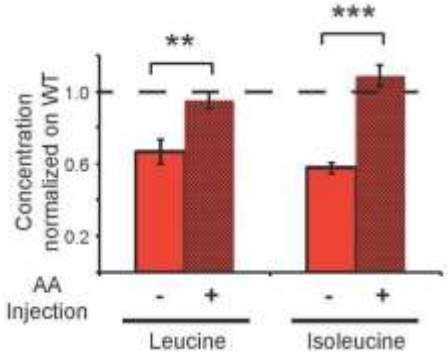


Tarlungeanu et al, 2016

$Tie2^{Cre}$;$Slc7a5^{fl/fl}$ adult mice

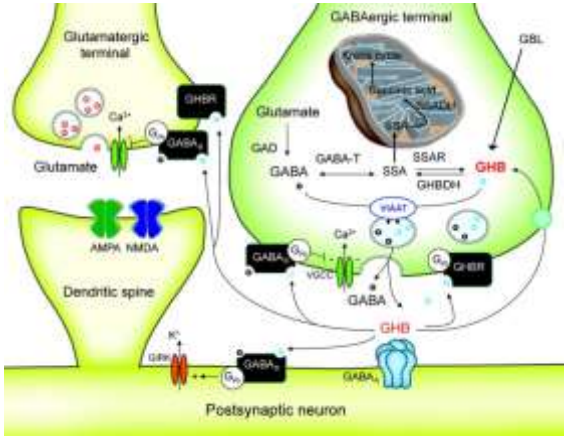
- Low Brain BCAA levels (similar to BCDK mice) and severe neurological abnormalities
- The levels of other LNAAs such as tyrosine and tryptophan were normal while a few other AA: serine, hystidine and phenylalanine where higher than controls
- Biochemical and clinical improvement after BCAA intraventricular injection

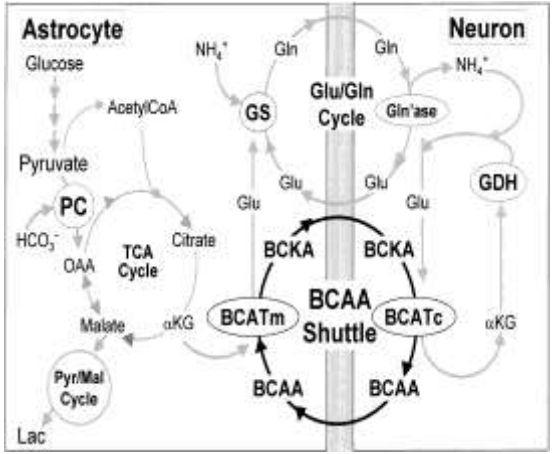




Low levels of BCAA (catabolism (BCKDK)/transport) are treatable developmental encephalopathies

- Early developmental delay, microcephaly, hypotonia, ID, autism, +/-epilepsy, +/-motor problems
- Long-term outcome? : Improvement although behavioural problems +++ (BCKDK)
- A natural history study is needed

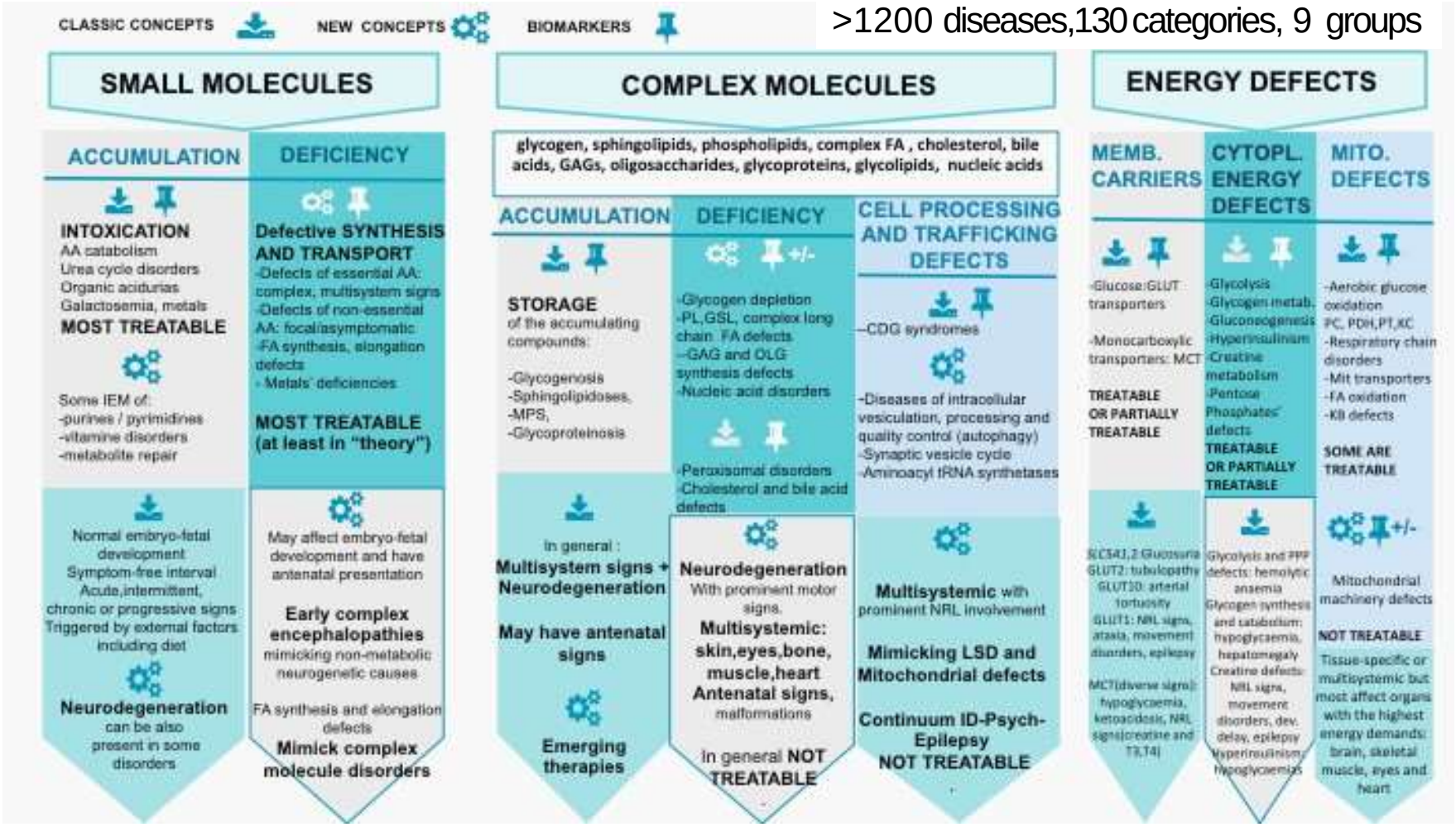




IEM of BCAA are diseases of small molecules

ACCUMULATION
"INTOXICATION"

DEFICIENCY



Proposal of a simplified classification of IEM. Saudubray et al, 2019

SJD
Sant Joan de Déu
Barcelona · Hospital

European Reference Network
for rare or low prevalence complex diseases

Networek
Hereditary Metabolic Disorders (MetabERN)

ciberer isciii

Unión Europea
Fondo Europeo de Desarrollo Regional
"Una manera de hacer Europa"