MEDICAL BIOCHEMICAL GENETICS
CLINICAL CORE
SEMINAR SERIES

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University of Colorado
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Fatty Acid Oxidation, Carnitine, Ketone disorders – Part 2

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22 July 2022
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DISCLOSURES

Company	Financial relationship type
Aeglea	Clinical Trial Support
Alnylam	Advisory Board
Amicus Therapeutics	Clinical Trial support, Advisory Board
ACI Clinical	Data Safety and Monitoring Chair (Applied Ther, Taysha)
Audentes/Astellas	Clinical Trial Support
AvroBio	Clinical Trial Support
BioMarin	Clinical Trial Support, Advisory Board, Travel support
BridgeBio/CoA Ther	Advisory Board
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CTI-Clinical Trial	Data Safety and Monitoring Board (Vtesse)
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Hemoshear	Clinical Trial Support, Advisory Board
Homology	Clinical Trial Support
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Pfizer	Clinical Trial Support
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Stealth Therapeutics	Clinical Trial Support
Synlogic	Clinical trial support, Consultant
Ultragenix	Clinical Trial Support, Advisory Board

Conflict of interest: managed by the University of Utah Institutional Review Board.

DISORDERS OF KETONE BODIES SYNTHESIS AND UTILIZATION

Objectives

Understand why and where ketones are synthesized

Define enzymes involved in ketone synthesis and utilization

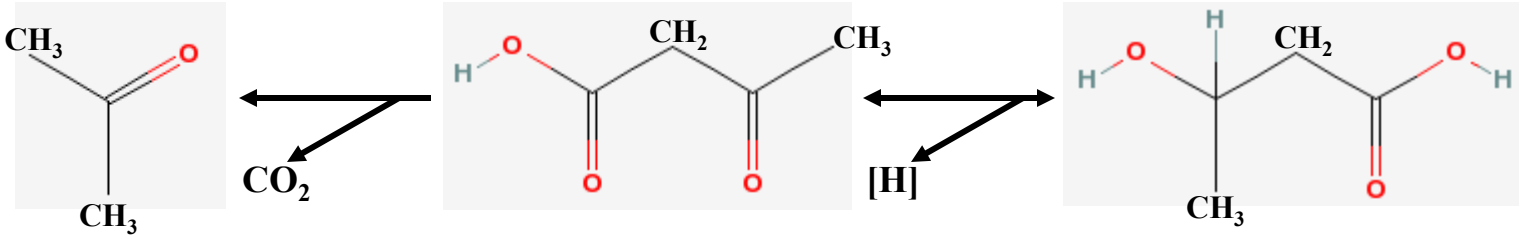
List therapies for disorders of ketone body synthesis and utilization

KETONE BODIES METABOLISM

- Ketone bodies are important in energy transfer during fasting or other lypolitic stresses.
- They derive from beta-oxidation of fatty acids and from ketogenic amino acid (leucine, lysine, isoleucine) catabolism.
- They are produced in liver mitochondria and are transported to extrahepatic tissues where they are utilized.
- Ketogenesis (hepatic ketone body formation) and ketolysis (extra hepatic ketone body utilization) are important processes, especially for the brain, to provide energy when glucose can not meet the metabolic need.
- Physiological levels of ketone bodies in plasma range from <0.1 mM (post-prandial) to 6 mM (prolonged fasting), they can reach 25 mM in diabetic ketoacidosis.
- Most of the ketone bodies are taken up by the extra hepatic tissues, 10-20% are lost in the urine during ketosis.

KETONE BODIES

- Three compounds are usually listed as “ketone bodies”: 3-hydroxybutyrate, acetoacetate, acetone.
- Acetoacetate is the main ketone body, acetone derives from its decarboxylation, while 3-hydroxybutyrate derives from its reduction.



Acetone **Acetoacetic acid** **3-Hydroxybutyric acid**

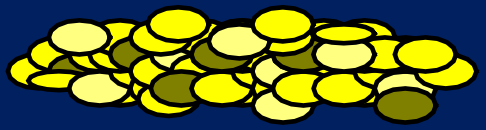
KETONE BODIES METABOLISM

- Rate of utilization of ketone bodies is proportional to their circulating levels.
- Heart and kidney have the greatest capacity for ketone utilization.
- The ketogenic pathway provides fat-derived fuel for the brain when glucose is low.
- Patients with defects in ketone synthesis or degradation are asymptomatic unless they are fasting:
  - **Defects of ketogenesis: hypoketotic hypoglycemia**
  - **Defects of ketolysis: ketoacidosis (severe) ± hypoglycemia**

HSL (Hormone Sensitive Lipase) Releases Fatty Acids from adipocytes. Transcription of HSL is increased during fasting and suppressed by insulin and glucose.

FATTY ACID OXIDATION DURING FASTING

ADIPOSE TISSUE



FATTY ACIDS

HEART

SKELETAL MUSCLE

LIVER

KETONES

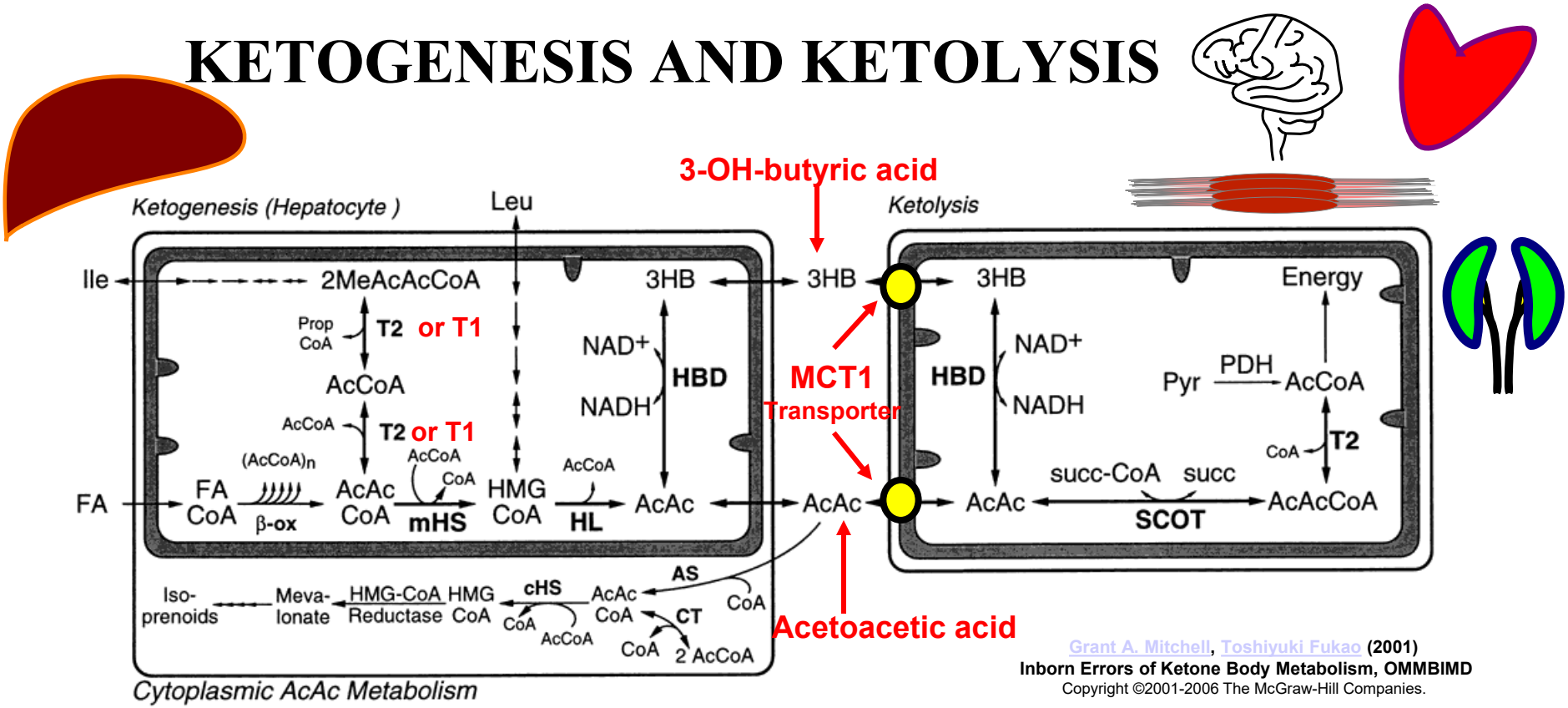
ß-hydroxybutyrate
acetoacetate

BRAIN

KETOGENESIS AND KETOLYSIS

- Ketogenesis is regulated by two hepatic mitochondrial enzymes:
  - **3-hydroxy-3-methylglutaryl-CoA synthase (mHS)**
  - **3-hydroxy-3-methylglutaryl-CoA lyase (HL)**
- Ketolysis in extra hepatic mitochondria is mediated by reversible reactions catalyzed by:
  - **The MCT1 transporter (*SLC16A1*): entry of ketones into tissues**
  - **SuccinylCoA:3-ketoacid(oxoacid) CoA transferase (SCOT)**
  - **Mitochondrial acetoacetyl-CoA thiolase (T2)(*ACAT1*)**
- Deficiencies of mHS or HL cause disorders of ketogenesis; deficiencies of MCT1, SCOT or T2 cause disorders of ketolysis.
- All are inherited as autosomal recessive traits

KETOGENESIS AND KETOLYSIS



Acetoacetate is synthesized from acetylCoA by cytosolic acetoacetyl-CoA thiolase (*ACAT2* gene, T1). Acetoacetyl-CoA (AcAc-CoA) and acetyl-CoA via two enzymatic steps (mitochondrial Hydroxy Methyl Glutaryl CoA synthase (mHS), a highly regulated enzyme, and Hydroxy Methyl Glutaryl CoA lyase (HL)) form ketones. The liver has both T2 (*ACAT1*, mitochondrial) and T1 (*ACAT2,* cytosolic) thiolase. R-3-hydroxybutyrate dehydrogenase (3HBD) catalyzes the reduction of Acetoacetate to 3-OH-butyrate.

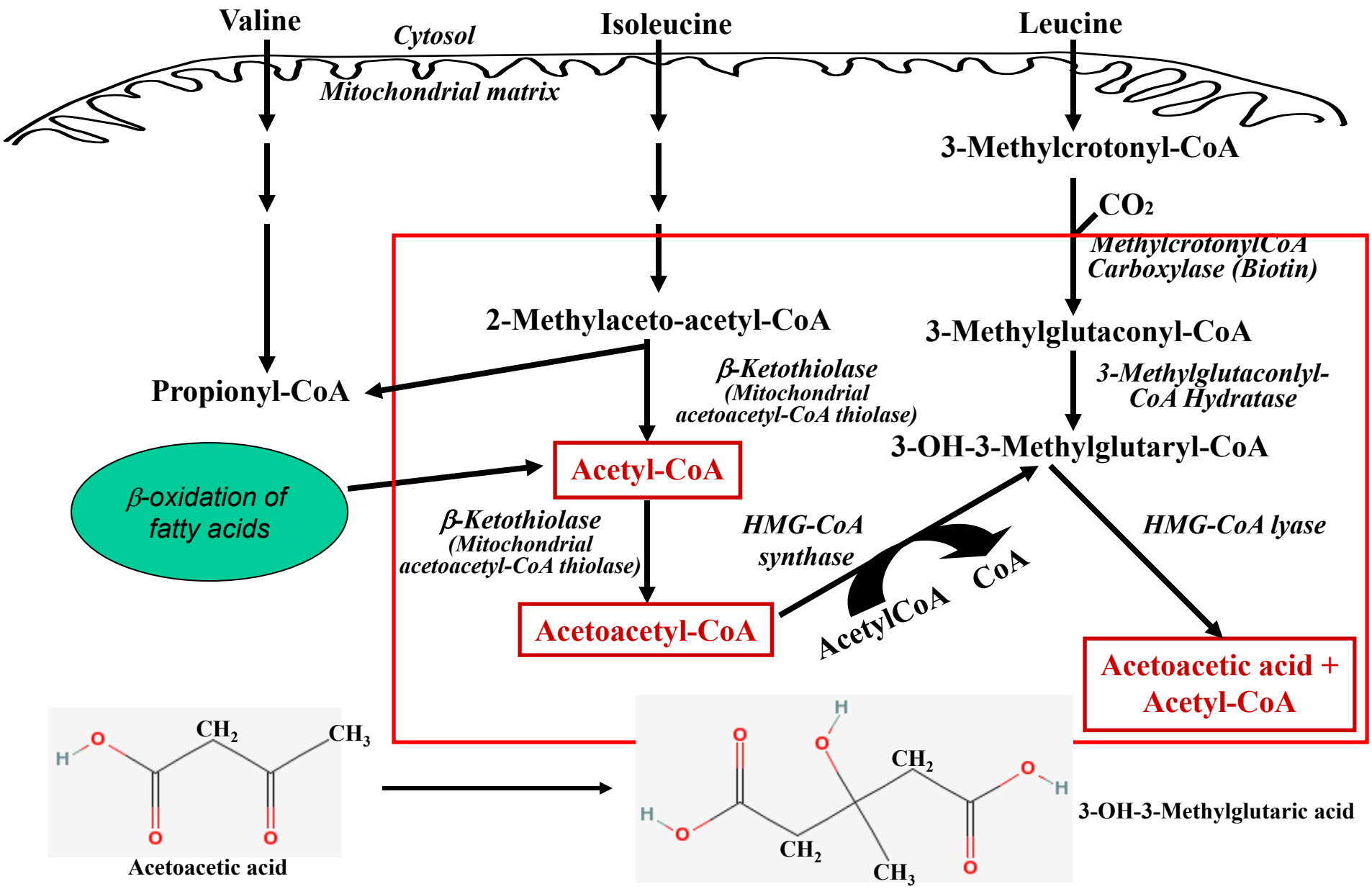
The MCT1 transporter mediates the uptake of ketones by peripheral tissues

HBD: 3-Hydroxy Butyrate Dehydrogenase

T1: *ACAT2*: cytosolic acetoacetyl-CoA thiolase

T2: *ACAT1*: mitochondrial acetoacetyl-CoA thiolase : MAT

BRANCHED-CHAIN AMINO ACID METABOLISM



DISORDERS OF KETOGENESIS

Mitochondrial 3-Hydroxy-3-Methyl-Glutaryl-CoA Synthase deficiency, mHS (OMIM 605911)

Frequency: rare

Presentation: hypoketotic hypoglycemia, metabolic acidosis, encephalopathy progressing to coma after fasting or infections, hepatomegaly. Can present without hypoglycemia.

Labs: Elevated serum free fatty acids and triglycerides at time of hypoglycemia, elevated acetylcarnitine, but acylcarnitines may be normal, dicarboxylic aciduria can be seen, 4-hydroxy-6-methyl-2-pyrone and 3-hydroxyglutarate can be present, ketones absent or barely present, normal lactate

Diagnosis: DNA testing: *HMGCS2* gene (1p13-p12)

Therapy: Fasting avoidance, cornstarch

DISORDERS OF KETOGENESIS

3-Hydroxy-3-Methyl-Glutaryl-CoA Lyase deficiency, HL (OMIM 246450)

Presentation early in life with vomiting, seizures, unconsciousness, hepatomegaly.

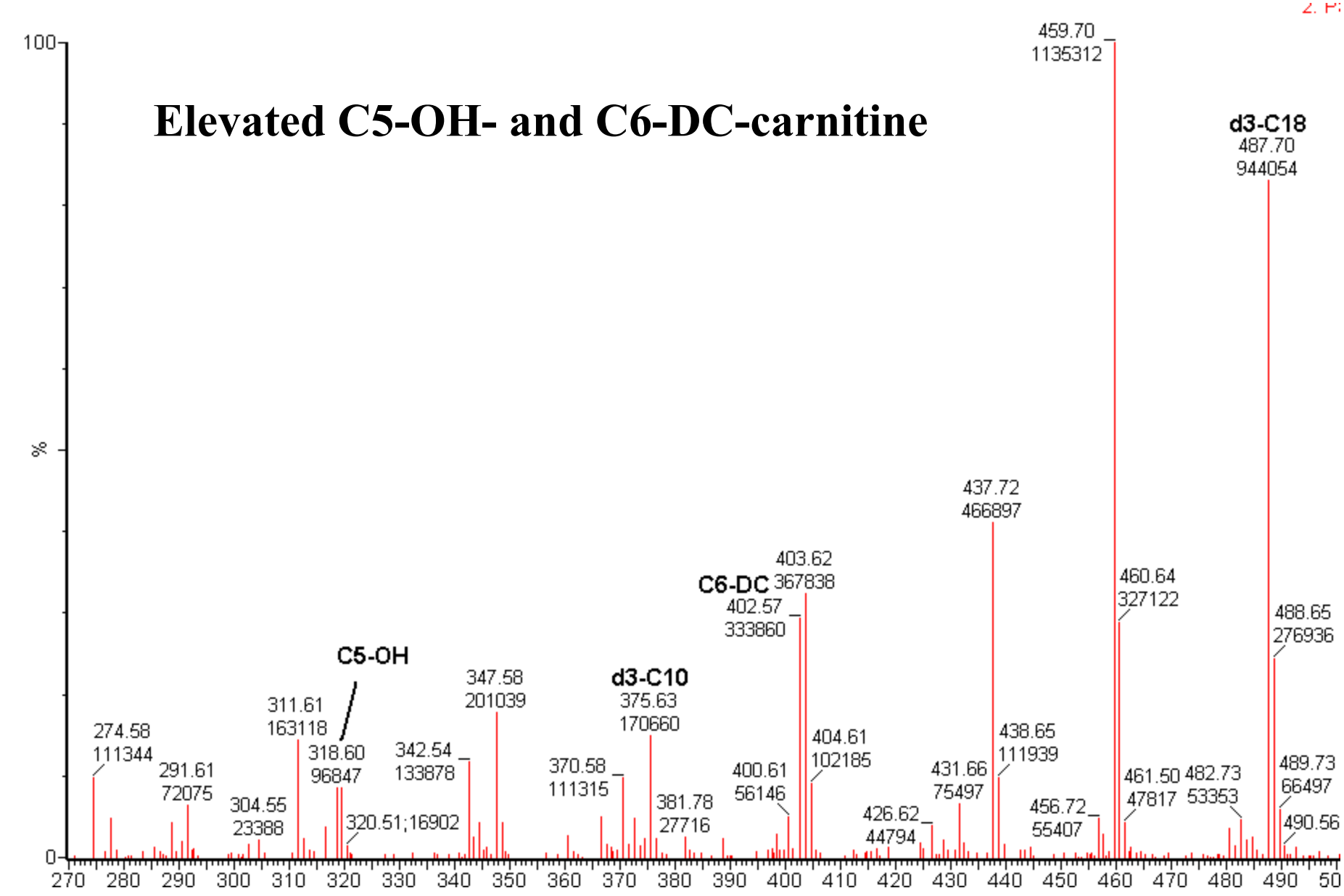
Labs: Hyperammonemia, acidosis, increased anion gap, elevated transaminases, hypoglycemia. Organic acids: Elevated excretion of 3-hydroxy-3-methylglutaric acid, 3-methylglutaconic acid, 3-methylglutaric acid, (3-hydroxyisovaleric acid, 3-methylcrotonylglycine); elevated 3-methylglutaryl (C6-DC) and 3-OH-isovaleryl- (C5OH) carnitine.

Diagnosis: DNA testing: *HMGCL* gene (1pter-p33)

Therapy: Fasting avoidance, carnitine, moderate protein restriction early in life, reduce fat calories to <30%, cornstarch supplements.

Grünert SC, Schlatter SM, Schmitt RN, Gemperle-Britschgi C, Mrázová L, Balcı MC, Bischof F, Çoker M, Das AM, Demirkol M, de Vries M, Gökçay G, Häberle J, Uçar SK, Lotz-Havla AS, Lücke T, Roland D, Rutsch F, Santer R, Schlune A, Staufner C, Schwab KO, Mitchell GA, Sass JO. 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency: Clinical presentation and outcome in a series of 37 patients. Mol Genet Metab. 2017 Jul;121(3):206-215. doi: 10.1016/j.ymgme.2017.05.014. Epub 2017 May 22. PMID: 28583327

3-Hydroxy-3-Methyl-Glutaryl-CoA Lyase deficiency



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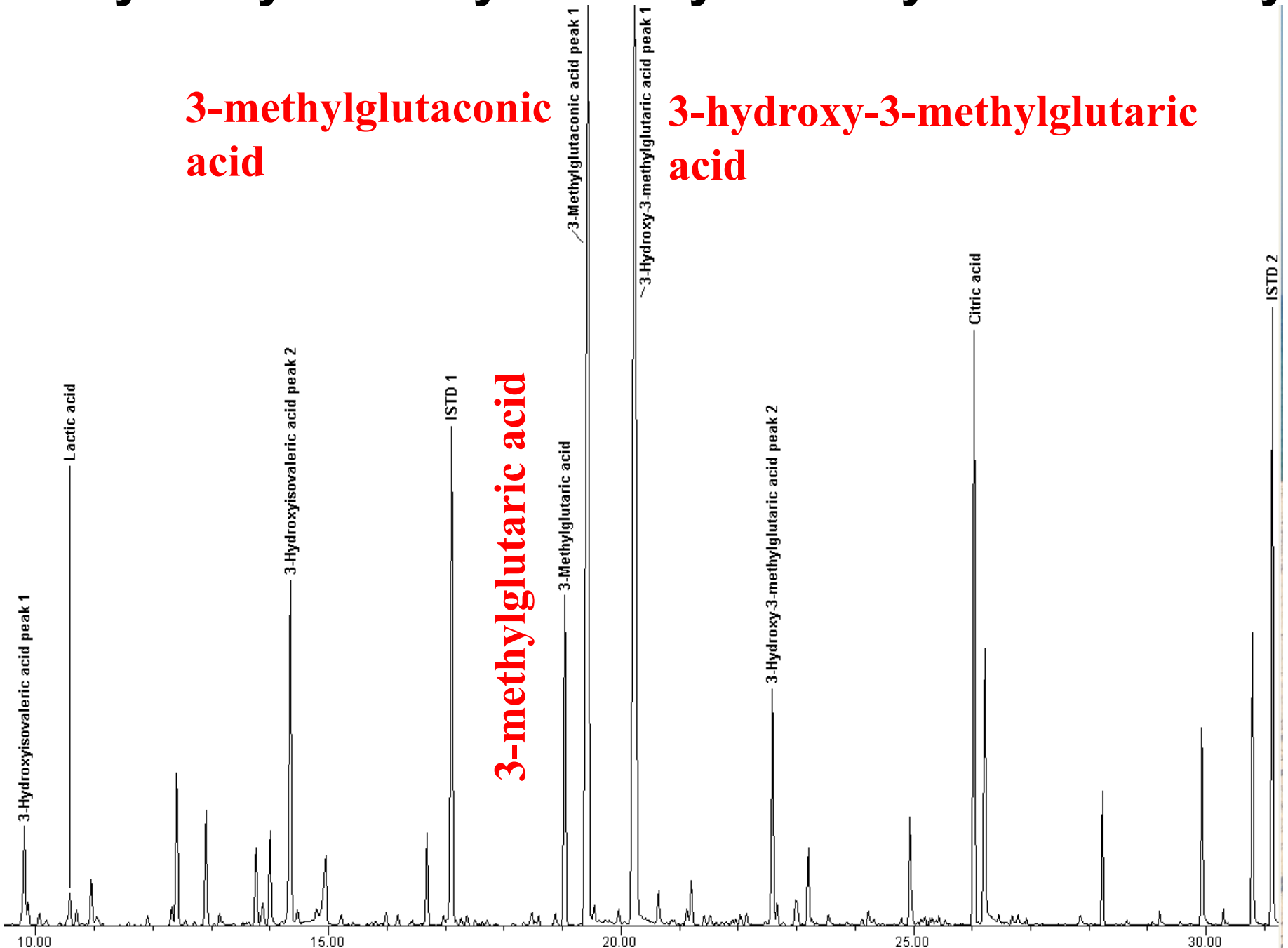
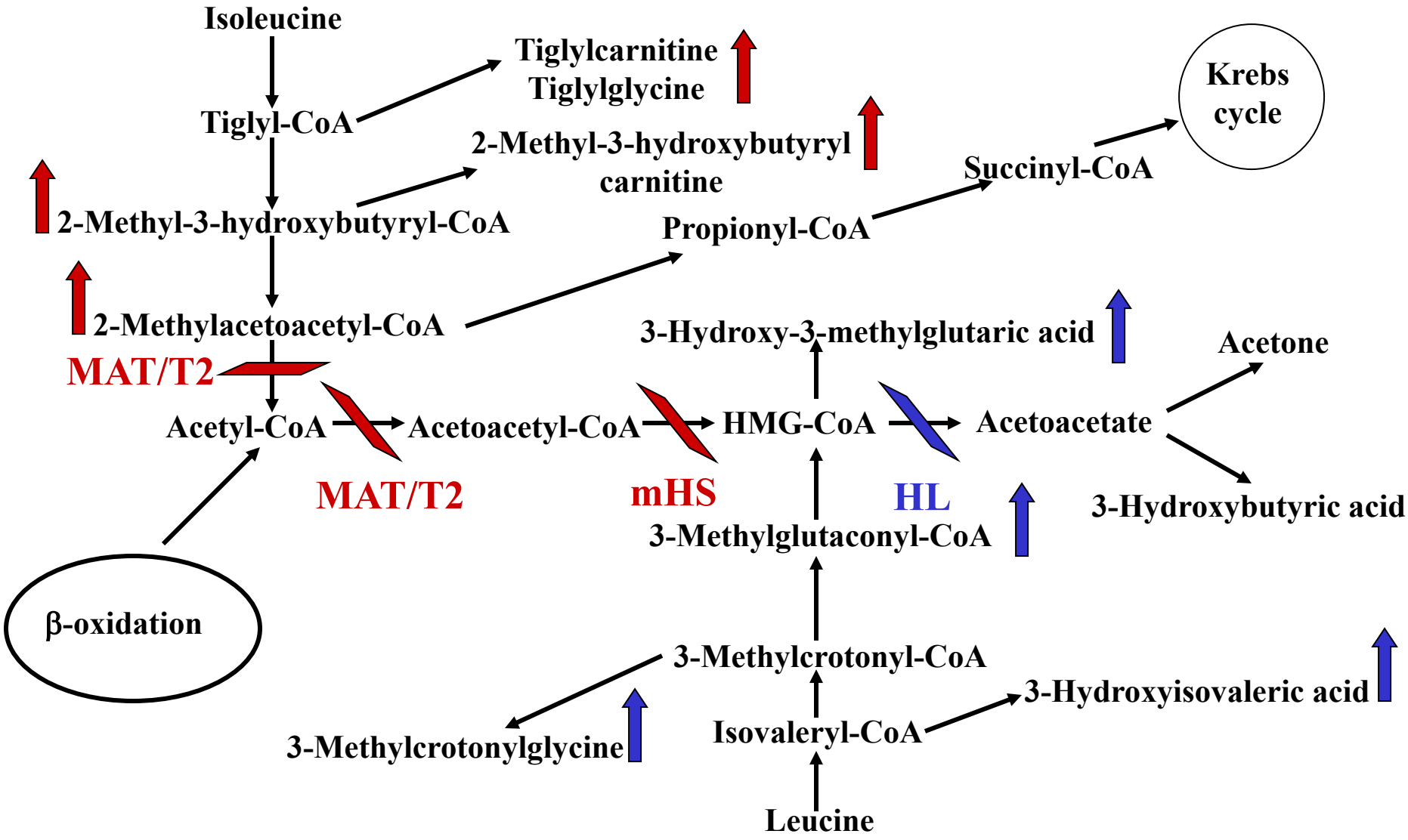
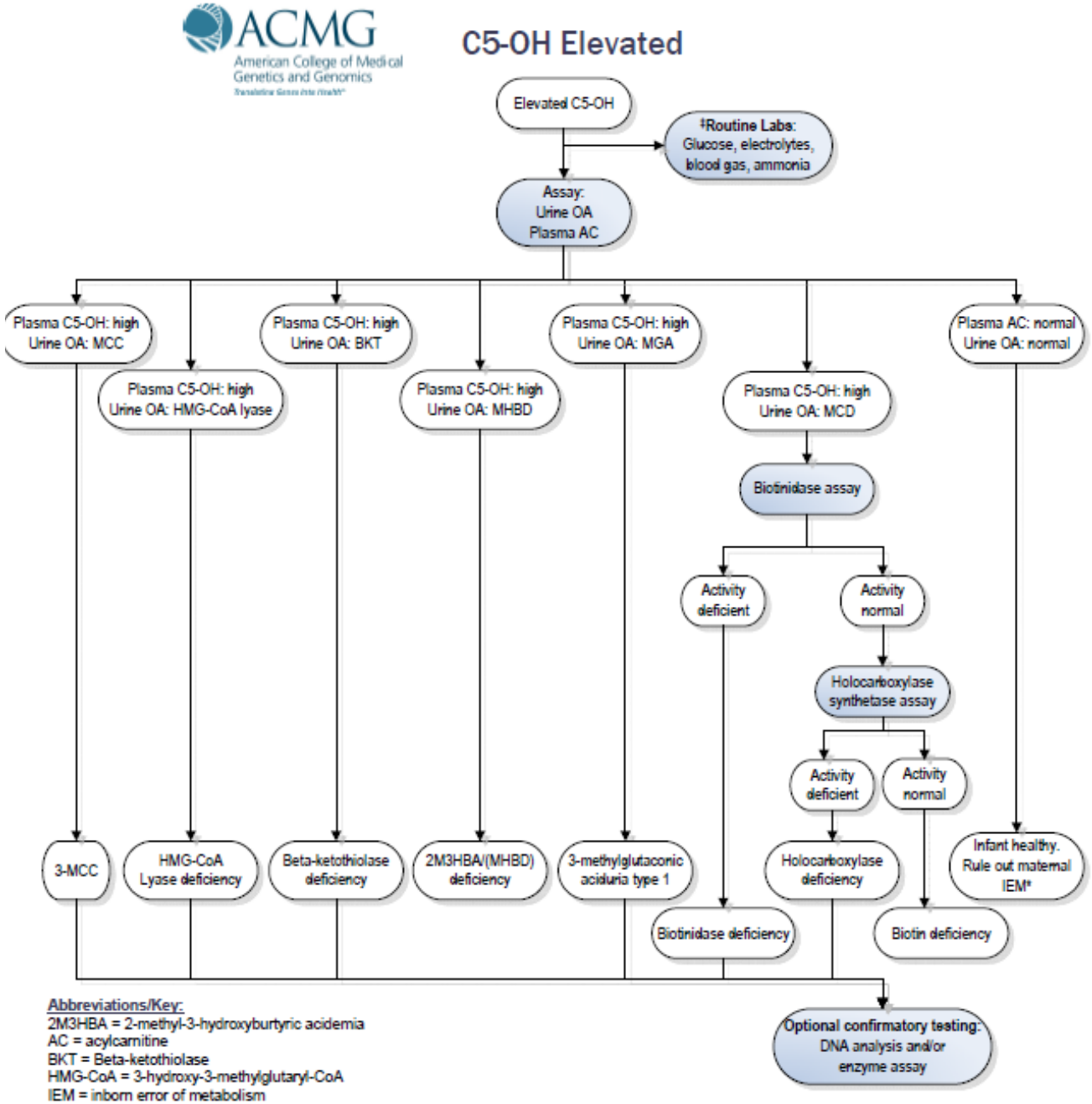


DISORDERS OF KETOGENESIS

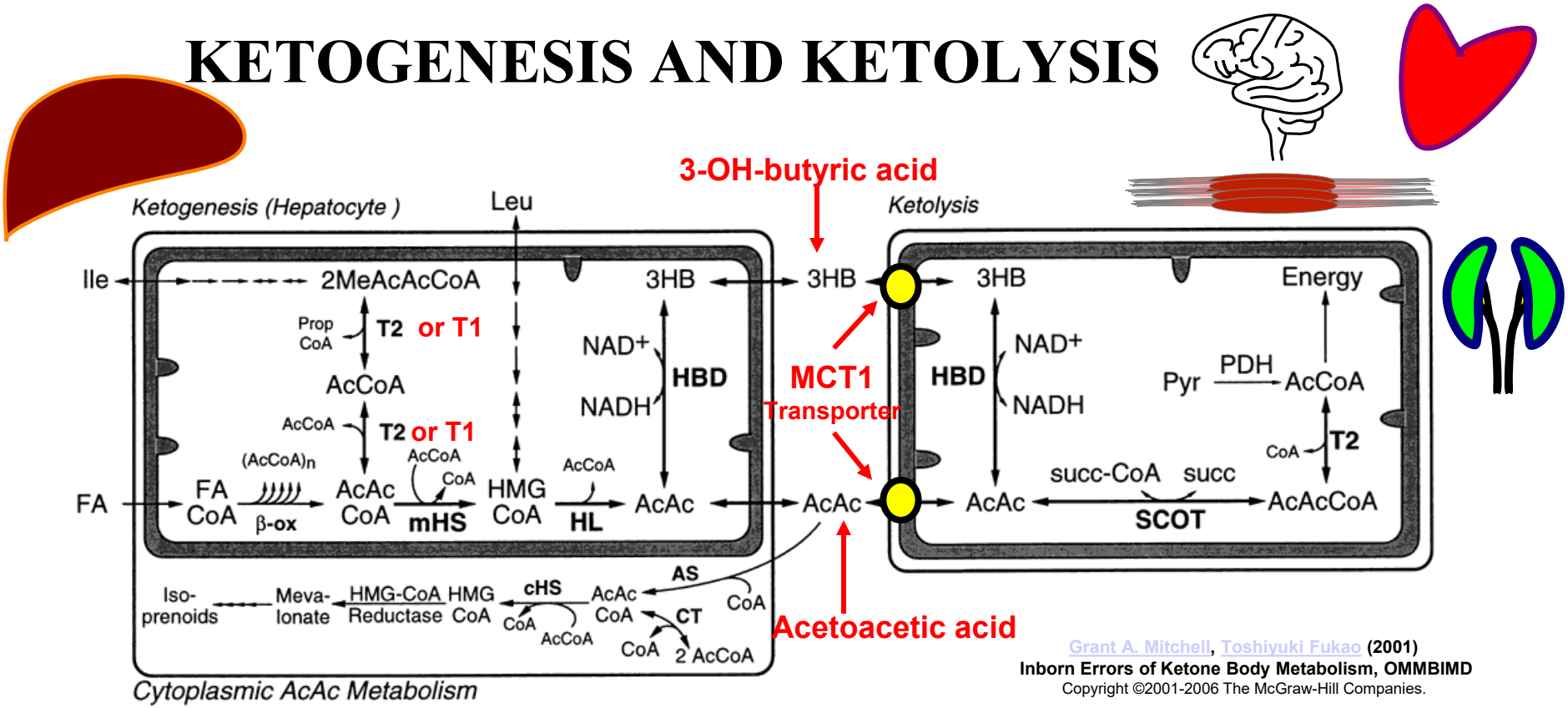




3-methylglutaryl (C6-DC) carnitine can be elevated as well in HMG-CoA Lyase deficiency.

DNA confirmation is necessary.

KETOGENESIS AND KETOLYSIS



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The MCT1 transporter mediates the uptake of ketones by peripheral tissues

HBD: 3-Hydroxy Butyrate Dehydrogenase

T1: *ACAT2*: cytosolic acetoacetyl-CoA thiolase

T2: *ACAT1*: mitochondrial acetoacetyl-CoA thiolase : MAT

MONOCARBOXYLIC TRANSPORTER 1 (MCT1) DEFICIENCY (OMIM 616095)

Frequency: AR, very rare (14 cases to 2022). Some only have one mutation in MCT1: lactate/pyruvate/monocarboxylate H+ transporter. Lack of ketones and lactate affect brain growth and function.

Presentation: episodic, non-physiologic or exaggerated physiologic ketoacidosis: Tachypnea, lethargy, coma, severe ketoacidosis with elevated anion gap. Cyclic vomiting. Psychomotor delay, epilepsy or corpus callosum agenesis. Resolution of metabolic crises after 8 years of age.

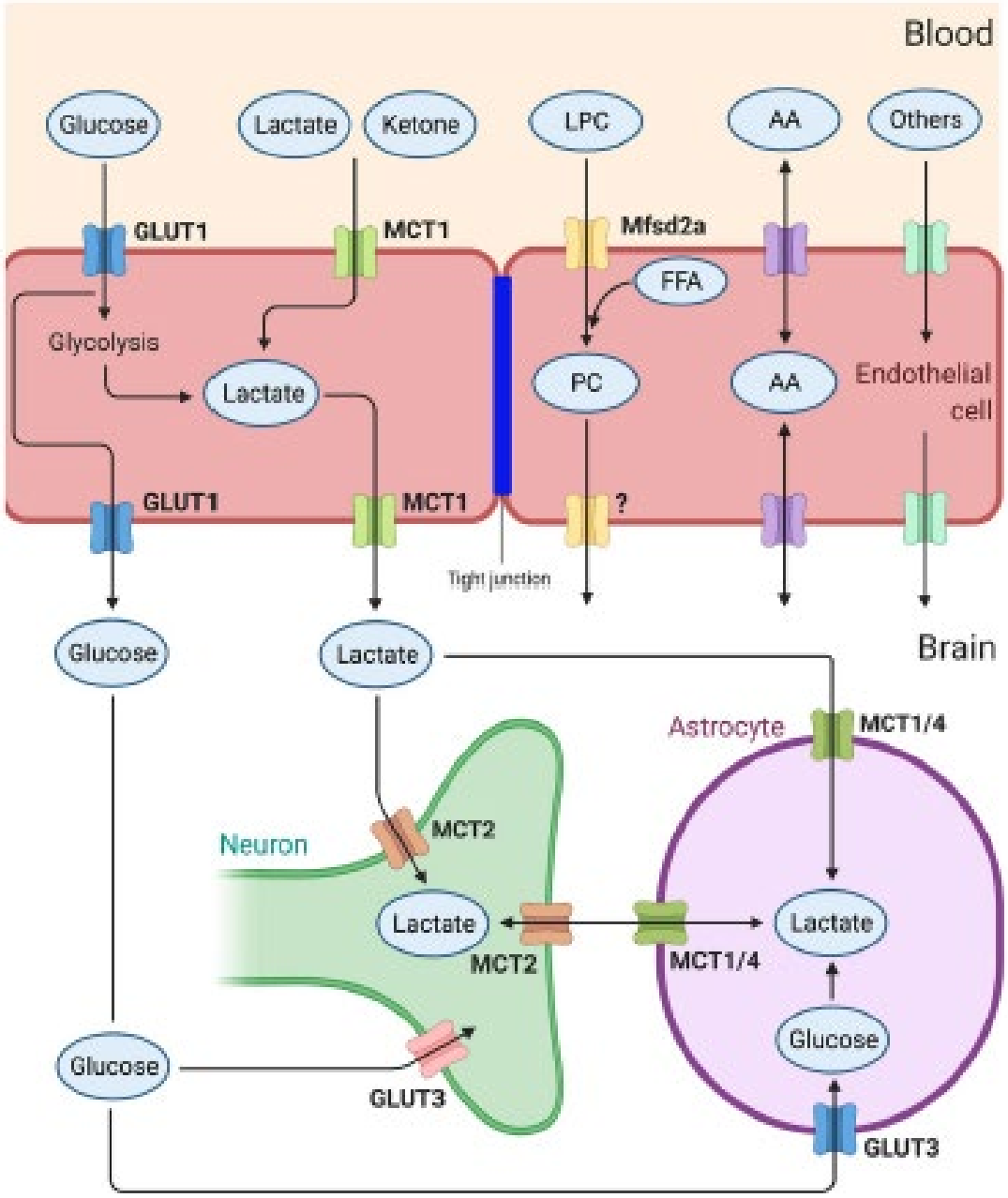
Diagnosis: Urine organic acids: increased Acetoacetate and 3-OH-Butyric acid, without abnormal urine organic acids. It is differentiated from physiological ketosis for the absence of adipic, suberic, and sebacic acids, usually seen during severe physiologic ketosis.

Confirmation: DNA testing *SLC16A1* gene on 1p13.2. Possible milder phenotype in heterozygotes with incomplete penetrance.

Therapy: prevention of fasting, alkali to prevent or reverse acidosis, mild protein and fat restriction, cornstarch, carnitine.

MJ, Duran K, Harakalova M, van der Zwaag B, Monavari AA, Okur I, Sharrard MJ, Cleary M, O'Connell N, Walker V, Rubio-Gozalbo ME, de Vries MC, Visser G, Houwen RH, van der Smagt JJ, Verhoeven-Duif NM, Wanders RJ, van Haaften G. Monocarboxylate transporter 1 deficiency and ketone utilization. van Hasselt PM, Ferdinandusse S, Monroe GR, Ruiter JP, Turkenburg M, Geerlings N Engl J Med. 2014 Nov 13;371(20):1900-7. doi: 10.1056/NEJMoa1407778. PMID: 25390740

Stanescu S, Bravo-Alonso I, Belanger-Quintana A, Pérez B, Medina-Diaz M, Ruiz-Sala P, Flores NP, Buenache R, Arrieta F, Rodríguez-Pombo P. Mitochondrial bioenergetic is impaired in Monocarboxylate transporter 1 deficiency: a new clinical case and review of the literature. Orphanet J Rare Dis. 2022 Jun 21;17(1):243. doi: 10.1186/s13023-022-02389-4. PMID: 35729663; PMCID: PMC9215049.

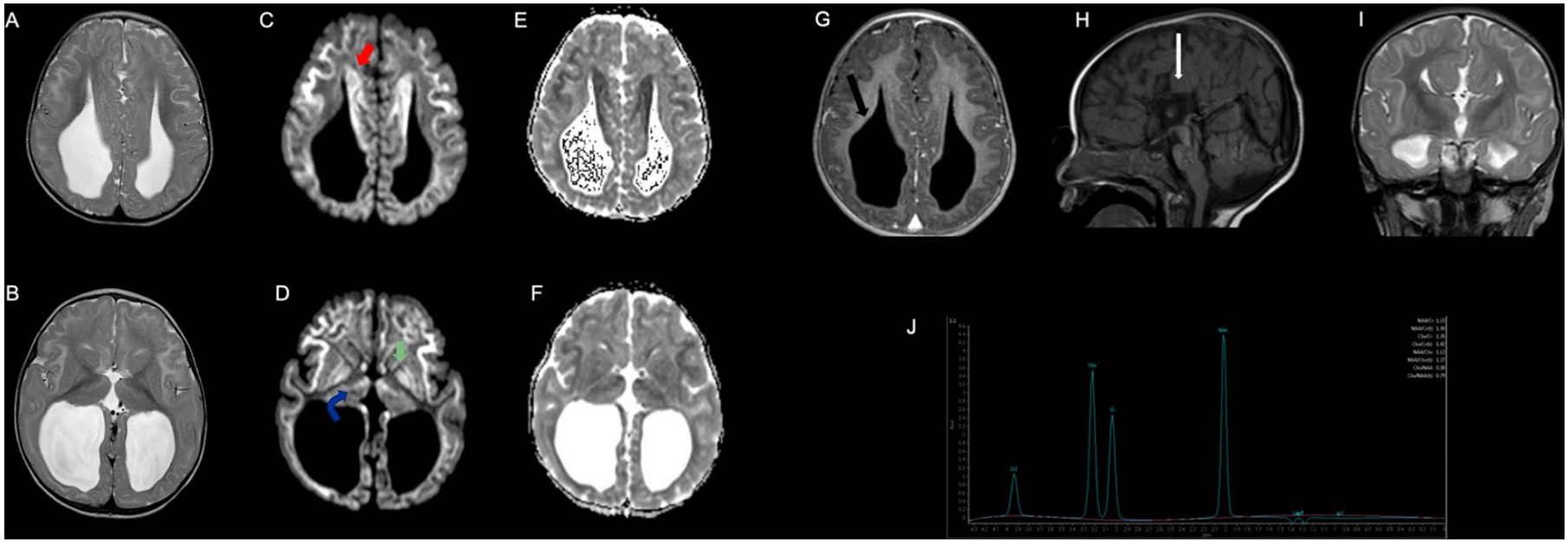


MCT1 is abundant in brain endothelial cells. Essential for the influx of lactate from blood stream into the brain and transfer of lactate to astrocytes and neurons.

Nguyen YTK, Ha HTT, Nguyen TH, Nguyen LN. The role of SLC transporters for brain health and disease. Cell Mol Life Sci. 2021 Dec 31;79(1):20. doi: 10.1007/s00018-021-04074-4. PMID: 34971415.

Lack of MCT1 impairs brain energy supply

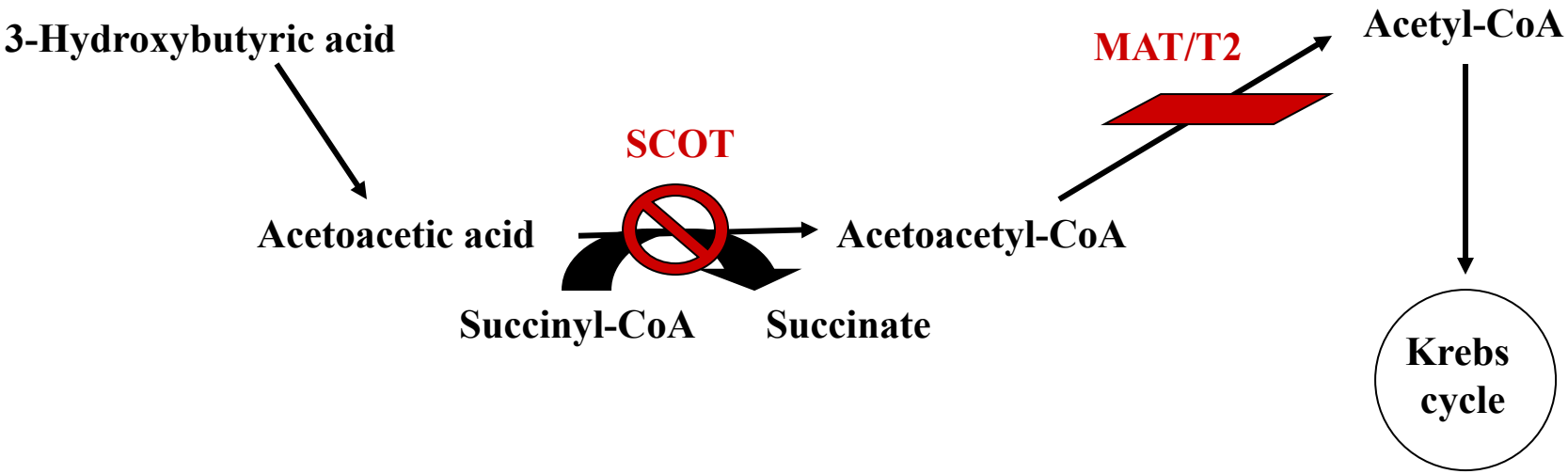
The brain becomes unable to use lactate (derived from glucose) in addition to ketones, with neuronal cell suffering.



Nicolas-Jilwan M, Medlej R, Sulaiman RA, AlSayed M. The neuroimaging findings of monocarboxylate transporter 1 deficiency. Neuroradiology. 2020 Jul;62(7):891-894. doi: 10.1007/s00234-020-02435-7. Epub 2020 Apr 21. PMID: 32318771.

Stanescu S, Bravo-Alonso I, Belanger-Quintana A, Pérez B, Medina-Diaz M, Ruiz-Sala P, Flores NP, Buenache R, Arrieta F, Rodríguez-Pombo P. Mitochondrial bioenergetic is impaired in Monocarboxylate transporter 1 deficiency: a new clinical case and review of the literature. Orphanet J Rare Dis. 2022 Jun 21;17(1):243. doi: 10.1186/s13023-022-02389-4. PMID: 35729663; PMCID: PMC9215049.

SCOT deficiency OMIM 245050



SCOT, Succinyl-CoA:3-ketoacid-CoA transferase (OMIM 245050) catalyzes the reversible rate-limiting step of ketolysis.

Cause: mutations in *OXCT* gene (5p12-p13).

OXCT gene not expressed in liver.

Frequency: very rare

SCOT deficiency OMIM 245050

Presentation: episodic, non-physiologic or exaggerated physiologic ketoacidosis: Tachypnea, lethargy, coma, severe ketoacidosis with elevated anion gap, persistent ketonemia/ketonuria even when stable or post-prandially, no diagnostic metabolites in urine or plasma. Ketones are present in fed state.

Diagnosis: Urine organic acids: increased Acetoacetate and 3-OH-Butyric acid, without other abnormal urine organic acids. It is differentiated from physiological ketosis for the absence of adipic, suberic, and sebacic acids, usually seen during severe physiologic ketosis.

Confirmation: DNA testing *OXCT1* gene on 5p13.

Therapy: prevention of fasting, alkali to prevent acidosis, mild protein and fat restriction, cornstarch, carnitine.

Alghamdi MA, Tohary M, Alzaidan H, Imtiaz F, Al-Hassnan ZN. Clinical variability and outcome of succinyl-CoA:3-ketoacid CoA transferase deficiency caused by a single OXCT1 mutation: Report of 17 cases. JIMD Rep. 2021 Sep 14;62(1):91-96. doi: 10.1002/jmd2.12248. PMID: 34765403; PMCID: PMC8574173.

METABOLIC ACIDOSIS

- **Hispanic female, the first child of first cousin parents. Born prematurely with birth weight of 1.96 kg. Hospitalized for the first two months to achieve normal birth weight and for unspecified respiratory problems.**
- **At 8 months of age she had tachypnea, vomiting and lethargy following fever (39C). Severe metabolic acidosis with pH of 6.98, low CO2 (<5 mEq/L), an elevated anion gap (22-27 mEq/L), and hypokalemia (1.4-2 mEq/L). Glucose and ammonia were normal. Urine ketones were strongly positive.**
- **Acidosis was corrected by intravenous bicarbonate and peritoneal dialysis was initiated. Acidosis reappeared when dialysis was discontinued, for which she was kept on a regimen of daily dialysis.**
- **At 15 months of age, her growth and development were only mildly delayed. Hypoglycemia (glucose 1.22 mmol/L – 22 mg/dL) after overnight fasting but not during daytime was noted, with hypokalemia (2.5 mEq/L), normal bicarbonate and elevated anion gap (23.5 mEq/L). Urinary organic acid analysis showed excess ketone bodies without dicarboxylic aciduria or other abnormal metabolites.**

LABORATORY FINDINGS

URINE ORGANIC ACIDS

ABNORMAL: Severe ketonuria suggesting severe catabolic state. No abnormal organic acids identified.

Organic acid quantitation in mmol/mol creatinine:

Analyte	Result	1 mo-12 yrs
Lactic acid	676	<370
Pyruvic acid	22	<34
Succinic acid	81	<80
Fumaric acid	31	<10
2-Ketoglutaric	180	<150
3-OH-butyric acid	10,563	<4
Acetoacetic acid	17,704	<4
2-Keto-3-methylvaleric	26	<10
2-Keto-isocaproic	9	<4
Ethylmalonic acid	8	<15
Adipic acid	23	<100
Suberic acid	14	<10
Sebacic acid	0	<3
4-OH-phenylacetic acid	81	<100
4-OH-phenylpyruvic acid	8	<2

SICK $CO_2 = 5$

URINE ORGANIC ACIDS

ABNORMAL: Severe ketonuria. Abnormal products of fatty acid oxidation are not present in this sample.

Organic acid quantitation in mmol/mol creatinine:

Analyte	Result	1 mo-12 yrs
Lactic acid	349	<370
Pyruvic acid	83	<34
Succinic acid	117	<80
Fumaric acid	33	<10
2-Ketoglutaric	577	<150
3-OH-butyric acid	6,380	<4
Acetoacetic acid	6,192	<4
2-Keto-3-methylvaleric	23	<10
2-Keto-isocaproic	8	<4
Ethylmalonic acid	21	<15
Adipic acid	28	<100
Suberic acid	11	<10
Sebacic acid	9	<3
4-OH-phenylacetic acid	216	<100
4-OH-phenylpyruvic acid	8	<2

WELL $CO_2 = 27$

Normal plasma and urine amino acids

Plasma carnitine: excess acylcarnitines while on supplements.

LABORATORY FINDINGS

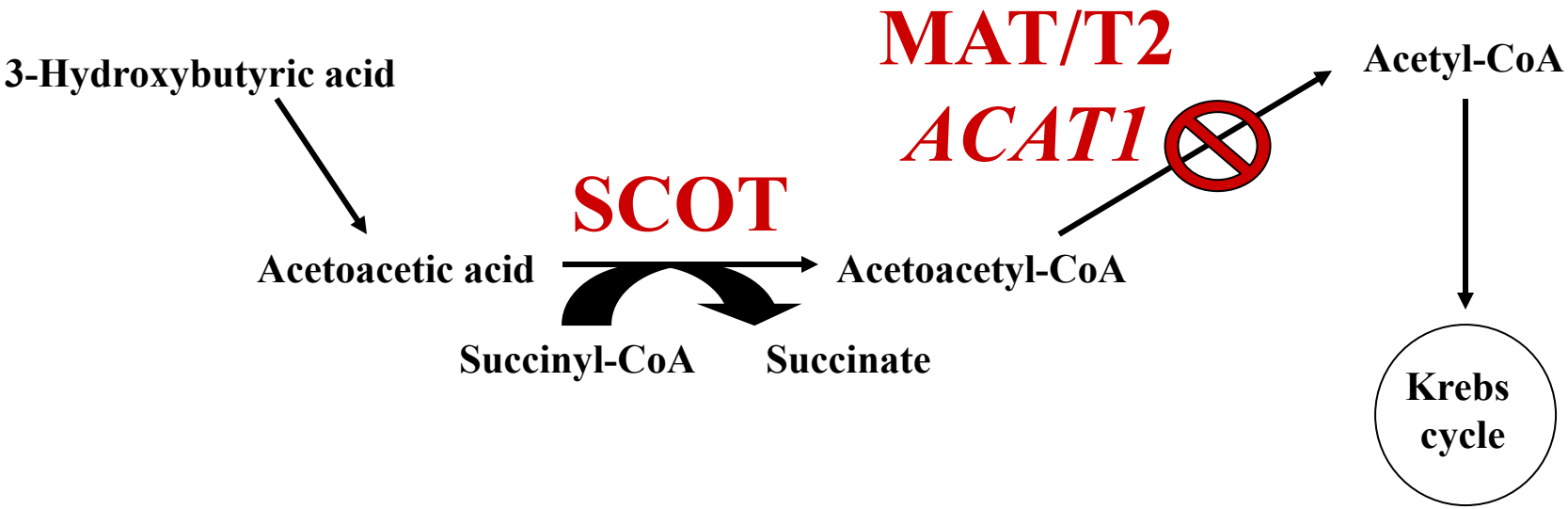
- **Ketolytic enzymes, Fibroblasts:**

Enzyme	Activity	Ref. range	Units
Beta-Ketothiolase	8.4	(5.6-15.9)	nmol/min/mg protein
Succinyl-CoA 3-ketotransferase	0.0	(4.1-8.1)	nmol/min/mg protein

- **Interpretation: SCOT deficiency.**
- **DNA *OXCT* gene: homozygous c.649C>T; p.R217X.**

Longo N, Fukao T, Singh R, Pasquali M, Barrios RG, Kondo N, Gibson KM. Succinyl-CoA:3-ketoacid transferase (SCOT) deficiency in a new patient homozygous for an R217X mutation. J Inherit Metab Dis. 2004;27(5):691-2. doi: 10.1023/b:boli.0000043023.57321.18. PMID: 15669687.

BETA KETOTHIOLASE DEFICIENCY



Mitochondrial acetoacetyl-CoA thiolase, MAT/T2 (OMIM 203750): has a ketolytic role (converts acetoacetyl-CoA and CoA in two molecules of acetyl-CoA) and a ketogenic role (converts 2-methylacetoacetyl-CoA and CoA in acetyl-CoA and propionyl-CoA).

Presentation: ketoacidosis, therefore the ketolytic process is more dependent upon adequate function of MAT/T2 (ACAT1): CAT/T1 (ACAT2) might bypass the defect in ketone body synthesis.

METABOLIC ACIDOSIS

3-Year-old male with a 24-hour history of vomiting, lethargy. In the ER, he had a blood glucose of 15 with 3+ ketones in the urine, metabolic acidosis (pH 6.8), bicarbonate <5, and BMP glucose of 7. Head CT was normal. Described as poor eater, very active in his sleep. No previous hospitalizations or surgeries. Has speech delay.

		Units	07/30/07	05/22/07
Na	137-146	mmol/L	139	144
K	3.4-4.7	mmol/L	3.9	4.4
Cl	98-109	mmol/L	106	120 H
CO2	18-24	mmol/L	24	* <5 L
Anion Gap	3-16	mmol/L	9	19 H
Glucose	60-115	mg/dL	91	95
BUN	5-17	mg/dL	12	32 H
Creatinine	0.3-0.7	mg/dL	0.4	0.6
Ca	8.7-9.8	mg/dL	9.4	7.8 L
Prot	5.9-7.0	g/dL	7.5 H	6.1
Alb	3.1-3.9	g/dL	4.7 H	3.6
Bili, Total	0.2-1.3	mg/dL	0.2	<0.1 L
Alk Phos	145-320	U/L	200	235
ALT	5-45	U/L	16	52 H
AST	20-60	U/L	55	69 H
Ammonia	21-50	umol/L	21	* 54 H

METABOLIC ACIDOSIS

Ketolytic enzymes, Fibroblasts:

Enzyme	Activity	Ref. range	Units
Beta-Ketothiolase	**10.3**	**(8.9-20.6)**	**nmol/min/mg protein**
Succinyl-CoA 3-ketotransferase	7.5	(2.6-8.6)	nmol/min/mg protein

- Interpretation: Beta-ketothiolase activity was in the low normal range, but not stimulated by potassium (normally K doubles enzyme activity).
- DNA *ACAT1* gene: c.T99A, p.Y33X; c.T155C, p.I52T
- Treatment: fasting avoidance, cornstarch and carnitine supplements.

BETA KETOTHIOLASE DEFICIENCY

Mitochondrial acetoacetyl-CoA thiolase deficiency

Presentation: intermittent ketoacidotic episodes during intercurrent illnesses, triggered by vomiting, fever.

Labs: Two groups of patients:

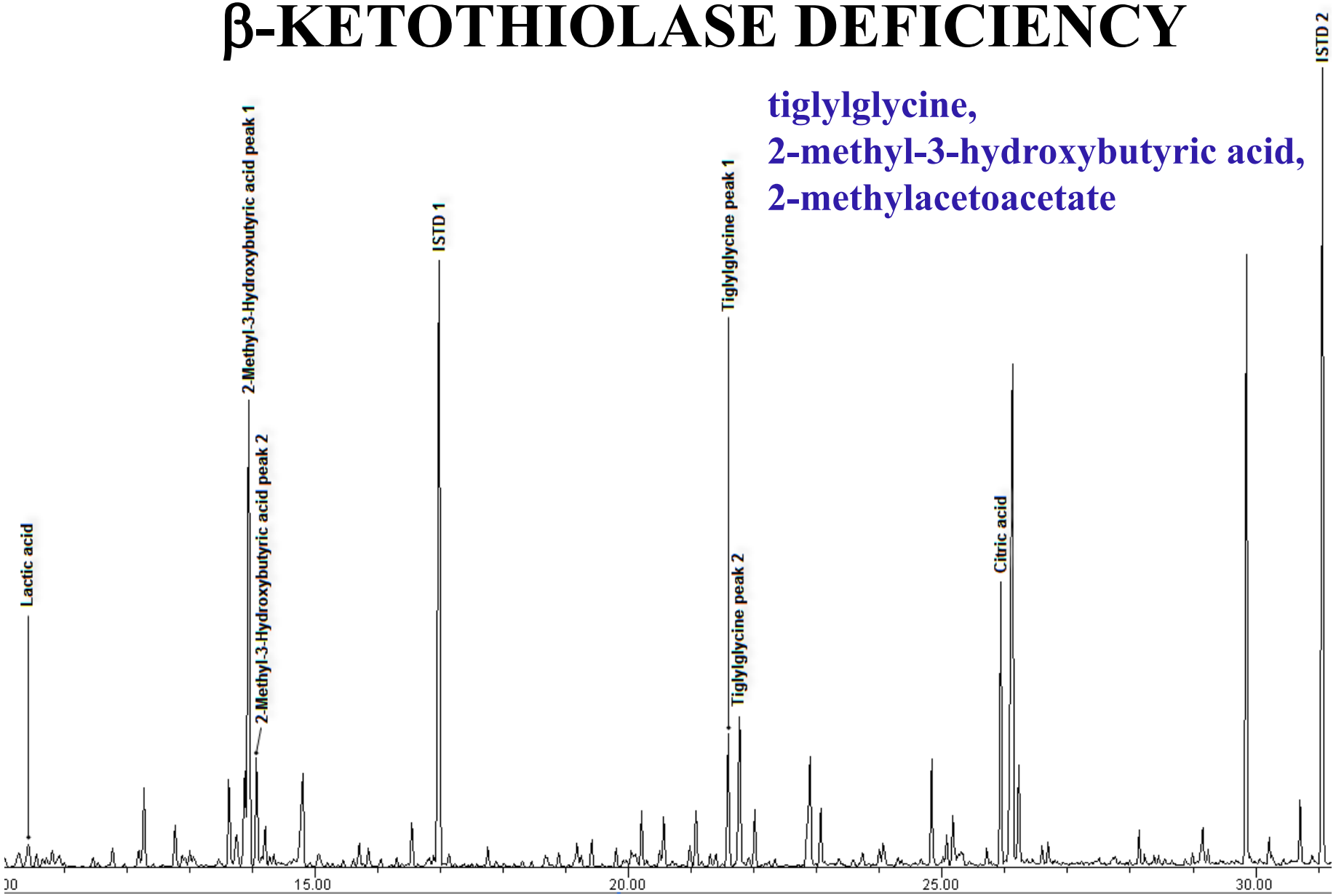
Group 1: no residual enzyme activity; urine organic acids ALWAYS show elevated tiglylglycine, 2-methyl-3-hydroxybutyric acid, 2-methylacetoacetate (unstable, rarely seen) with or without ketoacidosis; elevated tiglylcarnitine (C5:1) and 2-methyl-3-hydroxybutyrylcarnitine (C5OH).

Group 2: some residual enzyme activity; urine organic acids may be normal when stable; elevated tiglylcarnitine (C5:1) and 2-methyl-3-hydroxybutyrylcarnitine (C5OH). Newborn screening (and even acylcarnitine profile in plasma) can miss these patients .

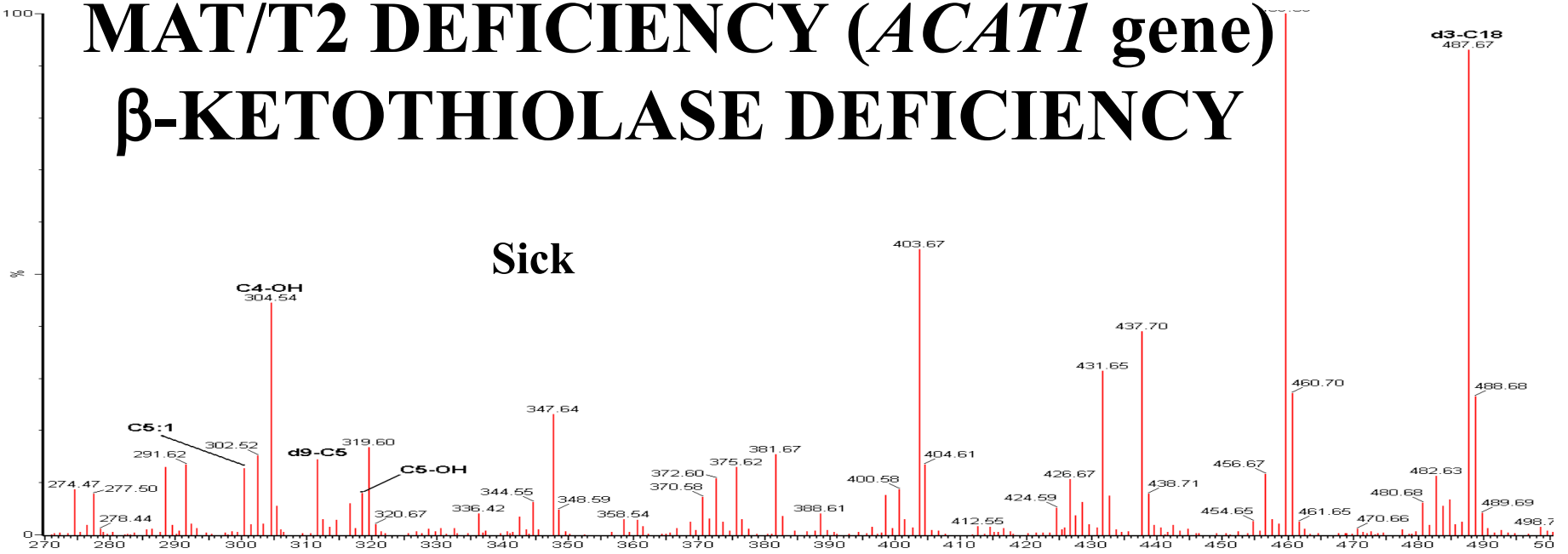
Diagnosis: DNA testing *ACAT1* gene (11q22.3-q23.1), enzyme assay

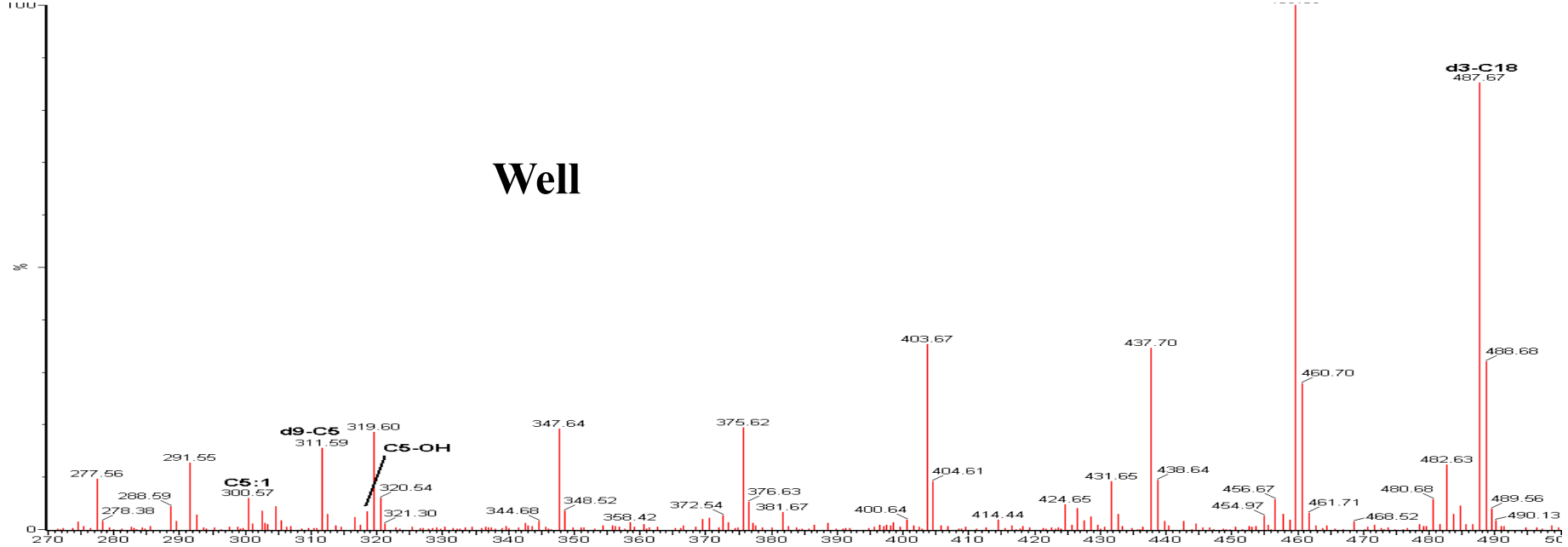
Therapy: Fasting avoidance, cornstarch, carnitine

MAT/T2 DEFICIENCY (*ACAT1* gene)
β-KETOTHIOLASE DEFICIENCY

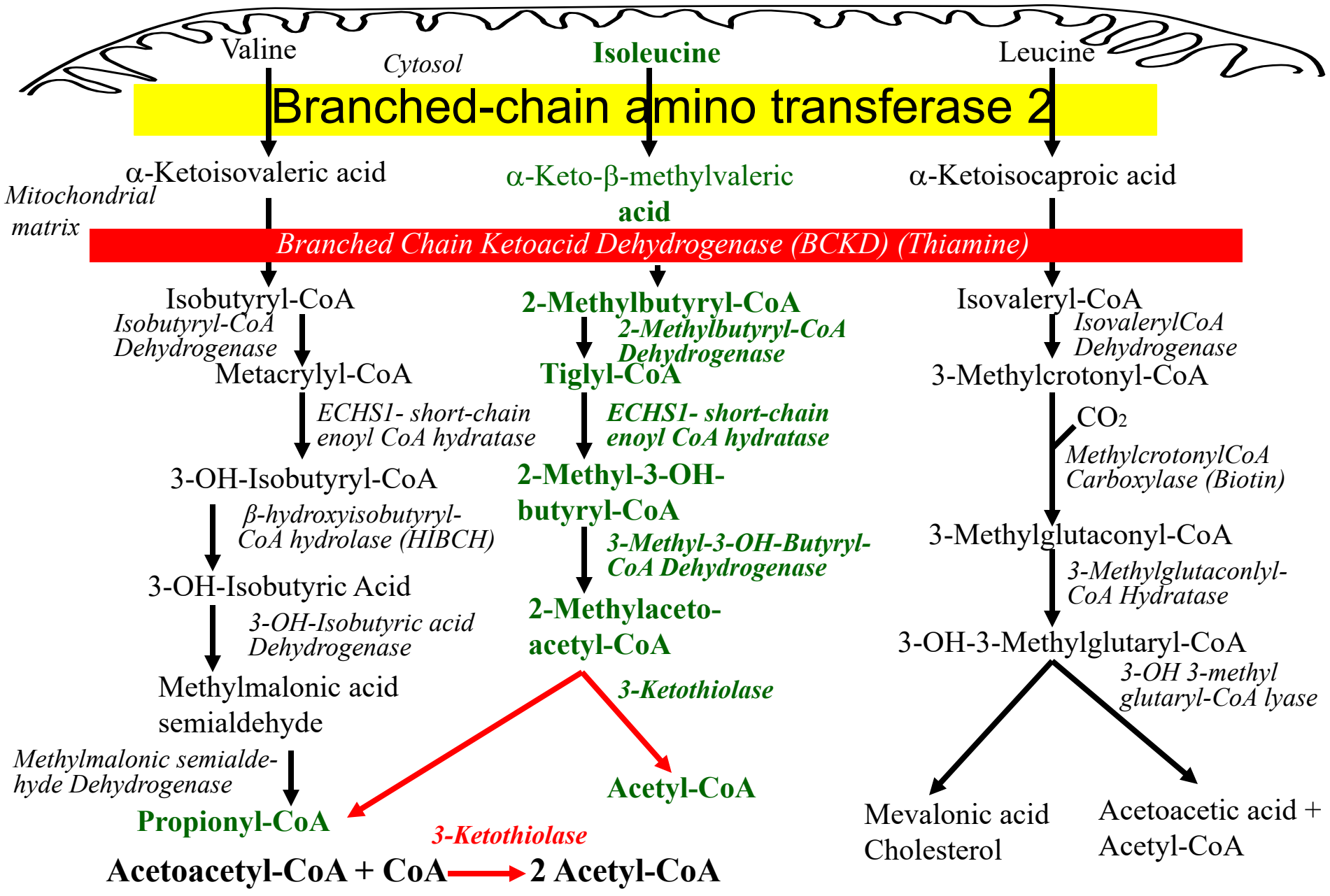


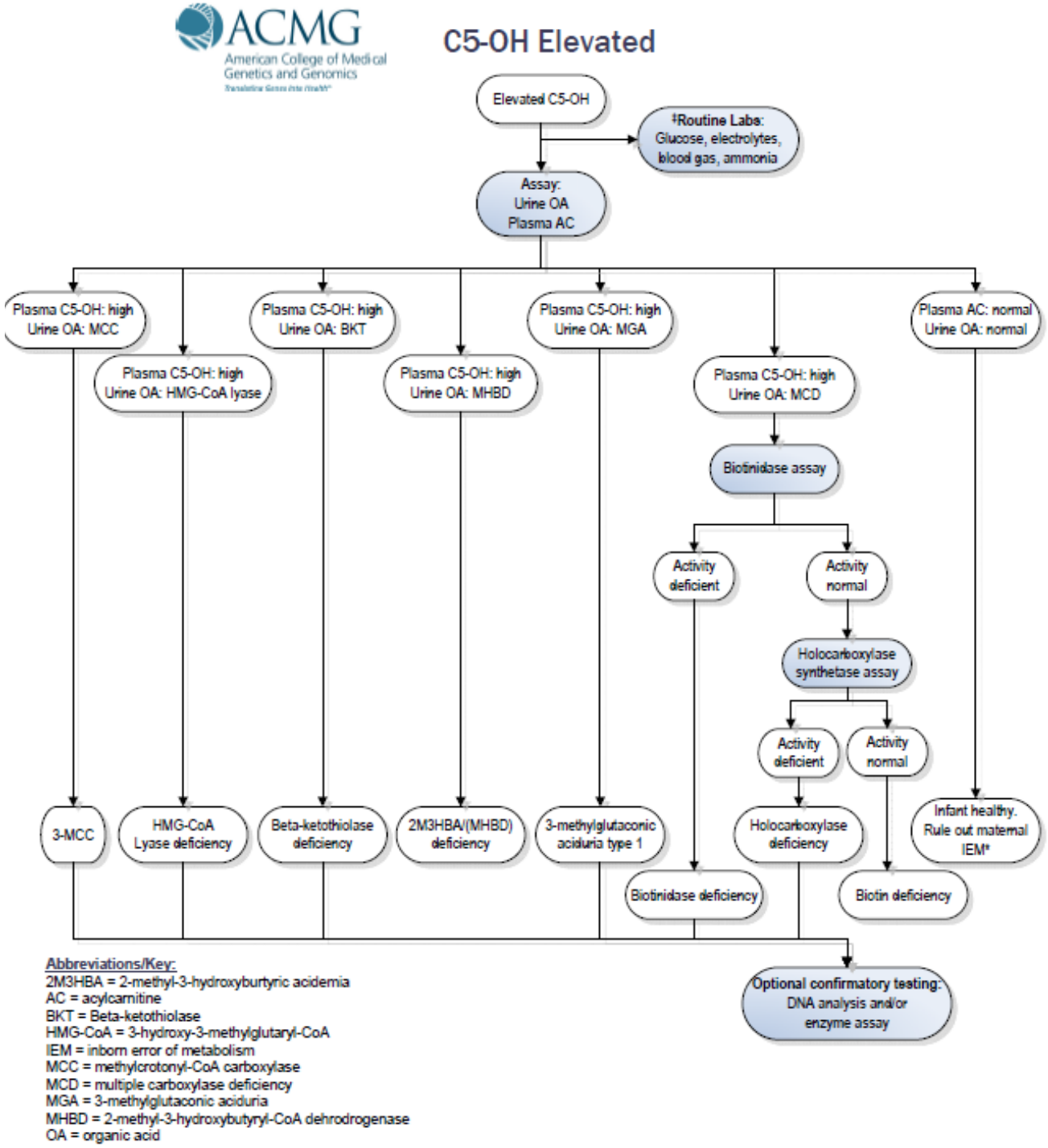
MAT/T2 DEFICIENCY (*ACAT1* gene)
β-KETOTHIOLASE DEFICIENCY





3-KETOTHIOLASE DEFICIENCY





C5-OH can be elevated, but we have seen combination of elevations of different species (C5:1, C4-OH) one at a time

SUMMARY

- **Fatty acids oxidation and ketogenic amino acids produce ketones (liver) that can be used by the body to produce energy.**
- **Disorders of ketogenesis (Mitochondrial 3-Hydroxy-3-Methyl-Glutaryl-CoA Synthase (mHs) and lyase (HL) deficiency) present as fatty acid oxidations defects with hypoketotic hypoglycemia.**
- **Disorders of ketolysis (MCT1, SCOT and MAT/T2 (*ACAT1*) deficiency) present with acute metabolic acidosis during fasting.**
- **Urine organic acids and plasma acylcarnitine profile can identify abnormal metabolites in HL and MAT/T2 deficiency. No diagnostic metabolites might be seen in mHS, SCOT and MCT1 deficiency. All require DNA studies for diagnosis.**

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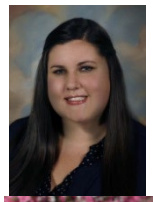



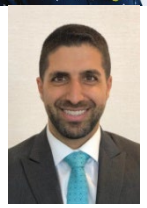
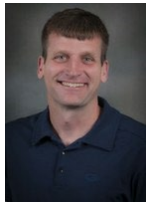
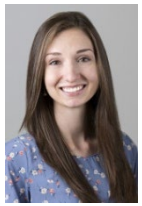
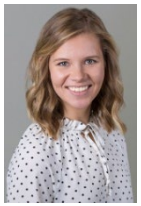

ARUP Laboratories

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Co-Author of all slides

All patients and their families.