Diagnostic approaches to rare genetic disorders

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Outline

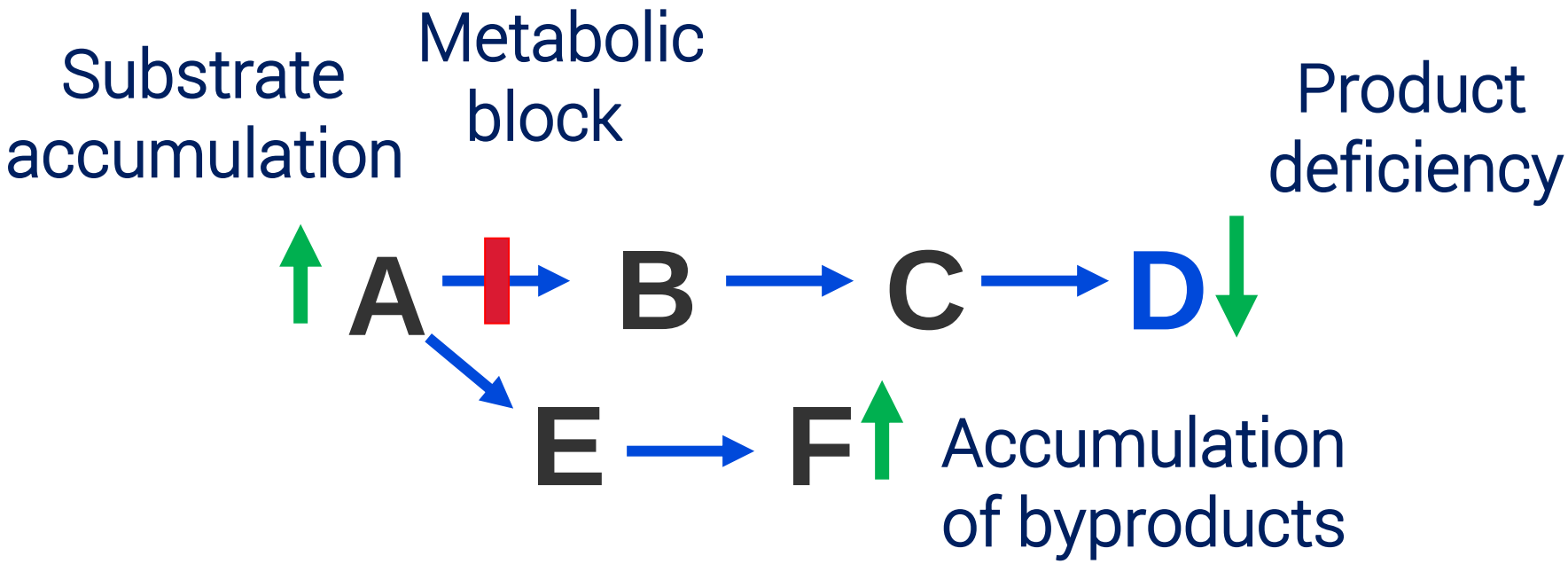
- Inborn errors of metabolism as examples of rare genetic disorders
- Diagnosis and treatment of inborn errors of metabolism
- Diagnostic approaches for inborn errors of metabolism

Inborn Errors of Metabolism (IEMs)

Clinical characteristics, diagnosis, treatment

Metabolism

- Metabolism: Transformation of energy and matter through a metabolic pathway
- Metabolic pathway: series of reactions catalyzed by different enzymes.



Inborn Errors of Metabolism (IEMs)

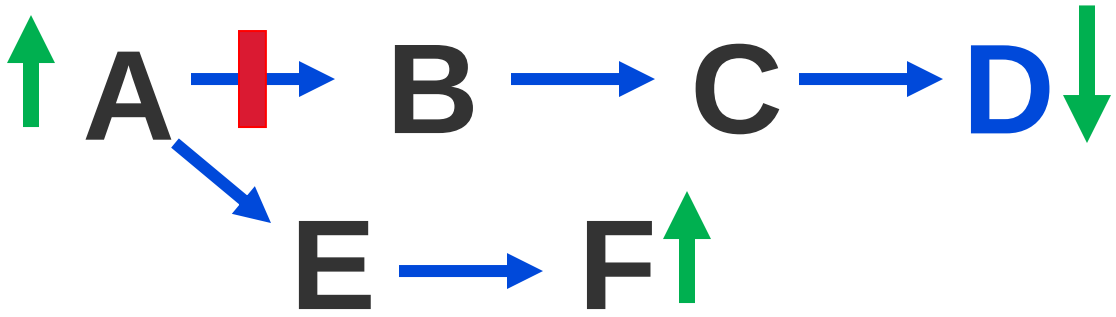
- IEMs affect the transformation of nutrients to obtain energy and growing.
- 3 Broad categories of IEMs:
  - » Intoxication: the metabolites accumulating in the body produce a toxic effect on different organs.
  - » Energy deficiency: symptoms are due to impaired energy production.
  - » Disorders of complex macromolecules.
- Individually, IEMs are rare, but their cumulative frequency is significant (>1:2,000 livebirths).

IEMs: Clinical presentation

- Symptom-free interval followed by signs of acute (vomiting, coma, liver failure) or chronic (failure to thrive, developmental delays, cardiomyopathy) intoxication.
- Intercurrent metabolic crises triggered by fever, catabolism, etc.
- Neurological/skeletal/liver/spleen abnormalities (storage disorders) with progressive symptoms, independent from external triggers.
- Hypoglycemia, lactic acidosis, hepatomegaly, hypotonia, failure to thrive, cardiomyopathy, myopathy.

IEMs: Treatment

- Dietary treatment:
  - » Restriction of toxic substrates
  - » Provision of products



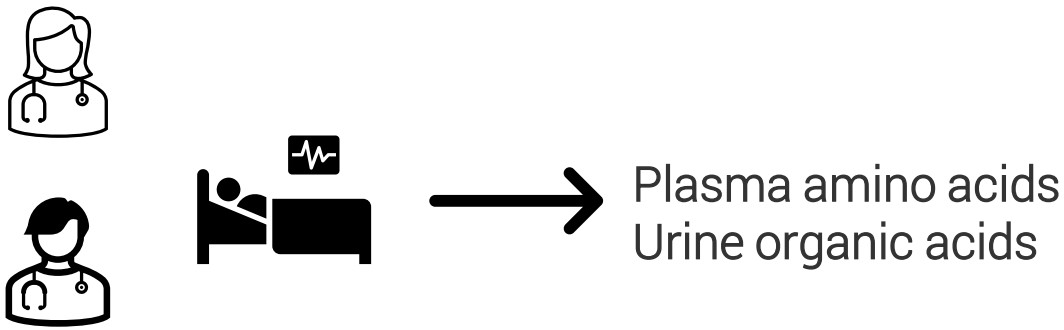
- Pharmacologic treatment:
  - » Inhibitors of formation of toxic products
  - » Drugs to bypass/reduce the effect of the metabolic block
  - » Vitamins
- Enzyme Replacement Therapy
- Organ transplantation
- Experimental therapy (gene therapy, mRNA therapy)

IEMs: Diagnosis

- Specialized “routine” tests to identify IEMs are the first example of clinical metabolomics, targeted and untargeted:
  - » Amino acids analysis (plasma, urine, CSF)
  - » Organic acids analysis (urine)
  - » Carnitine and acylcarnitine analysis (plasma, urine)
  - » Multiple tests targeted to specific conditions
- Enzyme/transporter assays: functional tests to confirm diagnosis
- Molecular tests

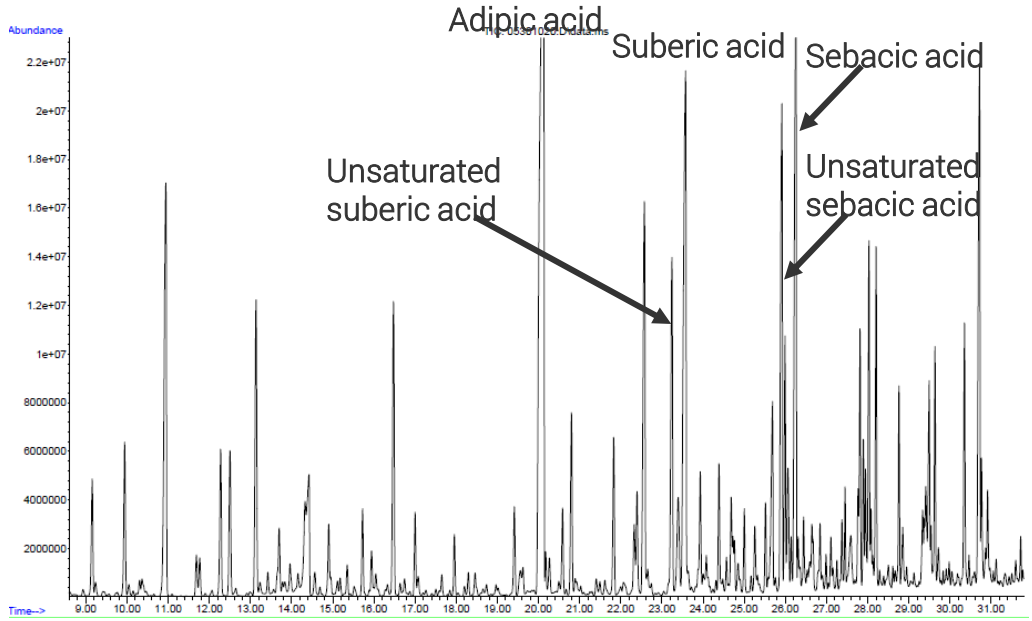
Clinical case #1: Sequential tests to reach the diagnosis

- Term infant developed hypothermia, low blood pressure, and severe hypoglycemia at 18 hours of age. He was intubated, then developed tachy- and bradycardia. On cardiac echo, cardiomyopathy was detected.
- He suffered cardiac arrest requiring chest compressions.
- He developed mild hyperammonemia and had increased liver function tests.
- He stabilized on IV glucose.



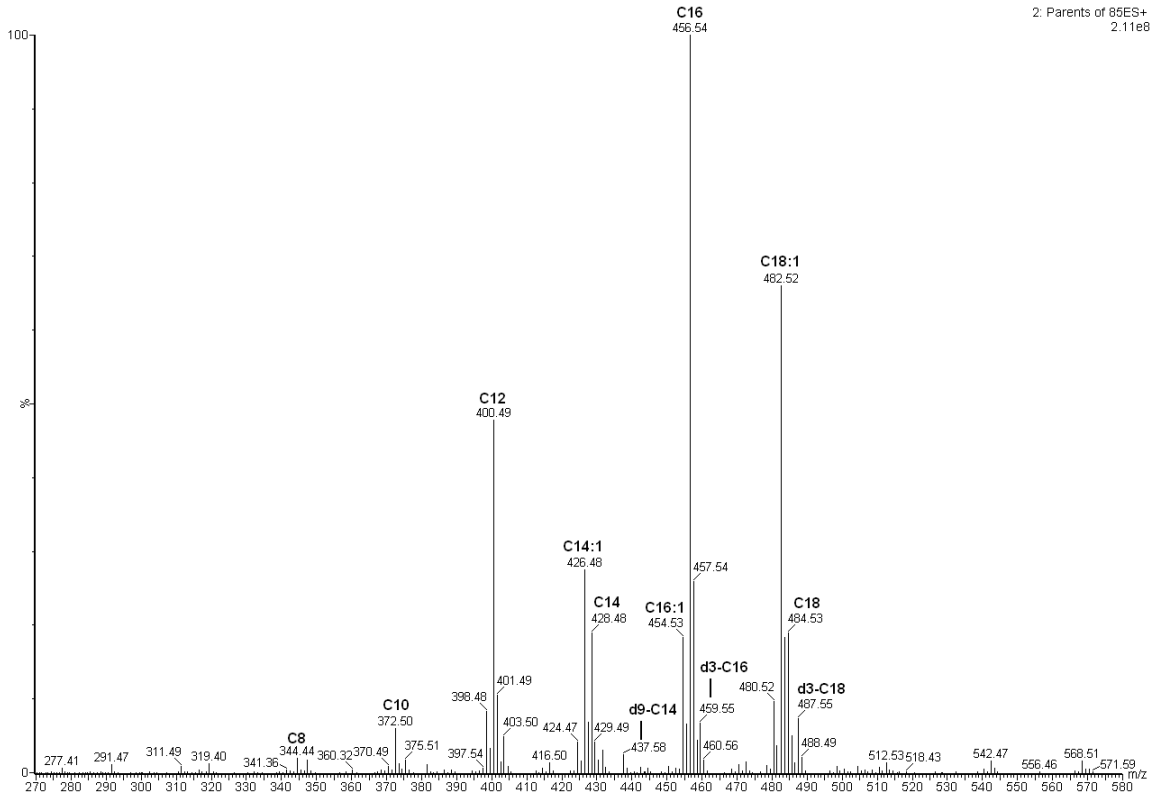
Clinical case #1: Sequential tests to reach the diagnosis

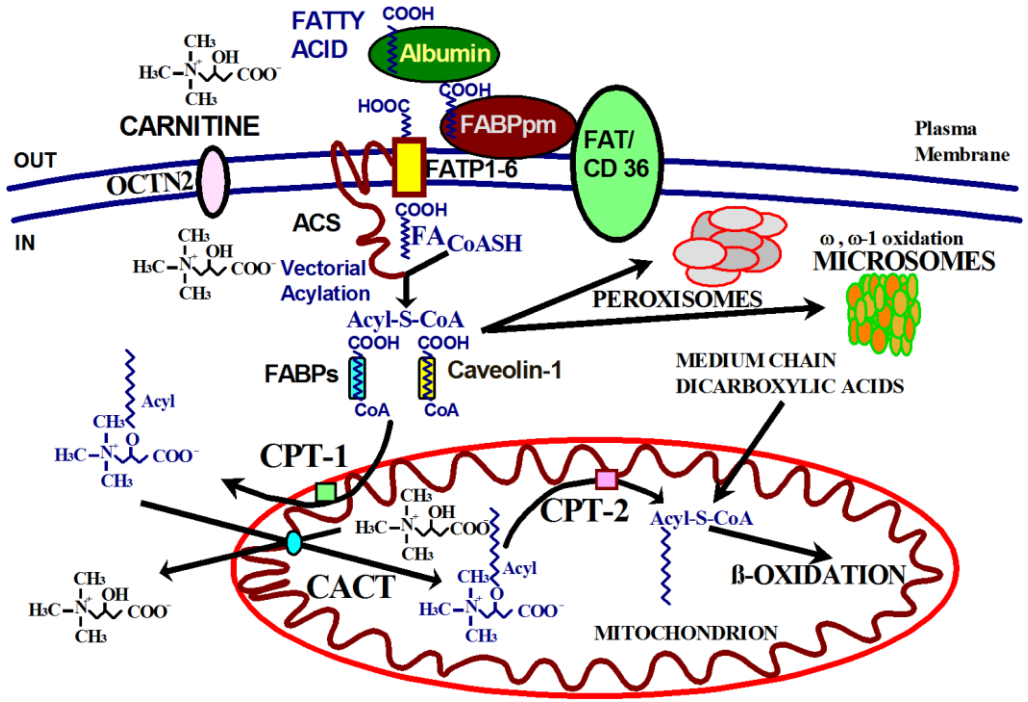
- Urine organic acids and plasma amino acids were performed STAT.
  - » Plasma amino acids were not informative
  - » Urine organic acids, although not diagnostic, showed a pattern consistent with a block in fatty acid oxidation.



Clinical case #1: Sequential tests to reach the diagnosis

- Plasma acylcarnitine profile confirmed impaired fatty acid oxidation, suggesting CPT2 or CACT deficiency.

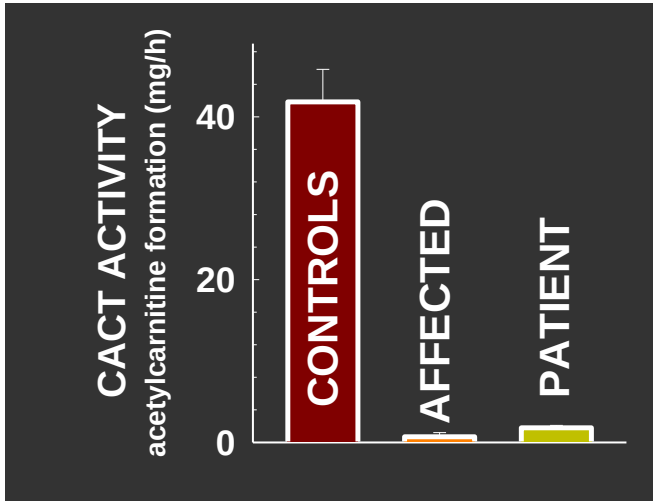




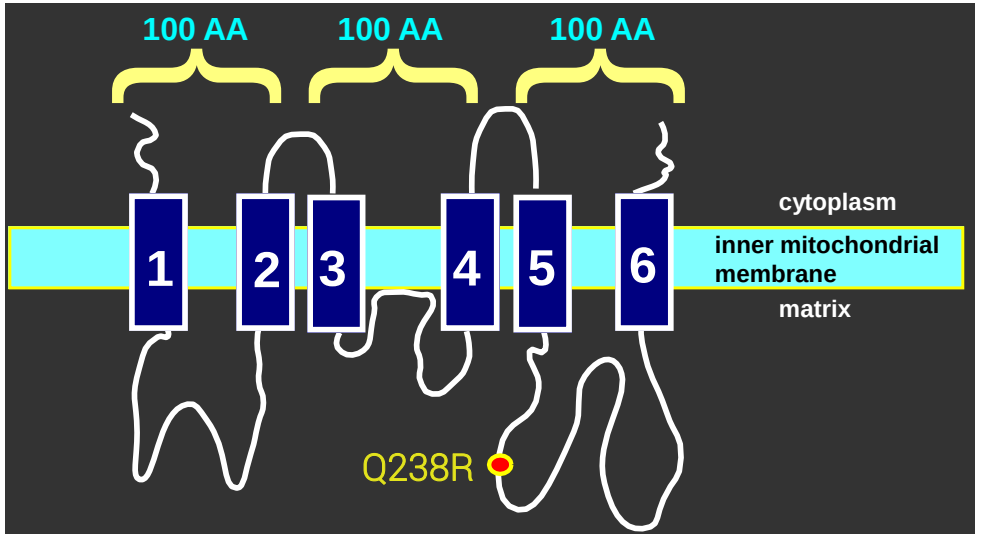
Modified from: Longo N, Amat di San Filippo C, Pasquali M (2006) Disorders of carnitine transport and the carnitine cycle. **Am J Med Genet C Semin Med Genet** 142(2):77-85

Clinical case #1: Sequential tests to reach the diagnosis

- Functional and DNA tests confirmed the diagnosis of Carnitine-Acylcarnitine Translocase (CACT) deficiency.



Iacobazzi V, Pasquali M, Singh R, Matern D, Rinaldo P, Amat di San Filippo C, Palmieri F, Longo N. Response to therapy in carnitine/acylcarnitine translocase (CACT) deficiency due to a novel missense mutation. Am J Med Genet A. 2004 Apr 15;126A(2):150-5.



Metabolomics is the study of metabolites present in cells, tissues, biological fluids

- Targeted metabolomics
  - »A known, defined set of metabolites is measured (Example: Newborn Screening)
- Untargeted metabolomics
  - »All metabolites present are analyzed (discovery)

Newborn Screening

- Tandem Mass Spectrometry changed the landscape of NBS and of biochemical genetics.
  - » Multiple analytes/enzymes can be analyzed simultaneously on the same sample (targeted metabolomics).
  - » Multiple IEMs can be detected.
  - » Diagnosis can be achieved before symptoms occur.
  - » Treatment can be initiated early → **Improved outcome**
- DNA analysis is used as a second-tier test for specific conditions (e.g. Cystic Fibrosis, Pompe Disease, MPS1).
- Functional/biochemical tests are still necessary for confirmation of diagnosis.

Clinical case #2: When enzyme and DNA testing are not sufficient

- 2-weeks old male with low iduronidase activity on NBS. DNA sequencing of the *IDUA* was performed on the same NBS dried blood spot. Results of DNA sequencing: Compound heterozygosity for a pathogenic variant and a VUS/possible pseudodeficiency.
- Suspected diagnosis: MPS 1
- Clinical examination of the infant: Normal

Mucopolysaccharidosis 1 (MPS 1)

Hurler/Hurler-Scheie/Scheie Syndrome

- Clinical Presentation:
  - » *Severe.* normal at birth, can have umbilical or inguinal hernia, frequent URI. Progressive coarsening of the facial features after age 1. Gibbus. Progressive skeletal dysplasia (dysostosis multiplex) involving all bones. Cessation of linear growth by age 3. Developmental regression. Hearing loss is common. Death (cardiorespiratory failure) by age 10.

  - » *Attenuated.* The severity and rate of disease progression range from serious life-threatening complications leading to death in the second to third decades to a normal life span complicated by significant disability from progressive joint manifestations. While some individuals have no neurologic involvement and psychomotor development may be normal in early childhood, learning disabilities can be present. Clinical onset is usually between ages three and ten years. Hearing loss and cardiac valvular disease are common.

Mucopolysaccharidosis Type 1

- Inheritance: Autosomal Recessive
- Cause: Impaired α-L-iduronidase
- Incidence: 1:100,000
- Diagnosis:
  - » Dysostosis multiplex by X-rays.
  - » Urinary glucosaminoglycans: Increased heparan and dermatan sulfate.
  - » Enzyme assay (α-L-iduronidase) in WBC or fibroblasts.
  - » DNA Sequencing.
  - » Newborn screening (on RUSP).

Mucopolysaccharidosis Type 1

Therapy:

- Supportive care
  - Physical therapy, CPAP, hearing aids, surgery
- Hematopoietic stem cell transplantation
  - Effective before delays appear in severe MPS 1
- Enzyme replacement therapy (ERT)
- Not shown to impact central nervous system
- Intratechal administration: experimental

Clinical case #2: When enzyme and DNA testing are not sufficient

- How to evaluate if the second variant could be pathogenic?
  - » Heparan sulfate and dermatan sulfate are degraded by iduronidase. Deficiency of iduronidase will result in accumulation of these glycosaminoglycans
- Pseudodeficiencies: variants in genes sequence that cause a decrease in enzyme activity without causing disease
- Biochemical tests measuring the metabolites substrates of the enzyme help in determining the pathogenicity of the variant:
  - » Accumulation of the substrate »» pathogenic
  - » Normal amount of substrate »» non-pathogenic (possible pseudodeficiency)

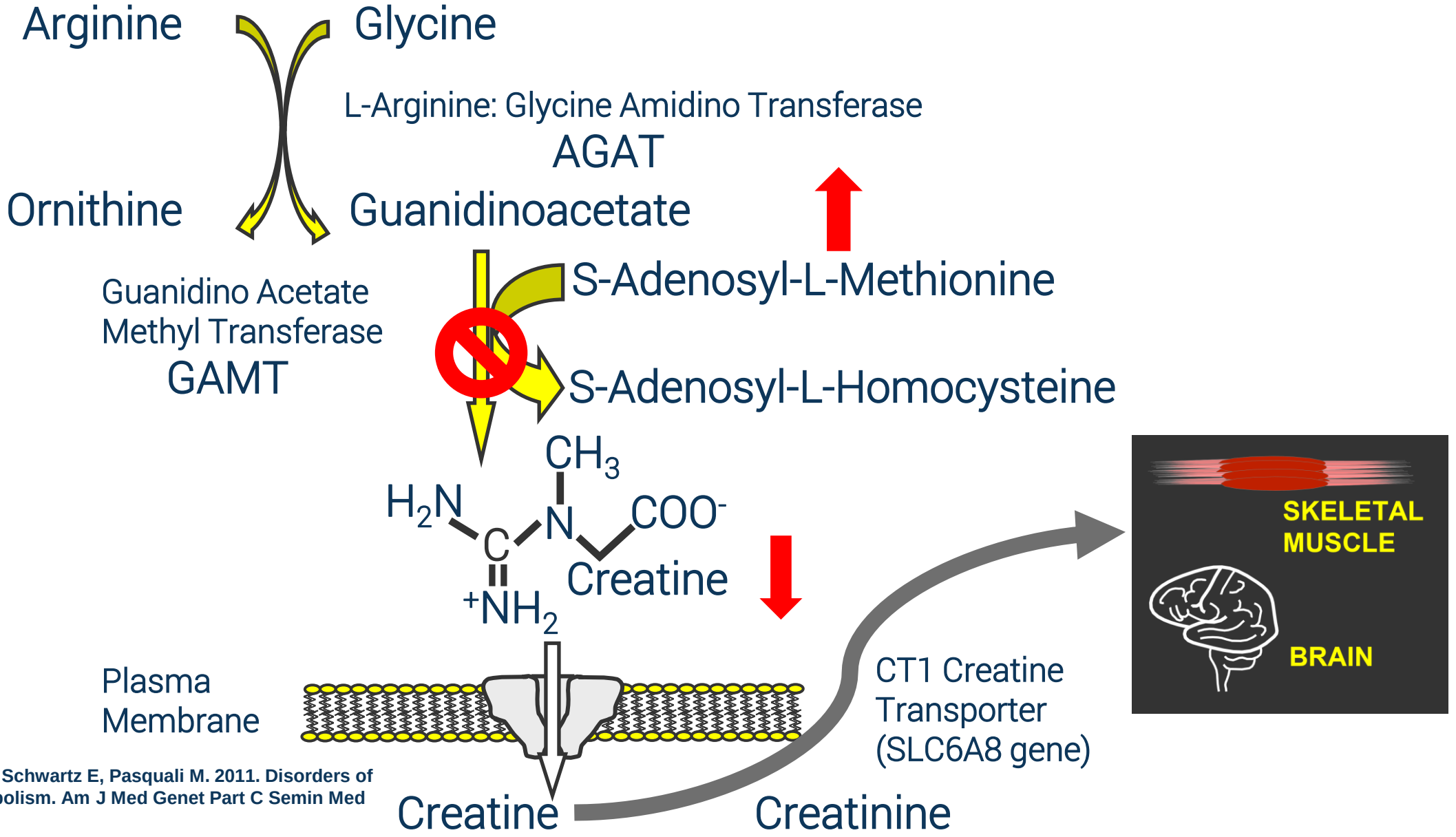
Clinical case #2: When enzyme and DNA testing are not sufficient

- Heparan sulfate and dermatan sulfate elevated in biological fluids:
  - » Mucopolysaccharidosis type 1
- Heparan sulfate and dermatan sulfate within normal range:
  - » False positive, second variant is most likely a pseudodeficiency

Newborn Screening

- "Flexible" platform: easy to modify
  - » Addition of GAMT screening to the RUSP (recommended Uniform Screening Panel)

GAMT Deficiency



Longo N, Ardon O, Vanzo R, Schwartz E, Pasquali M. 2011. Disorders of creatine transport and metabolism. Am J Med Genet Part C Semin Med Genet 157:72–78.

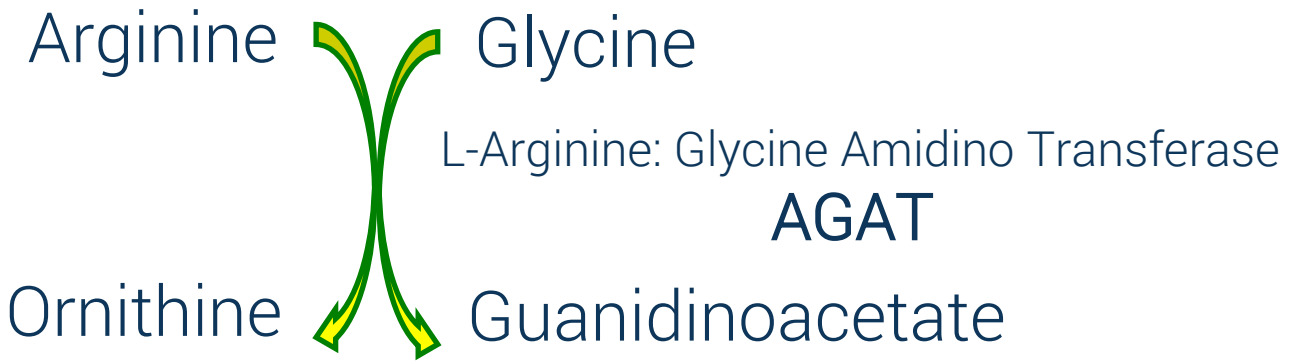
GAMT Deficiency

- **Cause:** autosomal recessive disorder caused by deficiency of guanidinoacetate methyltransferase (GAMT), encoded by *GAMT* gene on 19p19.3, and resulting in impaired creatine synthesis and accumulation of guanidinoacetate (GUAC).
- **Incidence:** estimated 1:275,000 (from variant frequency in genomic databases).
- **Presentation:** Developmental delays, hypotonia, seizures, autistic-like behavior, abnormal movements.
- **Diagnosis:** Lack of creatine peak in MR spectroscopy, increased plasma guanidinoacetate, decreased plasma creatine. Identified by newborn screening.
- **Therapy:** Creatine (400 to 800 mg/kg/day) initiated preferably early in life. GUAC levels can be reduced by ornithine supplementation (400-800 mg/kg/day) and Benzoate (50-250 mg/kg/day) to reduce glycine levels and GUAC synthesis.
- **Outcome:** Excellent if therapy is initiated before brain damage, intellectual disability can be prevented, but not reversed.

Stockler-Ipsiroglu S, van Karnebeek C, Longo N, Korenke GC, Mercimek-Mahmutoglu S, Marquart I, Barshop B, Grolik C, Schlune A, Angle B, Araújo HC, Coskun T, Diogo L, Geraghty M, Haliloglu G, Konstantopoulou V, Leuzzi V, Levtova A, Mackenzie J, Maranda B, Mhanni AA, Mitchell G, Morris A, Newlove T, Renaud D, Scaglia F, Valayannopoulos V, van Spronsen FJ, Verbruggen KT, Yuskiv N, Nyhan W, Schulze A. Guanidinoacetate methyltransferase (GAMT) deficiency: outcomes in 48 individuals and recommendations for diagnosis, treatment and monitoring. Mol Genet Metab. 2014 Jan;111(1):16-25. doi: 10.1016/j.ymgme.2013.10.018. Epub 2013 Nov 7. PMID: 24268530

GAMT Deficiency: Treatment

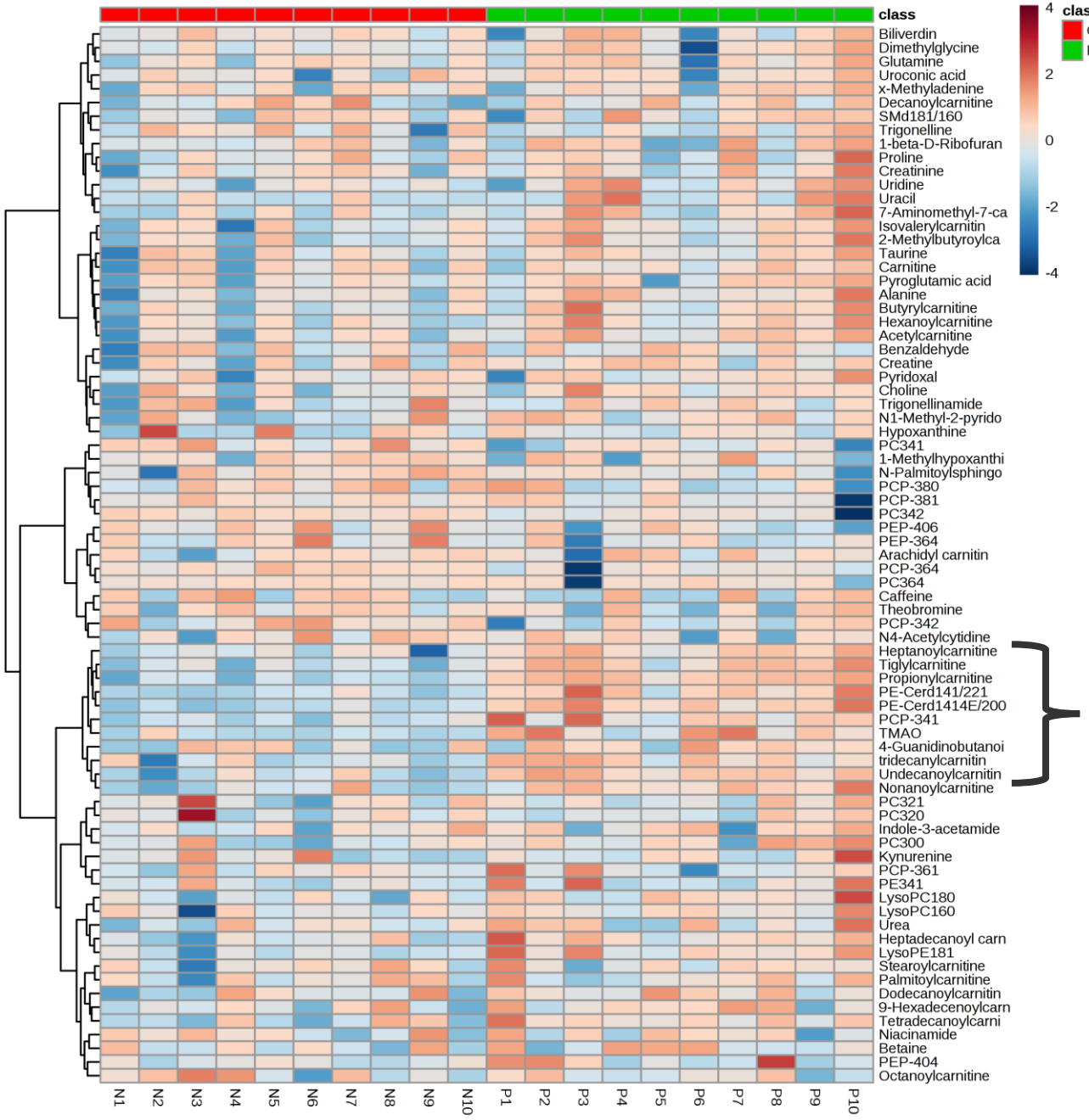
- Creatine, initiated preferably early in life
- Guanidinoacetate needs to be reduced:



- In GAMT deficiency, guanidinoacetate levels can be reduced by ornithine supplementation and benzoate to reduce glycine levels and guanidinoacetate synthesis.

Newborn Screening for GAMT deficiency

- Easy integration of additional targets, guanidinoacetate and creatine, to the platform routinely used
- Inexpensive
- No additional sample required
- Therapy is effective
- Approved to be added to the RUSP (Recommended Uniform Screening Panel) in May 2022

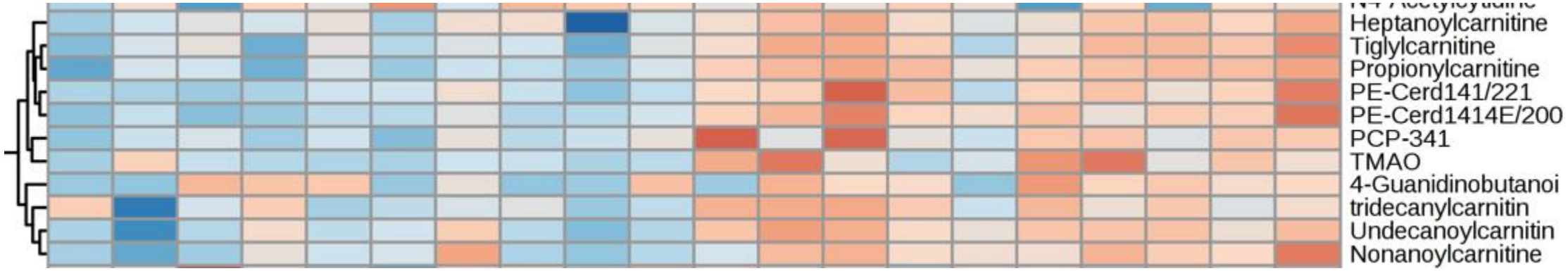


Research Untargeted Metabolomics Analysis

- Research tool
- Comparison between two sets of samples subject to a different treatment (e.g. propionic acidemia and controls)
- Propionic acidemia: autosomal recessive disorder of valine, methionine, isoleucine, threonine, odd-chain fatty acid metabolism.

Untargeted metabolomics

- Odd-chain acylcarnitines are consistently more abundant in propionic acidemia (due to increased odd-chain fatty acids)



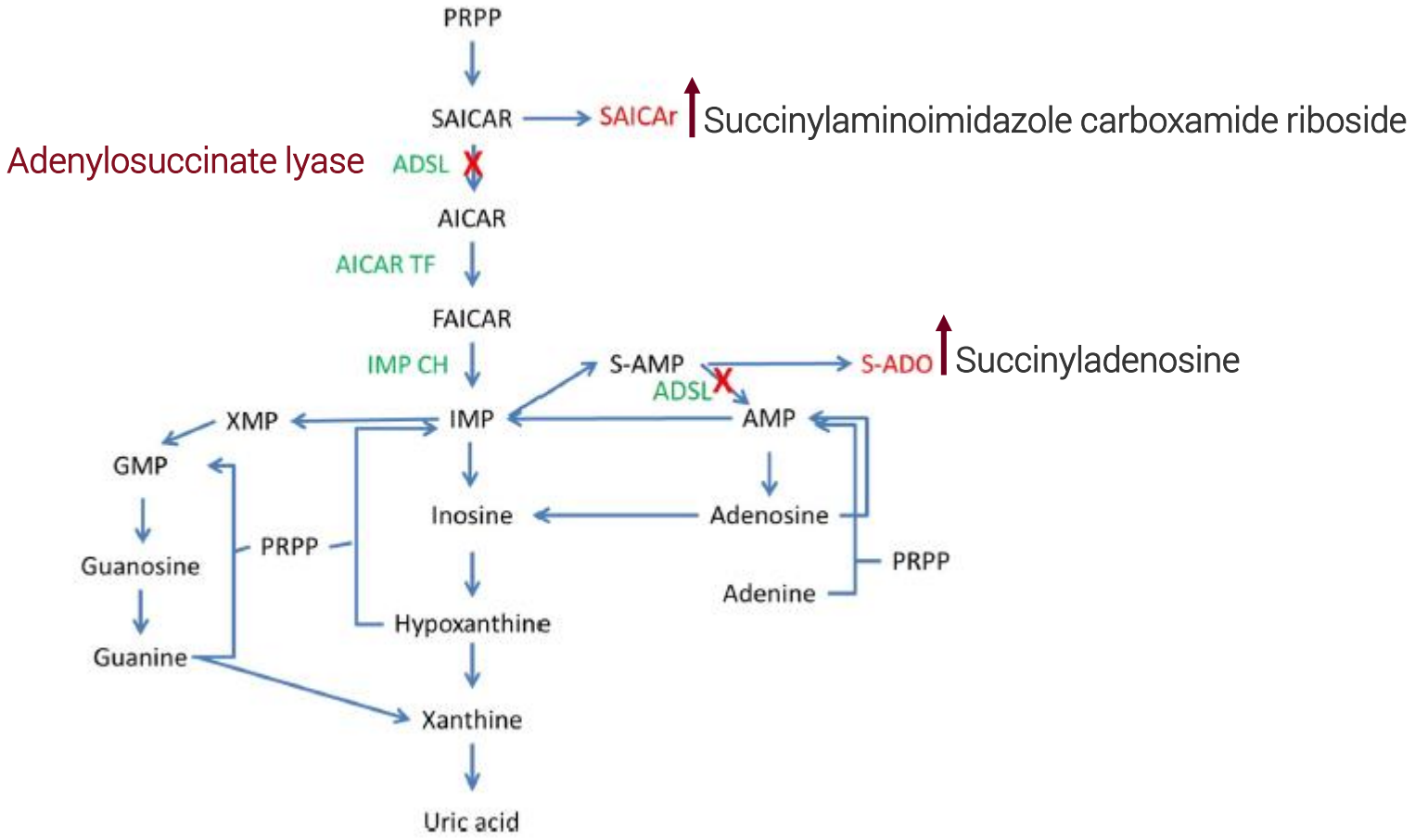
Clinical untargeted metabolomics

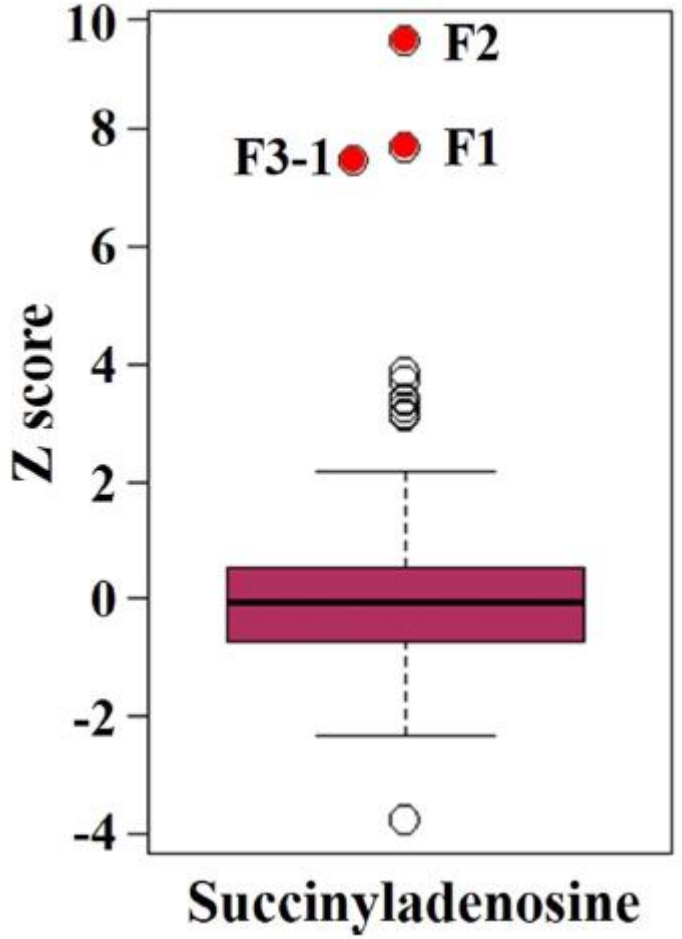
- Comparison between a patient and a reference population
  - » Non-specific clinical phenotype
  - » Broad differential diagnosis
  - » Not informative routine biochemical tests
  - » Shortens the time to diagnosis

Metabolomic profiling and adenylosuccinate lyase deficiency. Donti, RD et al; Mol Genet Metab Rep (2016) 8:61-66

- Clinical findings: developmental delay, seizures (+/-), ocular findings (strabismus, myopia), increased muscle tone, abnormalities in the brain MRI
- Age of onset of symptoms: 2-4 months
- Age at diagnosis: 7 months-3 years
- Standard biochemical tests (plasma & CSF amino acids, urine organic acids, plasma acylcarnitines) were not informative.

Metabolomic profiling and adenylosuccinate lyase deficiency. Donti, RD et al; Mol Genet Metab Rep (2016) 8:61-66





From: Donti, RD et al; Mol Genet Metab Rep (2016) 8:61-66

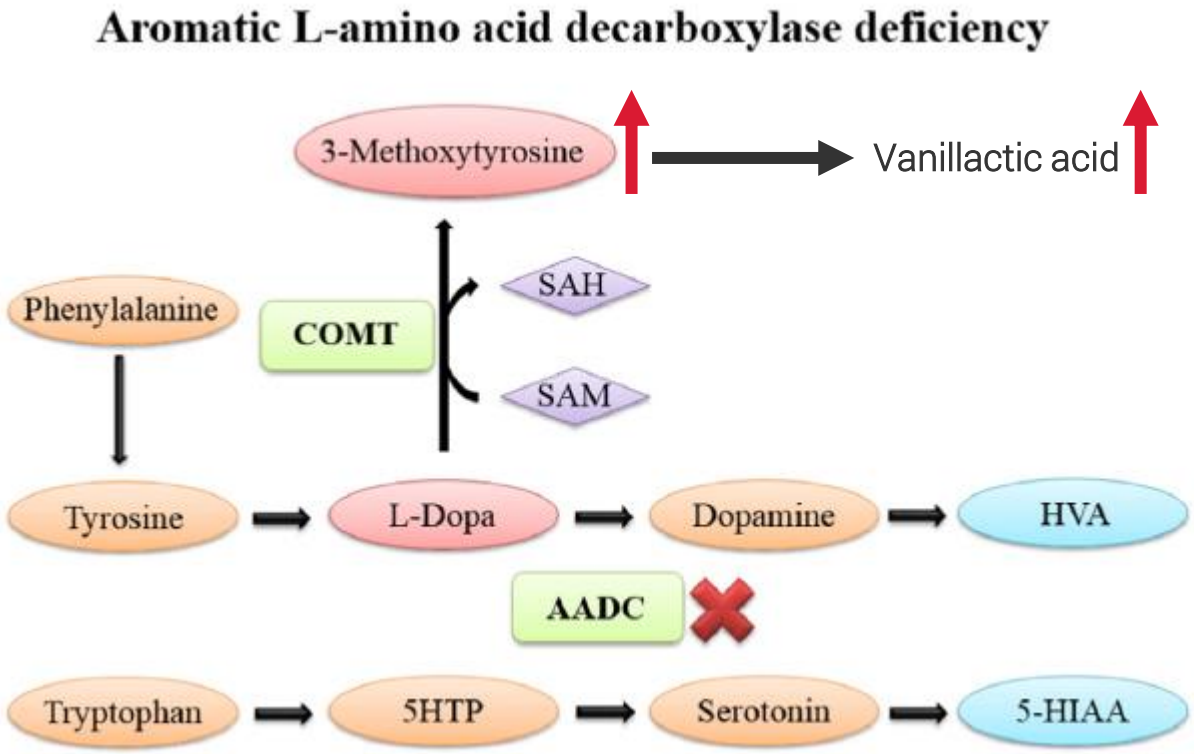
Metabolomic profiling and adenylosuccinate lyase deficiency. Donti, RD et al; Mol Genet Metab Rep (2016) 8:61-66

- Findings from the metabolic profiling were confirmed using a targeted urine purine panel and by DNA testing.
- The heterogeneity of the clinical findings did not suggest, in some patients, a purine disorder.
- Metabolomic profiling would accelerate the diagnosis.
- It is important for definitive confirmation to have functional/biochemical and molecular data.

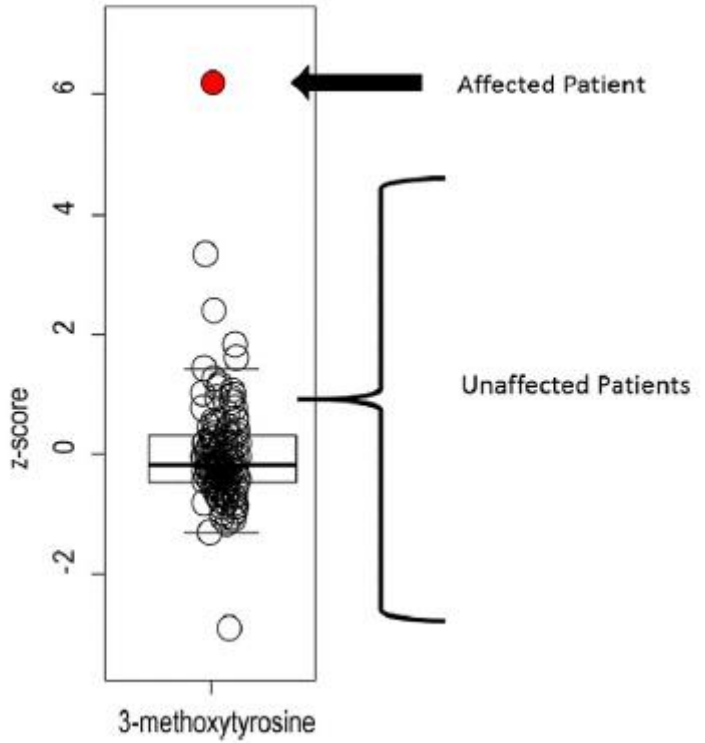
Clinical case #3: Integration of molecular and biochemical data. Atwal PS et al; Mol Genet Metab (2015) 115:91-94

- 11-months old boy: developmental delay (not rolling over, not babbling, poor head control), seizure-like episodes, hypotonia, bilateral ptosis, normal EEG.
- Lost to follow-up for 2 years.
- Upon return to clinic, whole exome sequencing was ordered.
  - » Compound heterozygosity for missense VUS (variants of uncertain significance) in the *DDC* gene (encoding for aromatic L-amino acid decarboxylase).

Clinical case #3: Integration of molecular and biochemical data. Atwal PS et al; Mol Genet Metab (2015) 115:91-94



Untargeted metabolomic analysis revealed elevated 3-methoxytyrosine, confirming the Diagnosis of AADC deficiency.



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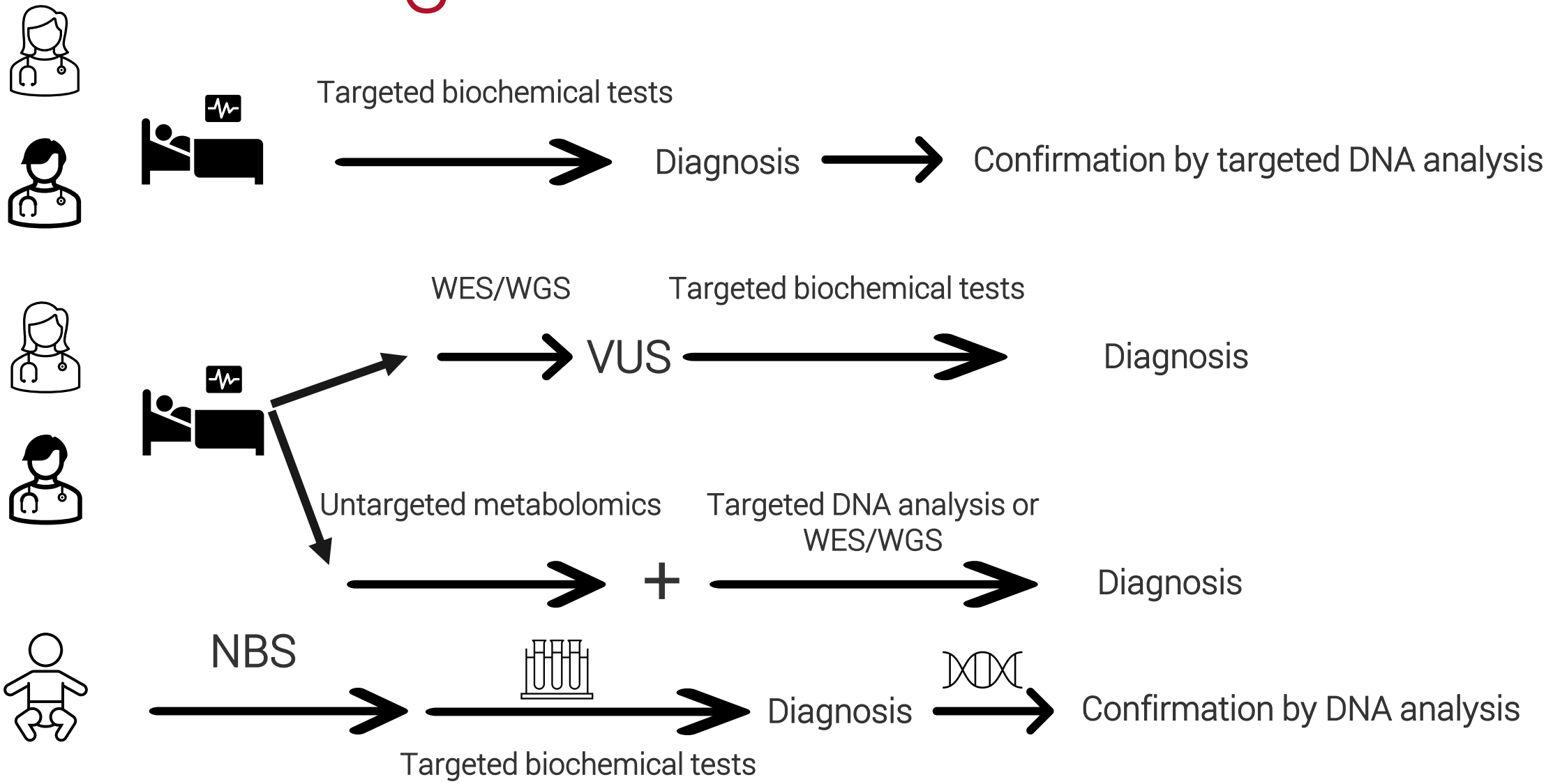
Path to diagnosis



Summary

- Many human diseases are caused by perturbations in metabolic pathways.
- Inborn errors of metabolism are amenable to intervention/therapy.
- Identifying new diseases and their metabolic cause paves the way to possible therapies for rare diseases and more common ones.
- The integration of biochemical/functional studies, molecular studies, and metabolomics analysis is of paramount importance for the confirmation of diagnosis and the assessment of variant pathogenicity.
- Metabolomics can be used for biomarkers discovery and is rapidly becoming a clinical tool.

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