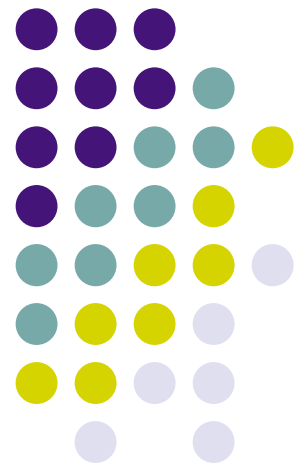


Expanded Newborn Screening

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Overview

- History of newborn screening
- Expanded newborn screening by tandem mass spectrometry
- Metabolic disorders detected by NBS
 - Amino Acidemias
 - Organic Acidemias
 - Fatty Acid Oxidation Disorders
- Follow-up of abnormal results
- Shortcomings
 - Case presentations
- Long-term Follow-up
- Future of newborn screening



History of NBS

- 1961...Development of Screening for PKU
 - Dr. Robert Guthrie developed a bacterial inhibition assay
 - Presence of phenylalanine on filter paper from heel prick
 - Diet intervention = good outcome
- 1963... Massachusetts passed legislation to mandate NBS for PKU
- By 1966, PKU screening was mandated in the majority of states
- Supporters included March of Dimes and National Association for Retarded Citizens
- Because PKU screening was a success, this led to development of other NBS tests



Sheila* is retarded. Kammy*
was spared because of new
medical research.

Retarded Children Can Be Helped

SUPPORT YOUR UNIT, NATIONAL ASSOCIATION FOR RETARDED CHILDREN



Expanded Newborn Screening



- Technology of tandem mass spectrometry applied to newborn screening beginning in 1990.
- Allows for multiple metabolic disorders to be screened for from one blood spot
- Previously, *each disorder required a separate test!*
 - Reasonable incidence
 - Significant morbidity and mortality
 - Proven and successful treatment
 - Cost effective
 - Adequate sensitivity and specificity

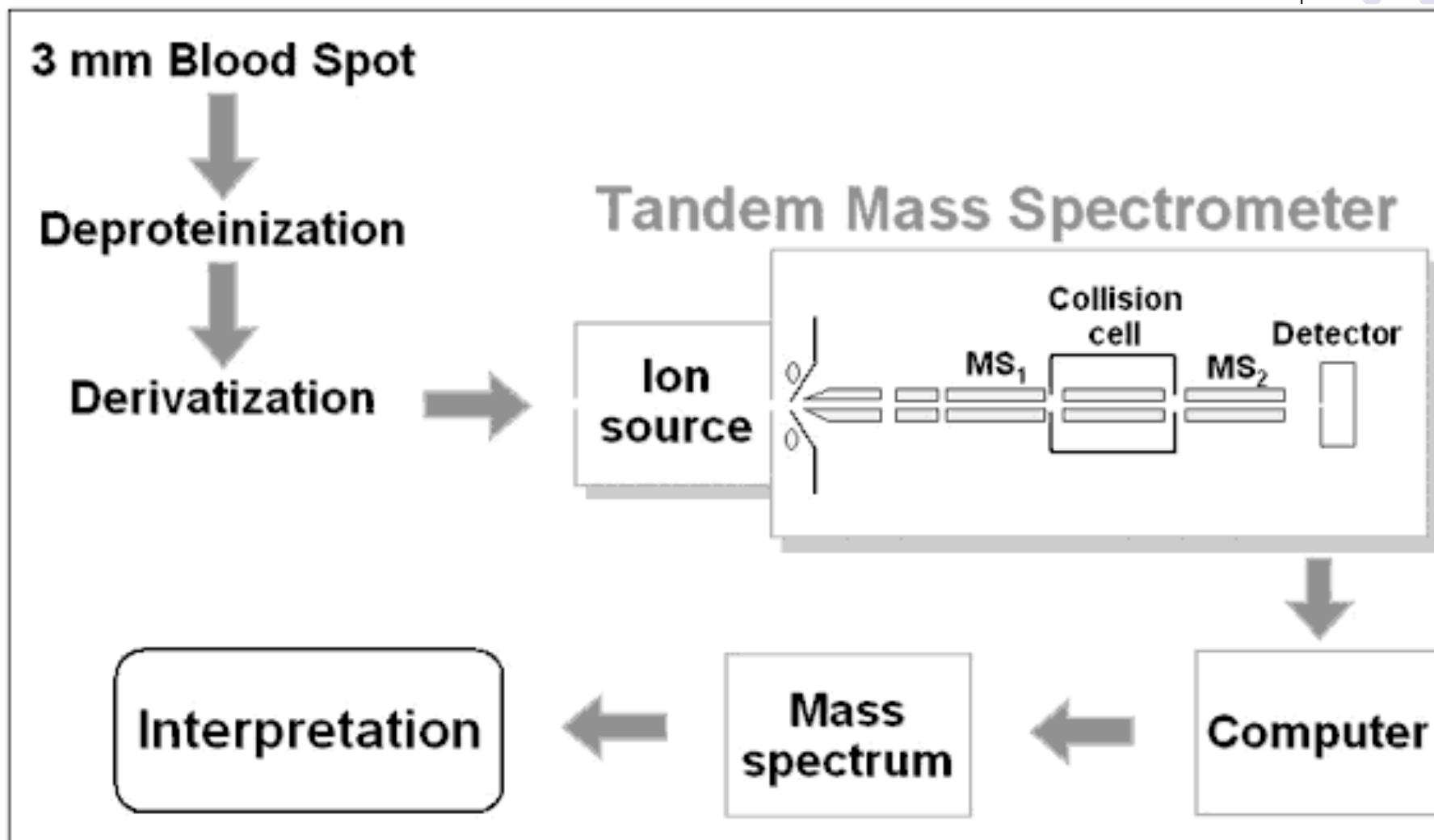
Tandem Mass Spectrometry (MS/MS)



- Uses electrical and magnetic fields to separate and measure the mass of the charged particles
- Why use MS/MS for NBS?
 - Multiple biochemical metabolites tested in a single run (1 blood spot)
 - Amino Acidemias /Urea Cycle Disorders
 - Organic Acidemias
 - Fatty Acid Oxidation Disorders
 - Quick
 - Less than 3 minutes
 - Inexpensive
 - If done in large numbers
 - Ideal for population screening



Diagram of MS/MS

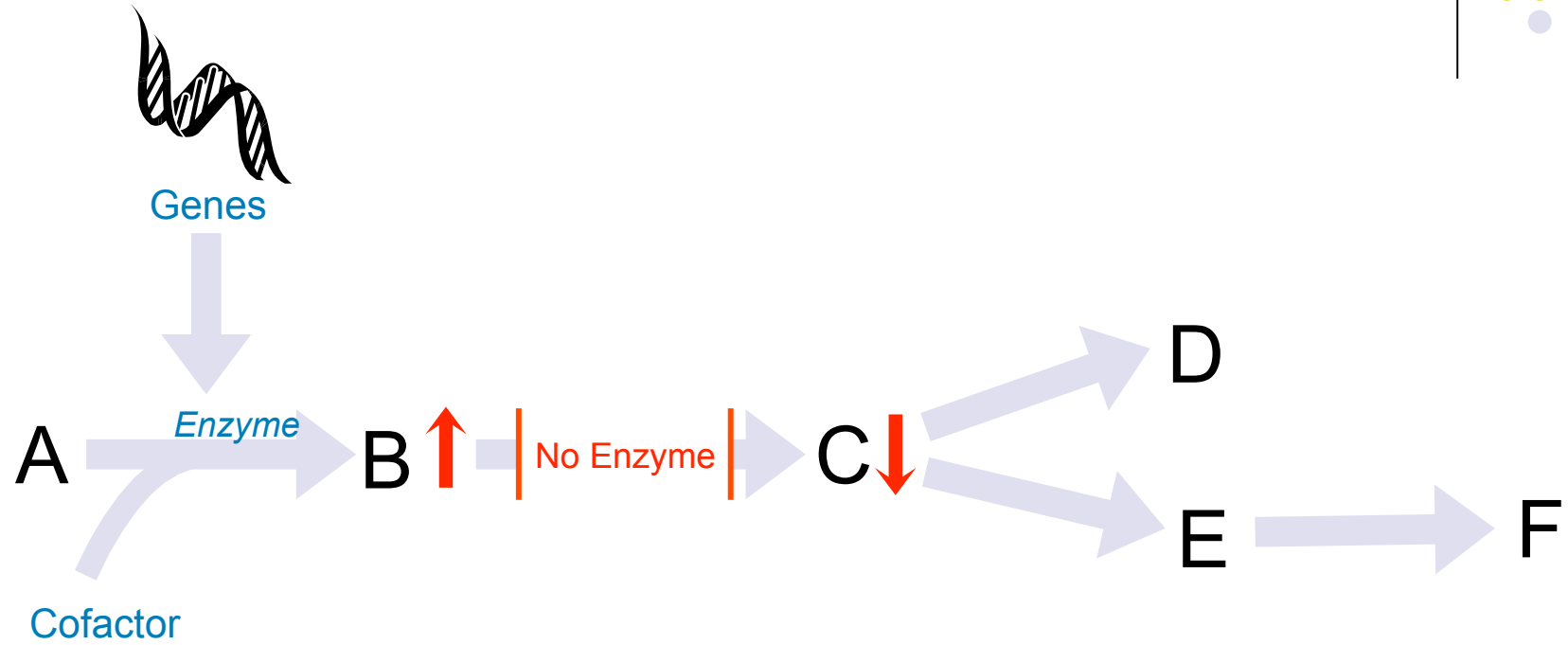


Metabolic Disorders 101



- Wikipedia -**Inborn errors of metabolism** comprise a large class of [genetic diseases](#) involving disorders of [metabolism](#). The majority are due to defects of single [genes](#) that code for [enzymes](#) that facilitate conversion of various substances ([substrates](#)) into others ([products](#)). In most of the disorders, problems arise due to accumulation of substances which are toxic or interfere with normal function, or to the effects of reduced ability to synthesize essential compounds.
- My definition – A problem breaking down food into energy

Enzymes





Amino Acidemias

- Enzyme deficiency in the breakdown of amino acids
- Most patients present after the first month of life
- PKU - build-up of Phe toxic to brain = MR, seizures, & spasticity
- Tyrosinemia – build-up of Tyr toxic to liver = liver failure
- Homocystinuria – build-up of Hcy affects brain (seizures and MR), eyes (lens dislocation), bones (marfanoid habitus), and clotting (strokes, pulmonary embolism)

...Amino Acidemias



● Treatment

- Protein restriction plus amino acids supplements (formula) that do not contain the amino acid that cannot be metabolized
- Medications
 - PKU – Kuvan® (tetrahydrobiopterin, cofactor for phenylalanine hydroxylase enzyme)
 - TYR – Nitisinone (NTBC, alternative pathway for Tyr)
 - HCY- Betaine (alternative pathway for homocysteine)
- Liver transplant
 - TYR –question if needed b/c of NTBC therapy



Urea Cycle Disorders

- Sub-category of amino acidemias
- Urea cycle used to excrete nitrogen waste (urea), a byproduct of protein metabolism
 - Ammonia detoxification
- Block in pathway results in ↑↑↑ Ammonia



...Urea Cycle Disorders

- Can present at any age, but most commonly in infancy
- Initially healthy for 24-48 hours...then poor feeding, vomiting, lethargy, irritability, and hyperventilation
- Progress to apnea, coma and death
- Labs can be relatively normal (no acidosis) but very increased ammonia



...Urea Cycle Disorders

- Treatment
 - Initially
 - Stop protein intake
 - Ammonia scavenging drugs
 - Dialysis (tertiary care)
 - Long-term
 - Protein restricted diet with supplemented essential amino acids
 - Prevention of catabolism
 - No fasting
 - Glucose during illness
 - Ammonia scavenging drugs
 - Liver transplant



Organic Acidemias

- Enzyme deficiency in intermediary metabolism of amino acids results in characteristic accumulations of metabolites in urine
- Present at any age:
 - Severe neonatal onset form
 - Initially healthy (hrs-weeks), then poor feeding, vomiting, & lethargy
 - Acidosis and ketonuria
 - Hyperammonemia
 - Bone marrow suppression
 - Chronic intermittent late-onset form (>1 year)
 - Present during illness or fasting
 - Chronic, progressive form
- Sometimes asymptomatic



...Organic Acidemias

- Treatment

- Initially

- Stop protein intake
- Ammonia scavenging drugs
- Dialysis (tertiary care)

- Long-term

- Protein restriction plus amino acids supplements (formula) that do not contain the amino acid that cannot be metabolized
- Possible vitamin supplementation
 - B12 in methylmalonic acidemia
 - Biotin in multiple carboxylase deficiency
- Supplementation with L-carnitine
- Prevention of catabolism
 - No fasting
 - Glucose during illness

Fatty Acid Oxidation Disorders



- Enzyme deficiency in fatty acid oxidation
- Fatty acids used most often during fasting and illness
- Used in liver, heart, and muscle
 - Liver – no ketones can be made = nonketotic hypoglycemia
 - Heart – cardiomyopathy and arrhythmia
 - Muscle – rhabdomyolysis and myoglobinuria
- Disorders named by the length of fat chain that can't be broken down
 - Longer the chain, the more severe the disorder
 - Short chain acyl-CoA dehydrogenase def = benign
 - Medium chain acyl-CoA dehydrogenase def = moderate
 - Very Long chain acyl-CoA dehydrogenase def = severe

...Fatty Acid Oxidation Disorders



- Milder types (MCADD)
 - Symptoms only when triggered by illness, fasting, and extreme exercise
 - Nonketotic hypoglycemia = poor feeding and lethargy during illness or fasting
 - Treatment: prevention of fasting/glucose during illness
- Severe types
 - Symptoms during first few days of life
 - Nonketotic hypoglycemia = poor feeding, lethargy, etc.
 - Cardiomyopathy and arrhythmia
 - In rare cases, multiple congenital anomalies
 - Labs: hypoglycemic, abnl LFTS, elevated CK, increased ammonia/lactate
 - Treatment:
 - diet low in long-chain triglycerides with supplementation of medium chain triglycerides (MCT)
 - prevention of fasting /glucose during illness



Follow-up of abnormal results

- Colorado State lab receives NBS sample
- Samples logged in immediately and cards are punched and prepped
- Multiple tests run
 - MS/MS
 - Biotinidase
 - GALT
 - Hemoglobinopathies
 - T4 /TSH
 - CAH
 - IRT for CF

...follow-up



- Abnormal results called in IMD clinic
 - MS/MS in a.m.
 - BIOT and GALT in p.m.
 - Mon-Fri –Erica (pager)
 - Sat- metabolic physician on call
- Urgent results
 - Called to IMD immediately, without being rerun
 - “metabolic emergencies”
 - High levels
- Borderline results
 - Rerun again, results of two runs are averaged together



...follow-up

- IMD clinic contacts PCP listed on NBS card
- Request that clinical status of baby be checked immediately.
 - Poor feeding, lethargy, vomiting
- Additional diagnostic recommendations are made:
 - Amino Acidemias
 - Quant serum amino acids and homocysteine (in HCY)
 - Possible LFTs
 - Urea Cycle disorders
 - Quant serum amino acids
 - Possible ammonia level
 - Organic Acidemias
 - Plasma Acylcarnitine profile
 - Urine organic acids
 - Possible metabolic panel, ammonia levels, lactate, ketones
 - Fatty Acid Oxidation
 - Plasma acylcarnitine profile
 - Possible glucose, LFTs, serum CK, ammonia, lactate

...follow-up



- Await follow-up labs (3-7 days)
- If consistent with disorder, see patient and family in clinic for treatment and education
- If normal or results not consistent with NBS result, review labs with metabolic physicians to determine course of action
 - Consider case closed “false positive”
 - Consider additional studies
 - May request 2nd tier testing for disorders where metabolites have been reported to normalize with time (glutaric acidemia, type I, fatty acid oxidation disorders)



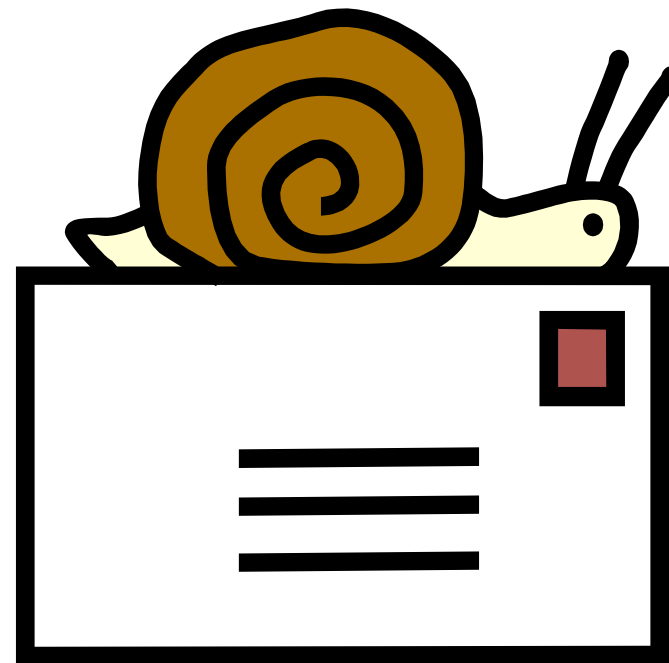
Shortcomings of NBS

- Disorders that present early
- Disorders that may be missed by NBS
- Disorders not detected by NBS

Early Presentation



- Multiple disorders on current panel have severe forms that may present within first few days of life
 - Organic acidemias
 - Fatty Acid oxidation disorders
 - “Metabolic Emergencies”
- Most NBS samples arrive at state lab via regular mail
- Average timing of NBS results = 7.5 days of age





Case #1

- 1st NBS obtained on day 3 of life (Friday).
- Baby in NICU at the time due to prematurity, product of a twin gestation.
- NBS card mailed via USPS
- NBS card arrived at state newborn screening lab the following Thursday, 6 days after it was obtained.
- NBS card ran overnight and showed elevated C3 with high C3/C2 ratio.

Propionic Acidemia (PA)



Isoleucine
Valine
Methionine
Threonine

Odd chain fatty acids
Gut flora

Occurring across tissues including liver...

Propionyl-CoA

Inhibits the urea cycle, citric acid cycle and others

Biotin

Propionyl-CoA Carboxylase

PA

Succinyl-CoA

Energy

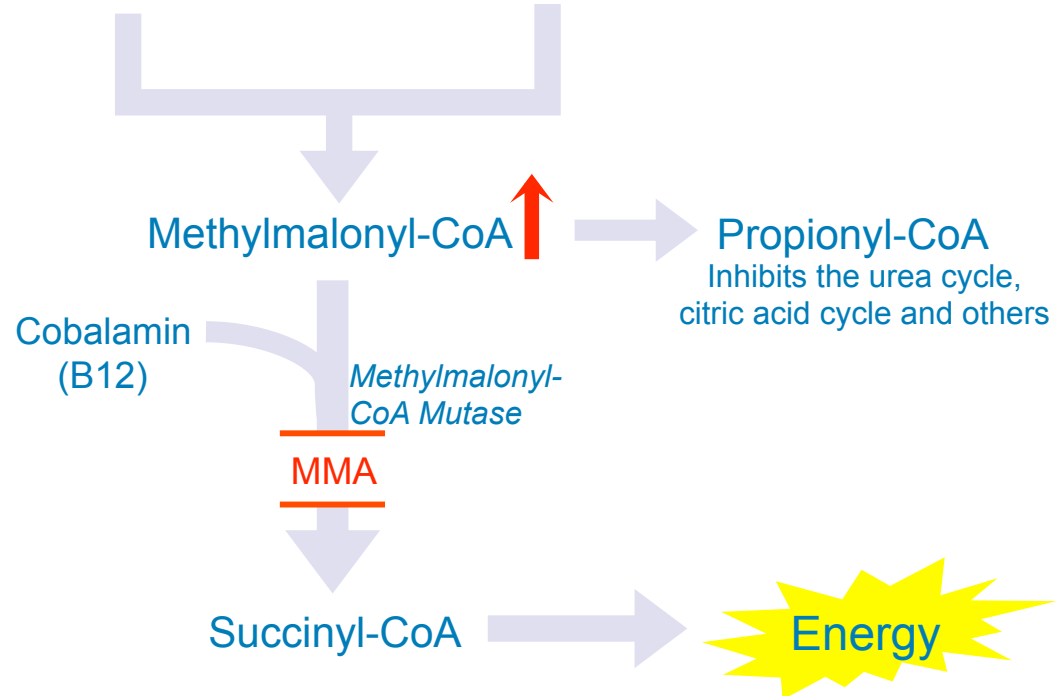
Methylmalonic Acidemia (MMA)



Isoleucine
Valine
Methionine
Threonine

Odd chain
fatty acids
Gut flora

*Occurring
across tissues
including liver...*





...Case #1

- Results reported to IMD Clinic on Friday morning.
- IMD clinic called NICU immediately. Requested that clinical status of baby be checked for poor feeding, lethargy, and vomiting. If concerns, NICU should check electrolytes (acidosis) and ammonia level.
- IMD clinic also requested urine organic acids and plasma acylcarnitine profile.

...Case #1



- Labs obtained immediately on baby due to concern that baby was not feeding well that day.
- Baby symptomatic with ammonia >1000 (<100).
- Airlifted to TCH within few hours of the positive NBS call.
- Due to extent of hyperammonemia/acidosis and very poor clinical status of the baby, care was terminated. Baby deceased at 23:09 p.m.



...Case #1

- Urine organic acids showed large peaks of methylmalonic acid and methylcitric acid.
- Complementation studies confirmed diagnosis of Cobalamin A, a type of methylmalonic acidemia.
 - Many C1b A patients respond to B12
- Another baby with severe MMA recently showed symptoms at 24 hours of life

False Negatives

- A “screen” is not diagnostic
- Cut-offs are established in order to capture all affected babies but also to limit the number of false positives
- Tremendous overlap exists for number of disorders
 - Tyrosinemia, Type I
 - Glutaric Acidemia, Type I
 - Methylmalonic Acidemias
 - Fatty acid oxidation disorders
- Multiple labs beginning to use 2nd tier testing (CO/WY using it for CF)



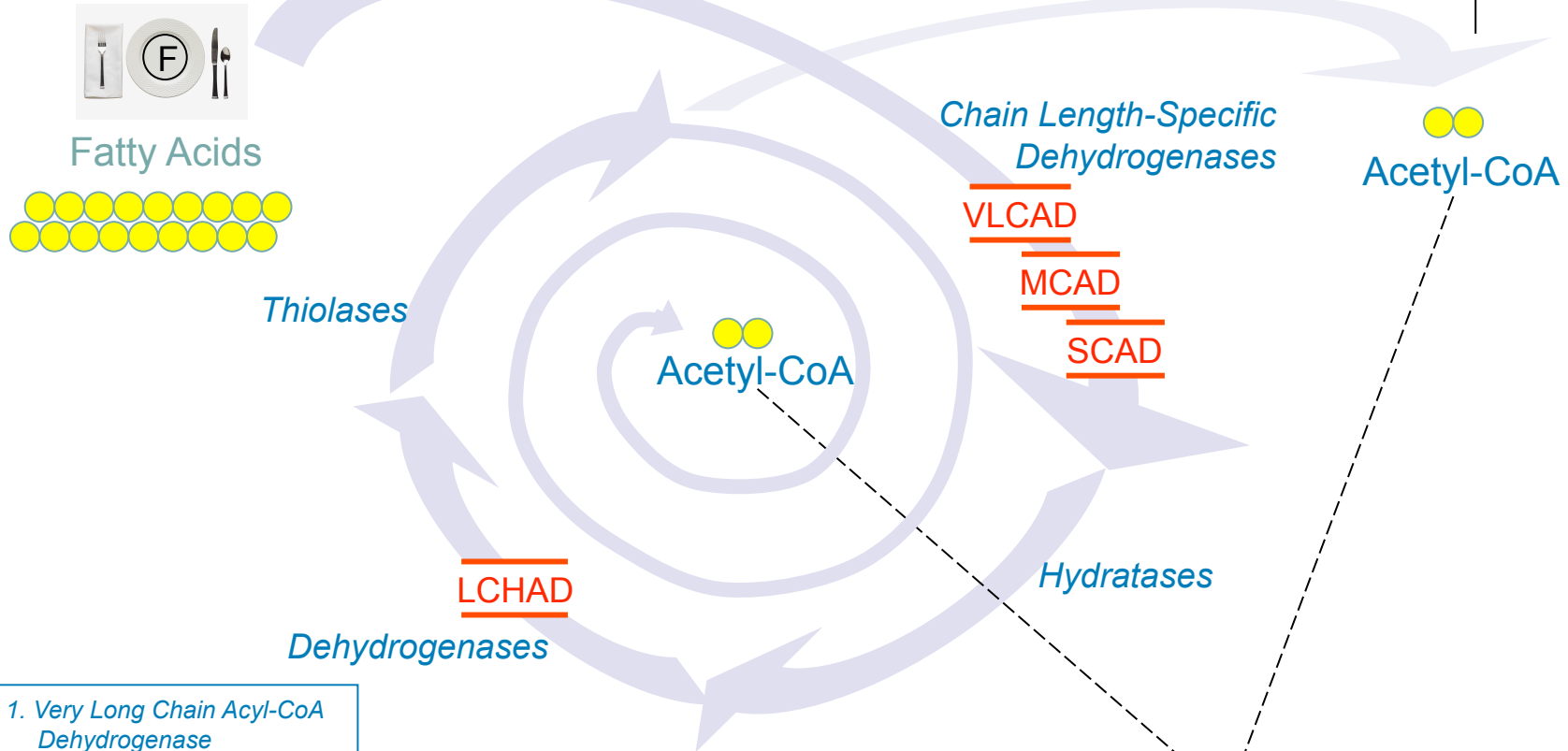


Case #2

- 6 month old female brought to the ER after parents found her limp and lethargic in her crib that morning.
- She had a two day history of low-grade fever and URI with limited oral intake and one episode of emesis the night before.
- Baby found to be hypoglycemic with a glucose of 17.
- CMP shows anion gap of 24 and extremely elevated bilis, AST and ALT. Ammonia elevated at 204. Urinalysis shows trace ketones.
- Parents report normal NBS to ER staff

Fatty Acid Oxidation

Occurring mainly in the heart, muscle, and liver...



1. Very Long Chain Acyl-CoA Dehydrogenase
2. Medium Chain Acyl-CoA Dehydrogenase
3. Short Chain Acyl-CoA Dehydrogenase
4. Long Chain 3-Hydroxyacyl-CoA Dehydrogenase

Energy for Body
 via ATP in muscle

Energy for Brain
 via ketones in liver



...Case #2

- Baby transferred to TCH in liver failure.
- Acylcarnitine profile confirms the suspicion of a FAO, namely LCHAD deficiency.
- Baby switched from breast milk to Lipistart (low in long chain fats, high in MCT oil)
- Liver function significantly improves during the hospitalization. No developmental concerns about child.
- At second look at NBS, C16:OH was normal at 0.14 umol/L (nl<0.17) and C18:1OH was elevated at 0.16 umol/L (nl<0.16). Abnormal screens only called out if both metabolites flag.
- State lab considering lowering the cut-off.

Disorders not included on NBS



- Expanded NBS = about 30 metabolic disorders
- Hundreds of metabolic disorders not on panel
- NBS is a very useful tool, but is a very small piece of the puzzle.
- If clinical concerns, don't rely on "normal" newborn screen results
- Have heard many doctors say "but the baby had a normal NBS..."

Case #3



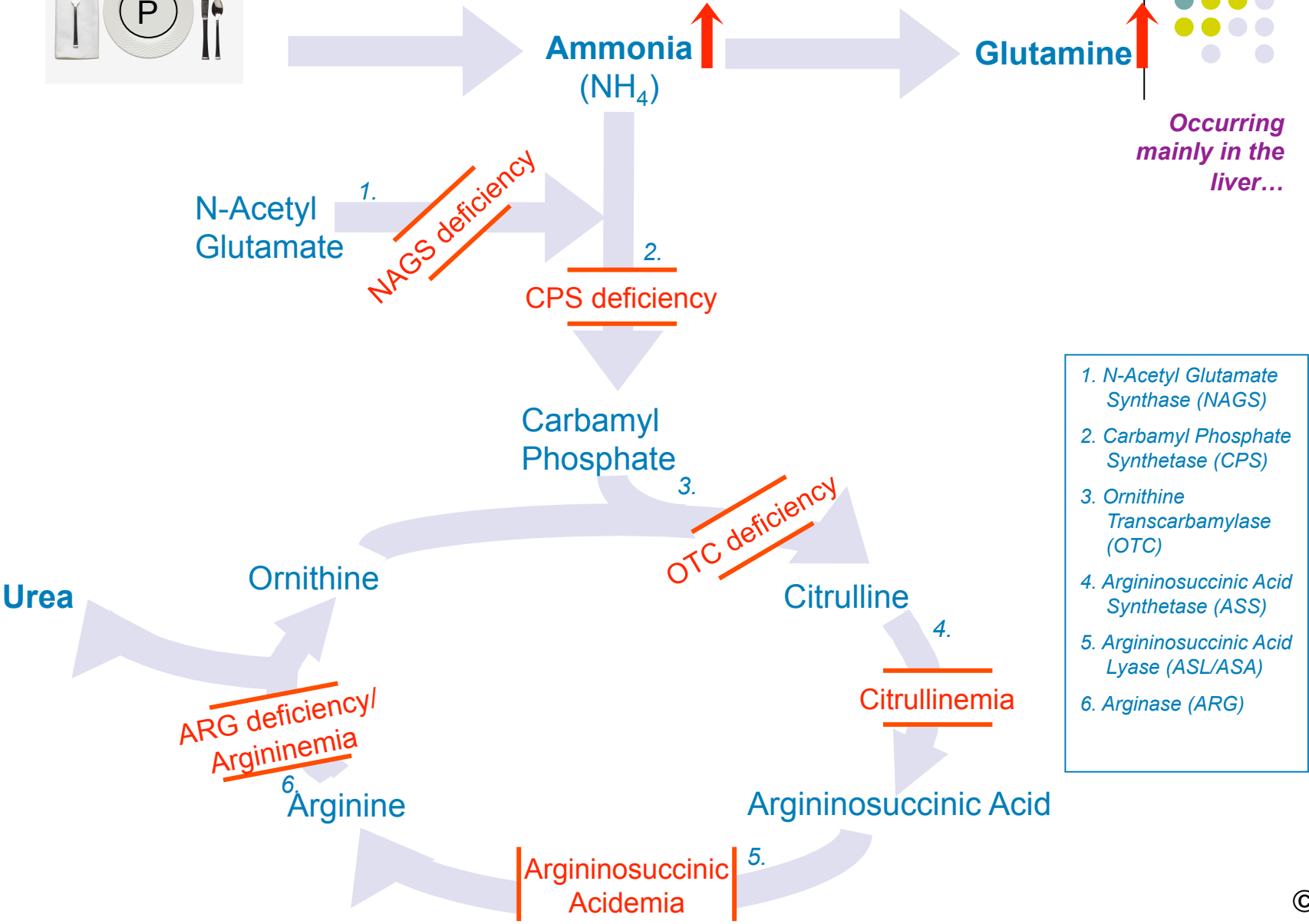
- Baby girl born to a G1, P0-1 mother after normal pregnancy and forcep assisted delivery
- Did well until DOL 3
- She was noted to have decreasing responsiveness but was still waking up for feeds.
- Due to lip smacking and hypertonia, there was a concern for seizures. CT scan showed cerebral edema, skull fracture, and moderate subarachnoid bleed
- Labs were notable for mild metabolic acidosis
- Ammonia level was 230 (<100)
- Baby transferred to TCH for further evaluation
- Ammonia rose to 440
- IMD team consulted



...Case #3

- Labs (Amino acids) pointed to OTC (*Ornithine Transcarbamylase*) deficiency
 - Not surprisingly, NBS was normal!
- Baby treated protein restricted diet, supplementation with AA arginine, & ammonia scavenging drugs
- OTC deficiency, the most common urea cycle disorder, it NOT on the NBS panel.
- OTC is X-linked, often lethal in affected males
- Female carriers are often symptomatic with elevated ammonia levels during illness or increased protein intake

The Urea Cycle



1. N-Acetyl Glutamate Synthase (NAGS)
2. Carbamyl Phosphate Synthetase (CPS)
3. Ornithine Transcarbamylase (OTC)
4. Argininosuccinic Acid Synthetase (ASS)
5. Argininosuccinic Acid Lyase (ASA)
6. Arginase (ARG)



When to be concerned?

- Signs and symptoms
 - Poor feeding, lethargy, and vomiting
 - Acidosis
 - Hyperammonemia
- If clinical suspicion exists
 - Forget NBS!
 - Call Metabolic physician on call
 - One call at TCH 720-777-3999
 - IMD clinic at 303-724-2338
 - Get pertinent labs
 - Comp metabolic panel (acidosis? hypoglycemia? LFTs?)
 - Ammonia
 - Urine organic acids, acylcarnitine profile, serum amino acids



Unanswered Questions

- Clinical outcomes of some of the disorders detected by NBS are not clear
- Treatment for some rarer disorders is also not clear
 - Vary from clinic to clinic
 - Vary from physician to physician
- Late complications might yet to be described
 - Maternal PKU
 - Ovarian failure in galatosemia
- Milder, possibly benign variants of disorders detected by NBS
 - Need for genotype-phenotype correlation
- Controversy of inclusions of certain disorders on NBS panels
 - SCAD deficiency and 3-MCC deficiency
- Long term follow-up needed!



MSGRCC

Metabolic Newborn Screening Long-term Follow-up Study

- A collaborative multi-state approach to newborn screening outcome research
- Biochemical geneticists, dietitians, genetic counselors and nurses throughout Mountain States region set out to develop a framework for LTFU of newborn screening
- Goal:
 - Develop LTFU program over a large population in a systematic manner to study the factors that affect outcome of all metabolic disorders detected by NBS

Mountain States Genetics Regional Collaborative Center



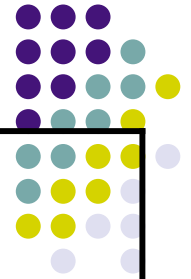
- 1 of 7 regional collaborative centers
- Arizona, Colorado, Montana, New Mexico, Nevada, Texas, Utah, and Wyoming
- Funded by US Dept of Health and Human Services, Health Resources, and Service Administration (HRSA) Genetic Services Branch
- Provide infrastructure to support regional genetics and NBS activities



Components of the Study

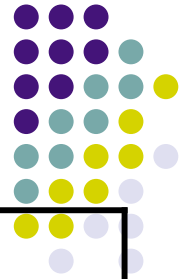
- Establishment of multi-state Metabolic consortium
 - Biochemical geneticists, genetic counselors, nurses, metabolic dietitians, and consumers
- Development of disease-specific care plans for majority of metabolic diseases detected by newborn screen
 - 28 in all
- Use of neuropsychological testing tools for long-term developmental outcome measures
 - Yearly questionnaires
 - Developmental evals at 3 and 6 years
 - Neuropsychological eval at 9 years
- Development of outcome measures for each disorder
 - “shared datasets”
 - Allows for systematic collection of data
- Database development

MSUD Care Plan



<p>Clinical Considerations</p> <ul style="list-style-type: none"> •Stabilizing neonate (essential AA versus hemodialysis) •Pancreatitis •Chronic demyelination from long-term elevated Leu •Type- intermittent, intermediate, classic 	<p>Initial labs (diagnostic & baseline)</p> <ul style="list-style-type: none"> •SAA +/- UOA •Basic metabolic panel •If symptomatic, osmolarity studies •BCKAD enzyme assay or molecular confirmation
<p>Diet considerations/ treatment</p> <ul style="list-style-type: none"> •Leu, Iso, Val restricted diet •BCAA-free formula •Avoid fasting •Supplementation <ul style="list-style-type: none"> •Thiamine trial •Consider valine/isovaline supplementation •Iron supplementation if low 	<p>Monitoring</p> <ul style="list-style-type: none"> •Quant serum branched chain AA •Targeted treatment range <ul style="list-style-type: none"> Leu <500µmol/L Isoleucine >100µmol/L Valine >100µmol/L •0-6 months Every week •6-12 months Every 2 weeks •1-3 years Monthly •>3 years Every 3 months
<p>Frequency of visits</p> <ul style="list-style-type: none"> •0-6 months Every 2 months •24 months Every 3 months •>2 yrs Every 6 months 	<p>Clinic visit labs</p> <ul style="list-style-type: none"> •See above

...MSUD Care Plan



<p>Emergency management</p> <ul style="list-style-type: none">• Immediate 10% dextrose with salts plus lipids• Cerebral edema risk-may need hemodialysis• Consider CT scan if edema present.• Track edema, Leu level ($>600 \mu\text{mol/L}$), Isoleucine ($>100 \mu\text{mol/L}$), valine ($>100 \mu\text{mol/L}$), use of dialysis, +/- mannitol, coma score, and osmolarity	<p>Labs to obtain during illness</p> <ul style="list-style-type: none">• Quant plasma amino acids• Basic metabolic panel• Osmolarity• Amylase and lipase
<p>Other evaluations</p> <ul style="list-style-type: none">• Brain MRI @ 1, 3, 6, & 9 yrs• Bone health<ul style="list-style-type: none">• DEXA-spine @ 9 & 18 y• Yearly developmental questionnaires (to be completed by parents)• Developmental eval @ 3 & 6 yrs• Neuropsych @ 9 & 18 yrs• Psychiatric screening @ 18y <p>• Consider referring to Liver for transplant</p>	<p>Yearly labs</p> <ul style="list-style-type: none">• Basic metabolic panel• Prealbumin• Plasma ferritin or transferrin• Amylase and Lipase• Consider essential fatty acid panel

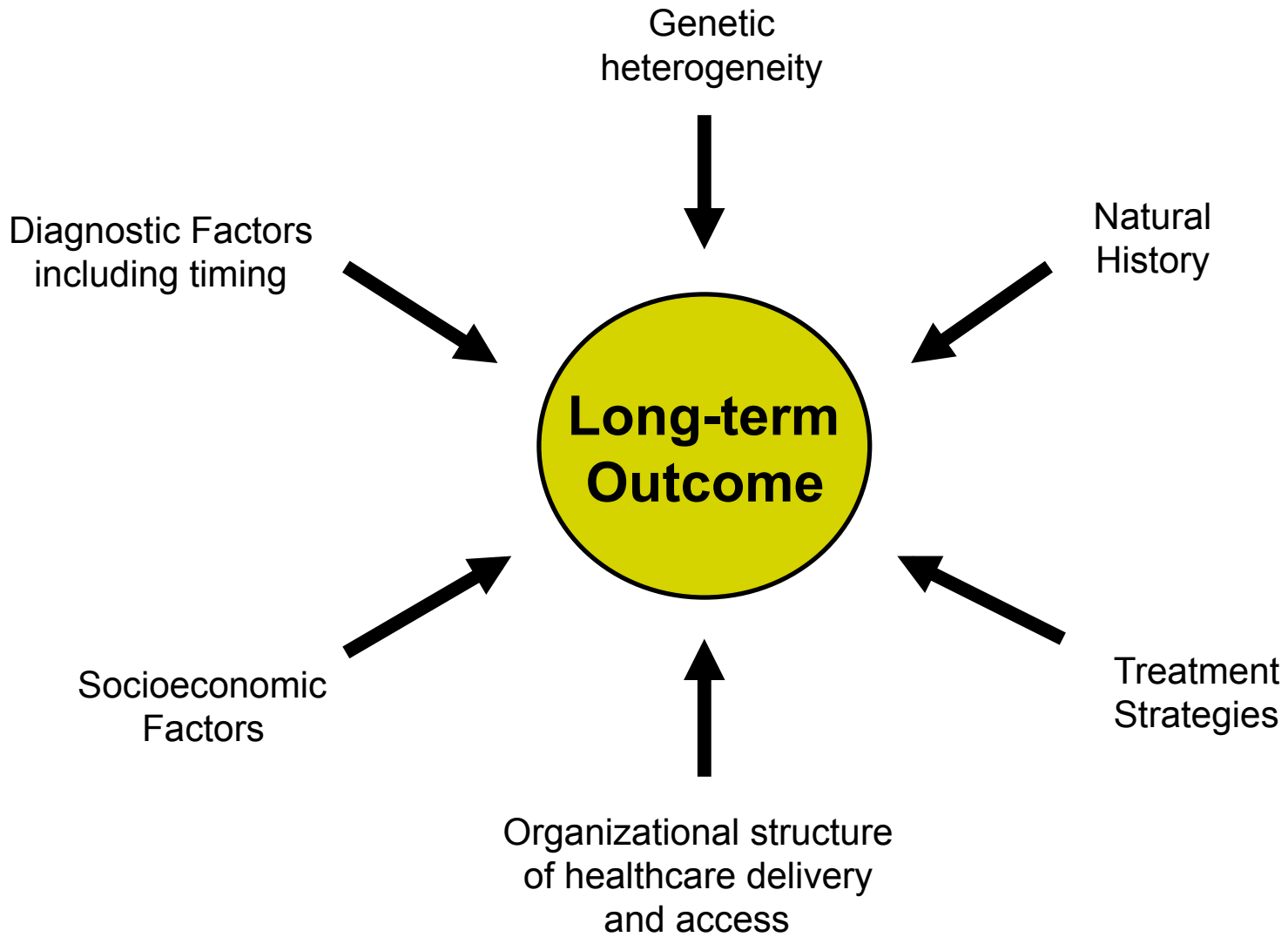
Performance Measures

1. Age of diagnosis (both positive NBS and confirmatory SAA)
2. Presence of illness at time of diagnosis.
3. Days until Leucine is within treatment range (<500µmol/L)
4. Therapy during initial care
 1. Enteral MSD formula vs. dialysis
 2. Track edema, Leu level (>600 µmol/L), use of dialysis, +/- mannitol, coma score, and osmolarity
5. Frequency of clinic visits and compliance with visits
6. Biochemical control
 1. Quantitative plasma amino acids
7. Laboratory studies
 1. Metabolic panel, prealbumin, ferritin or transferritin, amylase & lipase, fatty acid panel
8. Total decompensations and hospitalizations.
 1. Track edema, Leu level (>600 µmol/L), Isoleucine (>100µmol/L), valine (>100µmol/L), use of dialysis, +/- mannitol, coma score, and osmolarity
9. DEXA results and number of fractures
10. Neuropsychology evaluation results
11. Growth parameters
 1. Ht, wt, OFC, BMI
12. Type of MSUD
 1. Classic
 2. Intermediate
 3. Intermittent
 4. Thiamine responsive
 5. Lipoamide dehydrogenase (E3) deficiency
13. Diet
 1. Frequency of Dietician visits
 2. Frequency of dietary analysis (3 day diet records)
 3. Natural protein intake (tolerance)
 4. Formula (Y/N)
 5. Medical foods (Y/N)
 6. Mode
14. Transplant (Y or N)
15. Developmental services (PT, OT, speech, & IEP)

Outcome measures

1. Mortality
2. Development
 1. IQ
 2. Level of functioning
 3. Decline in IQ or level of function
3. History and/or presence of ADHD and use of medication
4. History and/or presence of psychiatric issues (generalized anxiety, panic, and/or depression)
5. History and/or presence of osteopenia
6. History and/or presence of abnormal MRI findings
7. Outcome of liver transplantation
8. Growth
 1. Final adult parameters







Future of NBS

- Improving current NBS by MS/MS
 - Development of 2nd tier tests
 - Limit both false positives and false negatives
- Lysosomal disorders
 - Krabbe in NY
 - Requires stem cell transplant within first few weeks of life
 - Prognosis???
 - Pilot studies of MPS disorders in other states
 - Enzyme replacement therapy or bone/stem cell transplant
 - Legislation to add MPS disorders passed in two states
- Severe Combined Immunodeficiency (SCID)
 - Bone marrow transplant
 - Pilot study in Wisconsin last year
- Fragile X syndrome
 - Benefit of early intervention and genetic counseling for parents



Conclusion

- Multiple metabolic disorders are on the NBS panel in Colorado and Wyoming
- Newborn screening is an amazing tool but shortcomings exist
 - Disorders may present early
 - Disorders may be missed
 - Many metabolic disorders are not detected by NBS

...Conclusion

- LTFU needed and being conducted in Wyoming as part of regional project
- Future of NBS is wide open!



Bibliography



- ACMG Newborn Screening Expert Group. Newborn screening: Toward a uniform screening panel and system- Executive Summary. *Pediatrics* 2006; 117:296-307.
- Fearing Marsha et al (2003) *Expanded Newborn Screening*. *Pediatric Annals*. 32(8) 509-515.
- Jones and Bennett (2002) *The changing face of newborn screening; diagnosis of inborn errors of metabolism by tandem mass spectrometry*. *Clinica Chmica Acta* 324 121-128.
- Kolker, Stephan et al (2006) *Natural History, Outcome, and Treatment Efficacy in Children and Adults with Glutaryl-CoA Dehydrogenase Deficiency*. *Pediatric Research* 59 (6) 840-847.
- Kolker, S. et al (2006) *Guideline for diagnosis and management of glutaryl-CoA dehydrogenase deficiency (glutaric acidemia Type I)*. *J Inherit Metab Dis* (2006).
- Shulze et al (2003) *Expanded Newborn Screening for Inborn Errors of Metabolism by Electrospray Ionization-Tandem Mass Spectrometry: Results, Outcomes, and Implications*. *Pediatrics* 111(6)1399-1406.
- Waisbren S. et al. Effect of Expanded Newborn Screening for Biochemical Genetic Disorders on Child Outcomes and Parental Stress. *JAMA* 2003; 290 (19): 2564-2570.
- Wilcox et al (2002) Postmortem screening for fatty acid oxidation by analysis for Guthrie cards with tandem mass spectrometry in sudden unexpected death in infancy. *The Journal of Pediatrics* 141(6) 833-836.
- Wilken et al (2003) *Screening Newborns for Inborn Errors of Metabolism by Tandem Mass Spectrometry*. *N Engl J Med* 348(23) 2304-2312.



Websites

- <http://genes-r-us.uthscsa.edu>
- <http://www.modimes.org>
- <http://www.newbornscreening.info>
- <http://www.savingbabies.org>
- <http://www.acmg.net/resources/policies/ACT/condition-analyte-links.htm>