

# Status of Newborn Screening for Homocystinuria

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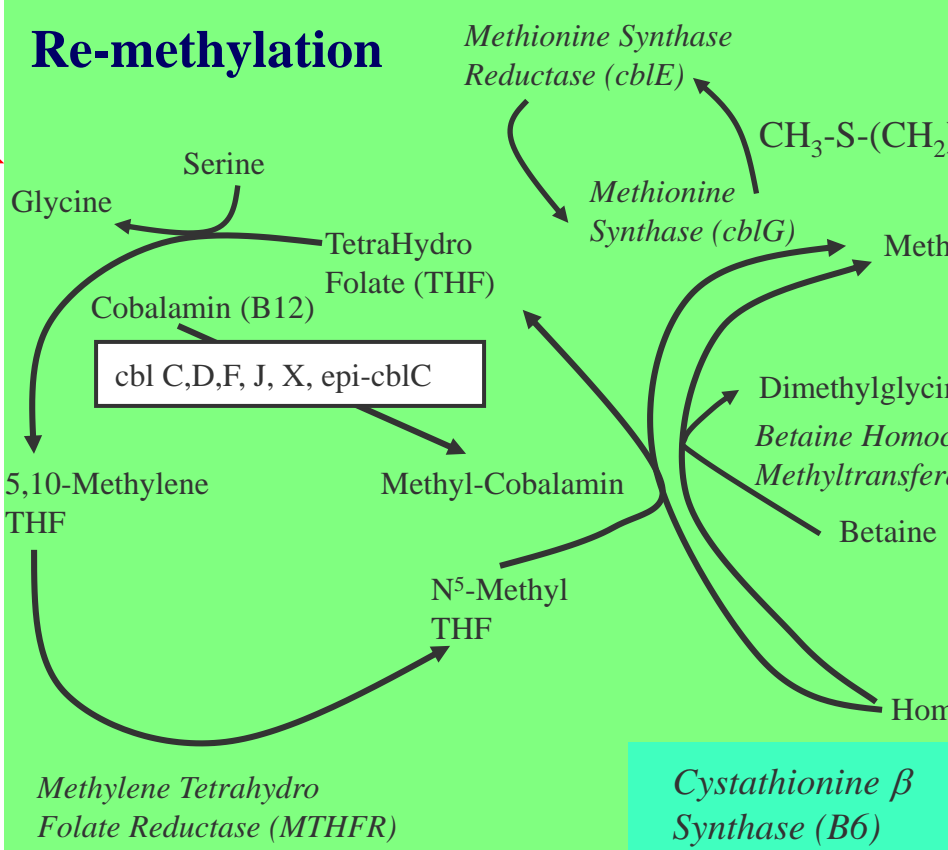
# Outline

- Homocystinurias
  - » Diagnostic biochemical patterns
- Clinical description of classic homocystinuria
- Newborn screening
  - » NBS markers for homocystinuria
  - » NBS second tier tests
- Recommendations for NBS for homocystinuria

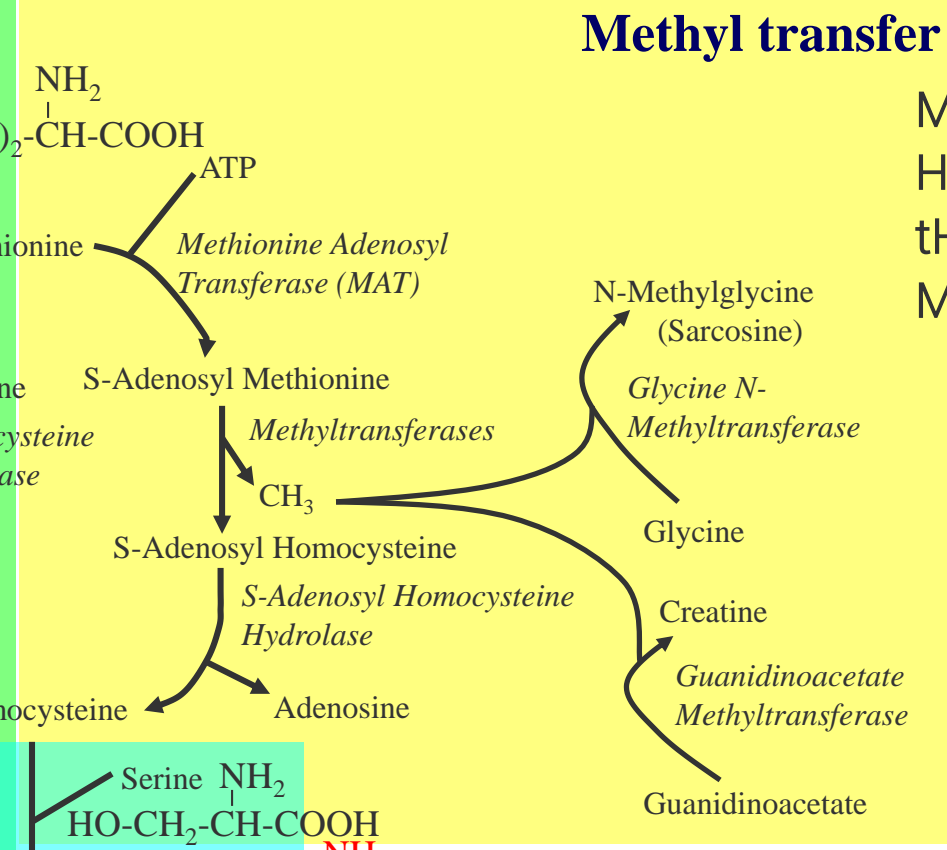
# Homocystinurias

- Homocystinurias are a group of disorders characterized by elevated homocysteine and, often, homocystine (two homocysteine molecules attached together).
- Only 1-2% of total homocysteine (HCY-SH) is present in its reduced form, the rest is bound to proteins through a disulfide bond (80%) or present as a homodimer, free homocystine, (HCY-S-S-HCY) or heterodimer with cysteine (HCY-S-S-CYS).
- Plasma amino acid analysis reveals only the homodimer free homocystine (HCY-S-S-HCY), which accounts for about 10% of the total homocysteine.
- Measurement of total homocysteine requires a step to reduce the disulfide bond.

## Re-methylation

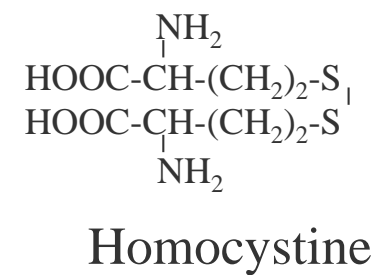
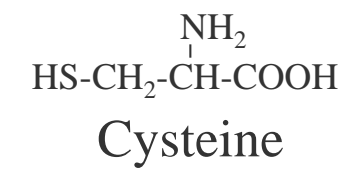
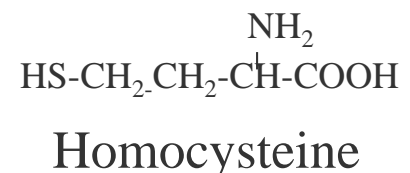
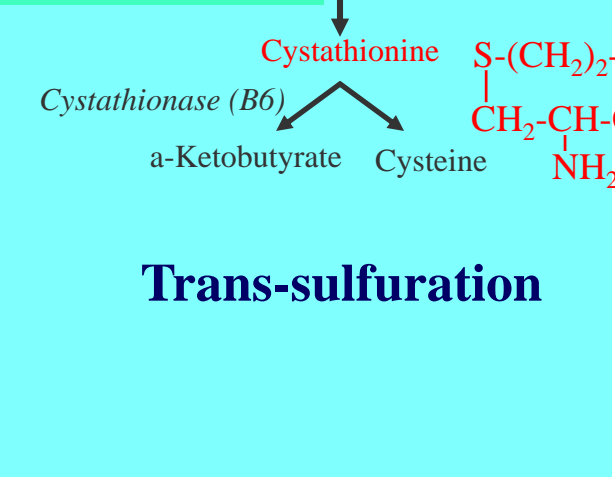


## Methyl transfer



Met ↑↑  
 HCY-S-S-HCY N  
 tHCY N - ↑  
 MMA N

## Trans-sulfuration



Met ↑-↑↑  
 HCY-S-S-HCY ↑  
 tHCY ↑↑  
 MMA N

Adapted from: Longo N. Inherited disorders of amino acid metabolism in adults. In Harrison's Principles of Internal Medicine, 20th Edition. 2018. McGraw-Hill Education, New York, NY.

# Homocystinurias: diagnostic patterns

- There are mainly 4 biochemical markers necessary for the diagnosis of homocystinurias:
- Methionine (Met)
  - » Elevated in cystathionine  $\beta$ -synthase deficiency (CBS, disorder of homocysteine trans-sulfuration)
  - » Low in homocysteine remethylation disorders
- Total homocysteine (tHCY)
  - » Markedly elevated in disorders of remethylation and in CBS
- Free homocystine (HCY-S-S-HCY)
  - » Elevated in disorders of remethylation and in CBS
- Methylmalonic acid
  - » Elevated in disorders of vitamin B12 metabolism impairing homocysteine remethylation and methylmalonic acid metabolism

# Classic Homocystinuria

- **Cause:** Deficiency of cystathionine  $\beta$ -synthase, a vitamin B6 (pyridoxine) requiring enzyme, resulting in the accumulation of methionine and excess homocyst(e)ine.
- **Incidence:** Incidence in the US by NBS=1:456,726 (Therrell BL, et al; Mol Genet Metab. 2014; 113(0):14-26) while the estimated prevalence is 1:200,000-1:335,000 (Sacharow SJ, Picker JD, Levy HL; GeneReviews® 2014 Jan 15; updated 2017 May 18)
  - » NBS can miss cases
- **Inheritance:** Autosomal recessive
- **Diagnosis:**
  - » NBS: increased methionine.
  - » Plasma amino acids: elevated methionine, presence of free homocystine. Markedly elevated total plasma homocysteine ( $>100 \mu\text{M}$ , normal  $<12 \mu\text{M}$ ).
  - » Confirmation: DNA sequencing.
- **Therapy:**
  - » Diet: Low protein diet with amino acid mixture lacking Met.
  - » Pyridoxine (100-500 mg/day) in responsive patients.
  - » Betaine (100-250 mg/kg up to 6 g/day divided BID) to favor homocysteine remethylation.
  - » Methylfolate, Vitamin B12.



# Classic Homocystinuria

- **Clinical Presentation:**
  - » Eye: lens dislocation and/or severe myopia
  - » Skeletal system: tall stature, long limbs, scoliosis, pectus excavatum, osteoporosis
  - » Vascular system: thromboembolism
  - » CNS: developmental delay/intellectual disability
- Thromboembolism is the major cause of early death and morbidity in untreated individuals (late childhood, young adults, typically)
- B<sub>6</sub>-responsive homocystinuria: milder phenotype than the non-responsive variant (mean IQ in untreated individuals=79 vs 57 for those B<sub>6</sub>-non-responsive)
  - » The majority of infants identified by NBS are B<sub>6</sub>-non-responsive; it is rare for a B<sub>6</sub>-responsive infants to have methionine elevated (above the decision limit/cutoff) at the time of the first NBS (24-48 hours of life)
- **Complications of homocystinuria can be prevented by early identification and treatment, therefore NBS is necessary**

# Newborn screening for cystathionine $\beta$ -synthase deficiency (classic homocystinuria)

- Tandem mass spectrometry (MS/MS) is almost universally used
  - » High throughput method
  - » Acylcarnitines and amino acids are detected and quantified
- Sensitivity of NBS for homocystinuria is dependent upon the choice of markers and decision limits (cut-offs)
  - » Methionine may not be above the cut-off in classic homocystinuria, especially for the B<sub>6</sub>-responsive variant at the time of the first NBS collection
    - Classic homocystinuria may be missed
- Ratios can be used as secondary markers to increase sensitivity
  - » Met/Phe accounts for protein intake



# Other causes of elevated methionine in newborn screening

- Dietary (high protein), low birth weight, prematurity (37% of infants with elevated methionine were premature)
- Liver disease
- S-Adenosylhomocysteine hydrolase deficiency (AHCY)
- Glycine N-Methyltransferase deficiency (GNMT)
- Methionine adenosyltransferase (MAT) deficiency
- Adenosine Kinase deficiency
- Citrin deficiency
- Tyrosinemia type 1

# Second tier tests and newborn screening

- Tests run on the same sample (DBS) used for the primary screen
- The targets are different analytes than those detected with the primary screen
- Often, a different methodology is used
- **What is the purpose of second tier tests?**
- Identify infants at risk of having a metabolic condition, while...
- Reducing false positives (proportion of non-affected individuals who test positive), and...
- Reducing false negatives (proportion of true affected individuals who test negative)

# Second tier tests and newborn screening

- Biochemical second tier tests (LC-MS/MS)
- Elevation of NBS markers is not due only to a metabolic condition (i.e. methionine and liver immaturity)
- Identify specific markers for metabolic conditions:
  - » Elevated methionine
    - Total homocysteine
- Molecular second tier tests

# Newborn screening for homocystinurias: recommendations

## **Newborn screening for homocystinurias: Recent recommendations versus current practice**

*J Inherit Metab Dis.* 2019;42:128–139.

- Revision of decision limits (cutoffs) with reference to the median
- Use a combination of markers (Met and/or Met/Phe)
- Use post-analytical tools (CLIR) which includes covariates (age at collection, BW)
- Implementation of second tier tests (tHcy and MMA)

# Summary

- Newborn screening for classic homocystinuria is possible and effective
- The primary marker (methionine) is not sensitive to detect all cases
  - » More sensitive and specific markers are needed
- Use of multiple markers increases the sensitivity
- Second tier tests are effective in reducing the number of false positives and false negatives, increasing both, sensitivity and specificity; however, it can be a burden to NBS laboratories
- Use of bioinformatics tools (e.g., CLIR) helps identifying samples needing 2nd tier tests, decreasing the burden to NBS laboratories



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