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.

Met ↓
HCY-S-S-HCY ↑
tHCY ↑↑
MMA N - ↑

Re-methylation

Methionine Synthase Reductase (cblE)

Methionine Synthase (cblG)

Serine

Glycine

TetraHydro Folate (THF)

Cobalamin (B12)

cbl C,D,F, J, X, epi-cblC

5,10-Methylene THF

Methyl-Cobalamin

N^5-Methyl THF

Methylene Tetrahydro Folate Reductase (MTHFR)

Methyl transfer

NH_2
CH_3-S-$(CH_2)_2$-CH-COOH

ATP

Methionine

Methionine Adenosyl Transferase (MAT)

S-Adenosyl Methionine

Methyltransferases

CH_3

S-Adenosyl Homocysteine

S-Adenosyl Homocysteine Hydrolase

Adenosine

Homocysteine

Dimethylglycine

Betaine Homocysteine Methyltransferase

Betaine

N-Methylglycine (Sarcosine)

Glycine N-Methyltransferase

Glycine

Creatine

Guanidinoacetate Methyltransferase

Guanidinoacetate

Met ↑↑
HCY-S-S-HCY N
tHCY N - ↑
MMA N

Trans-sulfuration

Cystathionine β Synthase (B6)

Serine NH_2
HO-CH_2-CH-COOH

Cystathionine

NH_2
S-$(CH_2)_2$-CH-COOH
CH_2-CH-COOH
NH_2

Cystathionase (B6)

a-Ketobutyrate

Cysteine

NH_2
HS-CH_2-CH_2-CH-COOH

Homocysteine

NH_2
HS-CH_2-CH-COOH

Cysteine

NH_2
HOOC-CH-$(CH_2)_2$-S
HOOC-CH-$(CH_2)_2$-S
NH_2

Homocystine

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 β-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 β-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 μM, normal <12 μM).
  - » 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.

Morris AA, Kožich V, Santra S, Andria G, Ben-Omran TI, Chakrapani AB, Crushell E, Henderson MJ, Hochuli M, Huemer M, Janssen MC, Maillot F, Mayne PD, McNulty J, Morrison TM, Ogier H, O'Sullivan S, Pavlíková M, de Almeida IT, Terry A, Yap S, Blom HJ, Chapman KA. Guidelines for the diagnosis and management of cystathionine beta-synthase deficiency. J Inherit Metab Dis. 2017 Jan;40(1):49-74. doi: 10.1007/s10545-016-9979-0. Epub 2016 Oct 24. PMID: 27778219

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_6-responsive homocystinuria: milder phenotype than the non-responsive variant (mean IQ in untreated individuals=79 vs 57 for those B_6-non-responsive)
  - » The majority of infants identified by NBS are B_6-non-responsive; it is rare for a B_6-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

Sacharow SJ, Picker JD, Levy HL. Homocystinuria Caused by Cystathionine Beta-Synthase Deficiency. 2004 Jan 15 [Updated 2017 May 18]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1524/

Newborn screening for cystathionine β-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_6-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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