Diagnostic follow-up of 47 infants with a positive newborn screen for Hurler syndrome: Identification of four recurrent IDUA sequence changes that significantly reduce enzyme activity

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Hurler syndrome (MPS I)

- Symptoms appear at ~6 months of age
- Coarse features
- Short stature
- ID / developmental delay
- Hepatosplenomegaly
- Cardiac problems
- Respiratory problems
- Corneal clouding
- Dysostosis multiplex
- Contractures/ restricted motility

- $\alpha$-iduronidase enzyme deficiency
- Autosomal recessive ($\text{IDUA}$ gene)
- Accumulation of heparan sulfate & dermatan sulfate in lysosomes of various body tissues
- Excretion of heparan sulfate & dermatan sulfate in urine
- Incidence = 1 / 100,000-150,000
- Severe (classic) OR mild (Scheie / Hurler-Scheie)

“classic” Hurler syndrome

Hurler-Scheie syndrome

Atlas of Inherited Metabolic Diseases
Newborn screening for Hurler syndrome

- State legislation mandating newborn screening for LSDs
  - Pompe, Gaucher, Fabry, Krabbe, Niemann-Pick A/B, Hurler
  - MS/MS substrates or fluorescent substrates via microfluidics
- Missouri started screening for four LSDs, including Hurler syndrome, January 2013 using Digital Microfluidics platform
- GGC diagnostic laboratory has performed confirmatory testing on 47 infants with positive NBS for Hurler syndrome in Missouri
- Measurement of alpha-iduronidase enzyme in leukocytes
  - “Gold standard” diagnostic assay
- Molecular analysis of IDUA gene in individuals with low enzyme activity
Correlation between alpha-iduronidase activity in leukocytes vs. NBS result

Current cut-off value

- **NORMAL**
- **AFFECTED**
Alpha-iduronidase activity in leukocytes from infants with positive NBS

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Graph illustrating the distribution of alpha-iduronidase activity (nmol/hr/mg) in leukocytes from infants with positive Newborn Screening (NBS), categorized into normal, affected, and gray-zone ranges.

- **Normal** (>6 nmol/hr/mg) - 26 infants
- **Affected** (<1 nmol/hr/mg) - 17 infants
- **Gray-zone** (1-6 nmol/hr/mg) - 3 infants

Total infants = 46
Characterization of 29 patients with reduced alpha-iduronidase activity

• No patients appear clinically affected
  ▪ all currently less than 21 months of age

• 24/29 had urine GAG studies performed
  ▪ No patients had qualitative urine GAGs consistent with Hurler
  ▪ 3 had slightly elevated total urine GAGs

• 28/29 had IDUA gene sequencing performed
  ▪ No patients were homozygous or compound heterozygous for two previously reported pathogenic mutations
Recurrent sequence alterations in patients with reduced alpha-iduronidase activity

- 9 patients homozygous for p.A79T
- 1 patient homozygous for p.H82Q
- p.H82Q identified in European Americans
- These four changes = **45/58** alleles in patients with reduced activity
Potential pseudo-deficiency alleles?

- **Pseudo-deficiency** = reduced *in vitro* enzyme activity in clinically unaffected individuals
- Documented for at least 7 different LSDs including Hurler syndrome
- Two possible explanations:
  1) Sequence alteration results in reduced, but not absent, enzyme activity towards both natural & artificial substrates
  2) Sequence alteration results in reduced enzyme activity towards artificial substrate only
Alpha-iduronidase activity in DBS using MS/MS substrate

25/26 patients with reduced leukocyte activity also had reduced activity in DBS using MS/MS substrate
Evidence for pseudo-deficiency

- **p.A79T**
  - Allele frequency of 2.8% in African Americans
  - 20/29 patients with reduced activity have at least one copy
  - Phenotypically normal adult is homozygous for this change

- **p.H82Q**
  - 0.74% allele frequency in European Americans

- **p.V322E**
  - 0.64% allele frequency in African Americans

- **p.D223N**
  - 0.53% allele frequency in African Americans

- None of these changes had been previously observed by our laboratory in clinically affected patients
3 patients with activity in affected range

- **Patient 1**
  - alpha-iduronidase activity = 0.252 nmol/hr/mg in leukocytes
  - normal urine MPS studies
  - homo p.S234T & p.T446N (both VUS)
  - normal alpha-iduronidase activity post-BMT

- **Patient 2**
  - alpha-iduronidase activity = 0.839 nmol/hr/mg in leukocytes
  - normal urine MPS studies
  - het p.G78D (VUS) + p.D223N

- **Patient 3**
  - alpha-iduronidase activity = 0.772 nmol/hr/mg in leukocytes
Conclusions

• Newborn screening has shown that alpha-iduronidase pseudo-deficiency is more common than previously realized
  ▪ Especially prevalent in African Americans
• Four new proposed pseudo-deficiency alleles for alpha-iduronidase
  ▪ Importance of IDUA molecular testing in patients with decreased enzyme activity
• Pseudo-deficiency alleles create many issues for clinicians/counselors:
  ▪ How do you explain this concept to families?
  ▪ Should asymptomatic newborns with “gray zone” activities be routinely followed or released from clinic?
  ▪ Should asymptomatic newborns with activities within the affected range but without a conclusive molecular diagnosis be treated?
Acknowledgments

• Greenwood Genetic Center
  Tim Wood, PhD
  Teresa Thompson
  Jenny Miller
  Christina Shouse
  Danica Carra

• Children’s Mercy Hospital (Kansas City, MO)
  Bryce Heese, MD
  Nicole Safina, MD
  Andrea Atherton, MS, CGC
  Meghan Strenk, MS, CGC
  Caitlin Lawson, MS, CGC

• University of Missouri
  Richard Hillman, MD
  Dawn Peck, MS, CGC

• Washington University School of Medicine (St. Louis, MO)
  Dorothy Grange, MD
  Marcia Willing, MD, PhD
  Marwan Shinawi, MD
  Linda Manwaring, MS, CGC

• SSM Cardinal Glennon Children’s Medical Center (St. Louis, MO)
  Stephen Braddock, MD
  Katherine Christensen, MS, CGC
  Deborah Boylan, RN

• Center for Disease Control
  Hui Zhou, PhD