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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Where Compassion Inspires Progress

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

- α-iduronidase enzyme deficiency
- Autosomal recessive (*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

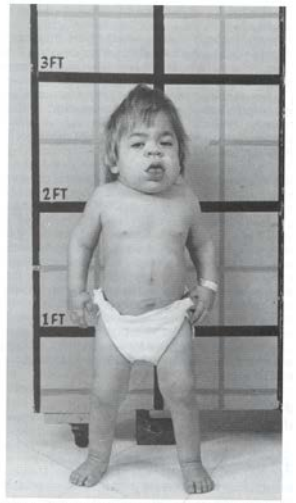


Fig. 136-6 MPS IH (Hurler syndrome) in a 4-year-old boy. Diagno

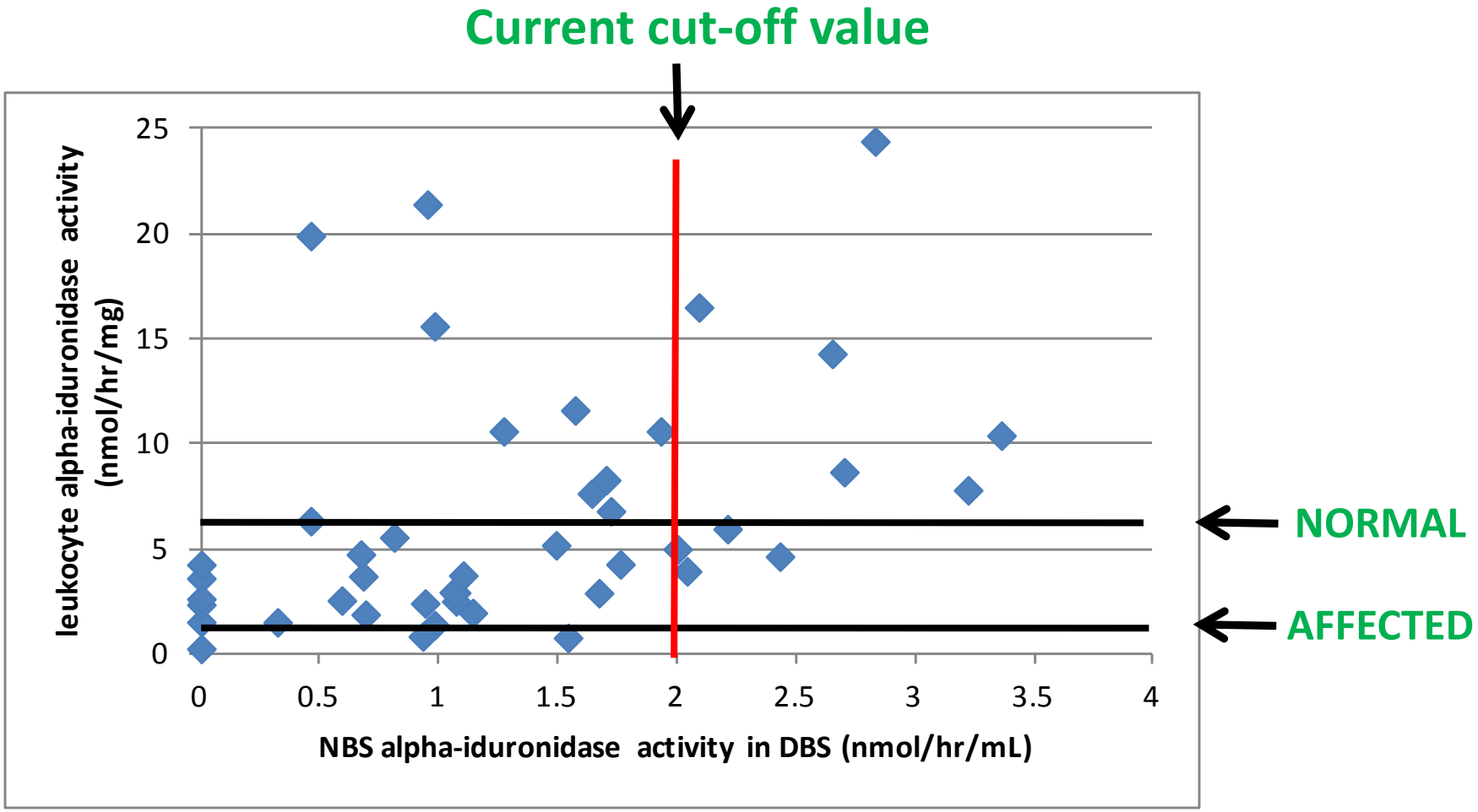
Hurler-Scheie syndrome

Atlas of Inherited Metabolic Diseases

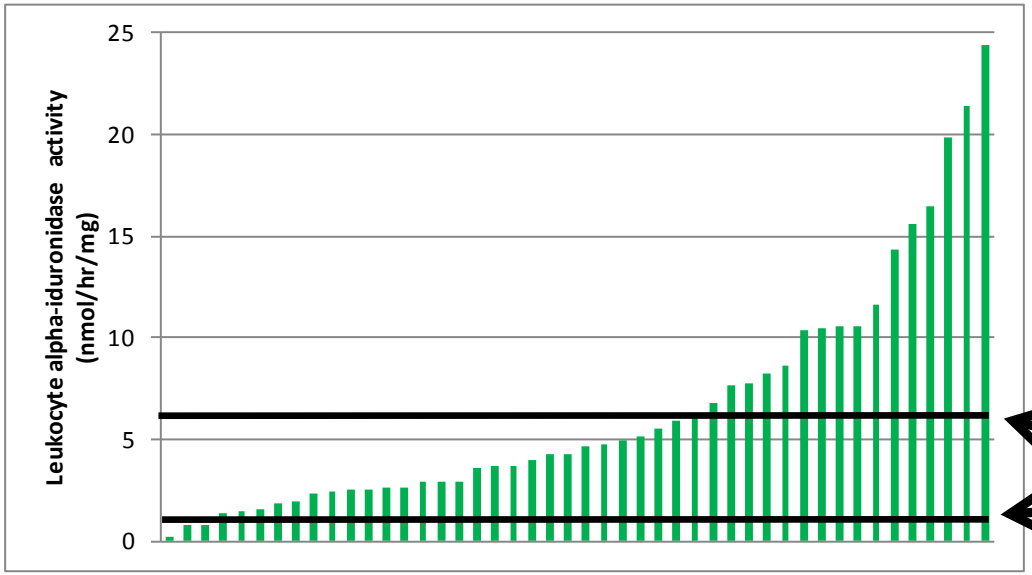
Newborn screening for Hurler syndrome

- 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

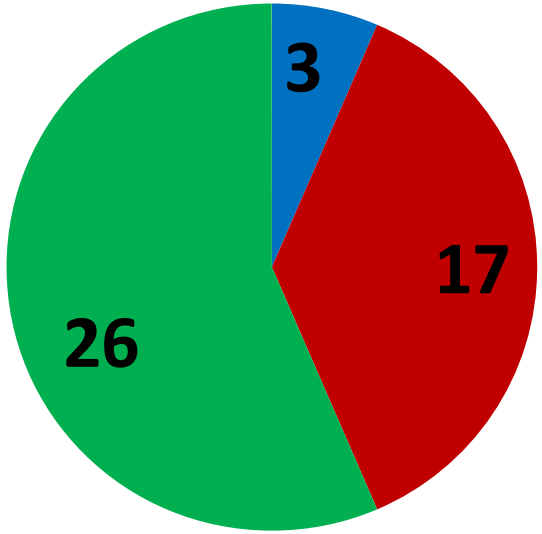
Correlation between alpha-iduronidase activity in leukocytes vs. NBS result



Alpha-iduronidase activity in leukocytes from infants with positive NBS



n= 46



Normal (>6 nmol/hr/mg)

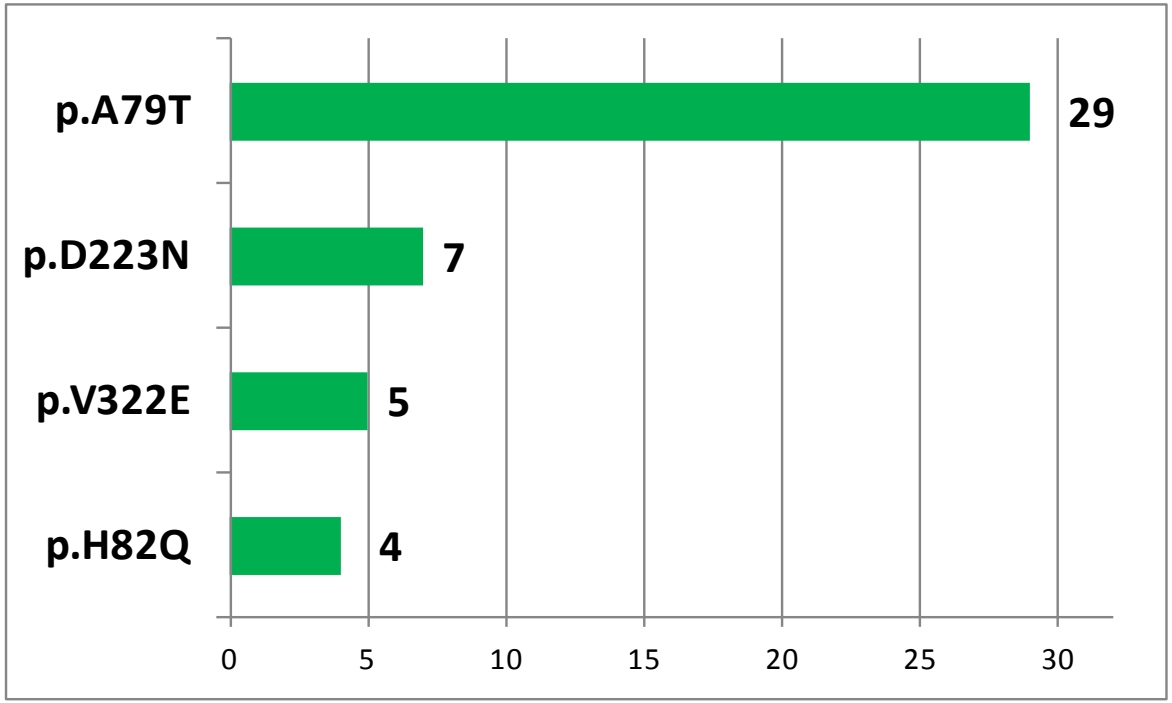
Affected (<1 nmol/hr/mg)

"Gray-zone" (1-6 nmol/hr/mg)

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.A79T, p.V322E & p.D223N identified in African Americans
- p.H82Q identified in European Americans
- These four changes = **45/58** alleles in patients with reduced activity

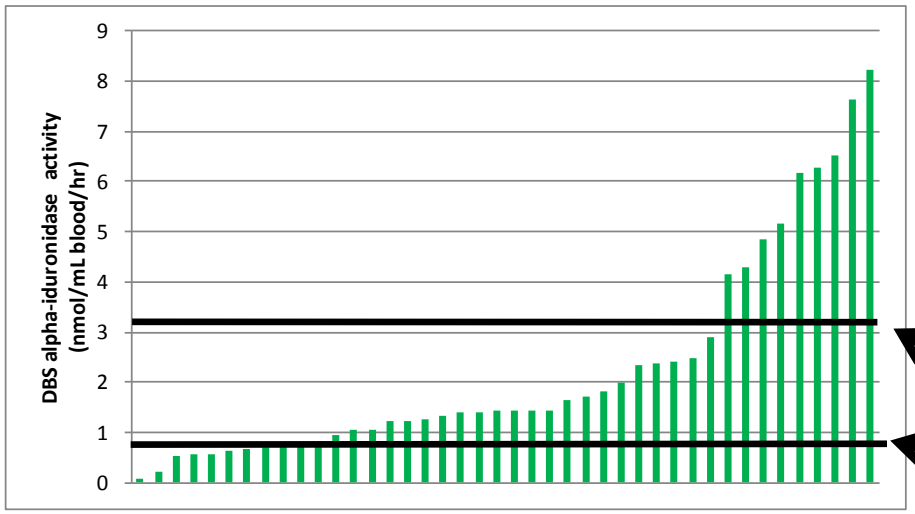
Potential pseudo-deficiency alleles?

- **Pseudo-deficiency**= reduced *in vitro* enzyme activity in clinically unaffected individuals
- Documented for at least 7 different LSDs including Hurler syndrome
- Two possible explanations:

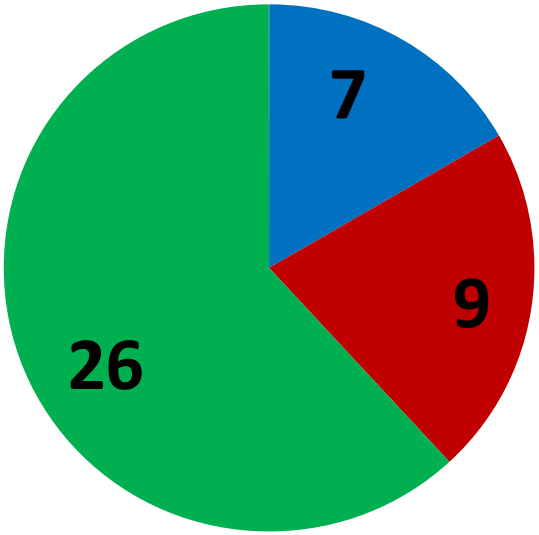
  1) Sequence alteration results in reduced, but not absent, enzyme activity towards both natural & artificial substrates

  2) Sequence alteration results in reduced enzyme activity towards artificial substrate only

Alpha-iduronidase activity in DBS using MS/MS substrate



n = 42



Normal (>3.2 nmol/mL/hr)
Affected (< 0.68 nmol/mL/hr)
"Gray-zone" (0.68-3.2 nmol/mL/hr)

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
  - 25% normal activity *in vitro* (Yogalingam *et al* (2004) *Hum Mutat.*)
- **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
  - het p.H240R (mutation) + **p.A79T**

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?

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