Neonatal Screening for Hemoglobinopathies

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Objectives

- Clinical relevance and purpose of neonatal/newborn screening for hemoglobin disorders
- Overview of NBS process
- Laboratory tests used for hemoglobinopathy screening and diagnosis
- Novel methods for hemoglobinopathy screening and diagnosis
  - Next generation sequencing
  - POC diagnostics in resource-limited settings

The speaker has no relevant financial disclosures
Normal hemoglobins in the newborn period & beyond

Normal hemoglobins:

Hb F: $\alpha_2\gamma_2$

Hb A: $\alpha_2\beta_2$

Hb A2: $\alpha_2\delta_2$
Classification of Hemoglobinopathies

• Qualitative (sickle cell disease)
  • Production of abnormal globin chains (e.g. Hb S, C, D, E)

• Quantitative (thalassemias)
  • Absent or decreased synthesis of globin chains
    • Alpha thalassemia = decreased alpha chain production
    • Beta thalassemia = decreased beta chain production
Why Screen for Hemoglobinopathies?

• Decrease incidence of disease
  – Premarital or pre-conceptual screening – identify carriers prior to pregnancy, genetic counseling
  – Prenatal screening – identify carriers early in pregnancy, offer PND, counseling and options

• Decrease mortality and morbidity in affected births
  – Newborn screening
    • SCD: prophylactic PCN, immunizations, early f/u
    • β thalassemias: f/u prior to development of severe anemia, education, chronic transfusions/HSCT
Sickle Cell Disease- Global Unmet Medical Need

- US Experience: 1000 births/yr
- NBS reduces infant mortality
  - pneumococcal prophylaxis, immunization
  - parent/caregiver education

- Africa /India ~ 300,000 births/yr
  - >50% die before age 5 yrs
  - SCD-related deaths are preventable

NBS decreases early mortality in US

Childhood Survival for SCD

NBS for Hemoglobinopathies in California

– In California,
  • 560,000 live births per year (>13% US births)
  • >99.9% screened
  • Race: 6% African-American, 13% Asian, 81% White
  • Ethnicity: 52% Hispanic
  • Screening panel includes Hb E, β thalassemia, Hb H disease

– Goal: early diagnosis
  • Sickle cell disease: penicillin prophylaxis, education
  • Thalassemia: early referral before development of severe anemia
Hemoglobin Disorders Identified by CA NBS, 2001-2010

- SCD, 59%
- Hb H, 33%
- β thalassemia, 8%

N = 5,419,093  Birth prevalence = 1:3300
49 different beta thalassemia mutations
(% affected newborns with specified \(\beta\) thal mutation)
Expected number of births with a hemoglobin disorder in the Philippines = 2,180
Components of NBS Program

- Reference Laboratory (Confirmatory Testing)
- Area Service Center
- Primary Care Provider
- Baby/Family
- Central Laboratory (GDL)
- SCD/Thal Center or Hematologist

1600 DBS samples/day
Primary Screening Test

F only
FS
FSC
FSA
FA Barts

Confirmatory Testing

β^0 thal
β^0 thal/HPFH
HPFH
SS
S/β^0 thal
S/HPFH
SC
S/β^+ thal

Report and Follow up

Hb H
α thal trait
Confirmatory Laboratory Testing

Biochemical (detect Hb variant)
- Electrophoresis (CAE, acid citrate)
- Isoelectric focusing (IEF)
- High Performance Liquid Chromatography (HPLC)
- Capillary electrophoresis (CE)

Molecular (identify mutation)
- Allele-specific PCR
- DNA sequencing
- Gap PCR (deletions, duplications)
- MLPA (deletions, duplications)

Second test required to confirm results of primary NBS test
Screening test is not diagnostic

- Disease or trait (carrier) ?
  - FSA: S/β+ thalassemia vs. FAS: sickle cell trait

- Disease or benign condition?
  - F only: β0 thalassemia vs. homozygous HPFH
  - FE: E/β0 thalassemia vs. EE

- Hb H disease or α thalassemia trait?
  - Barts >25% : Hb H (--/-α) vs. α thal trait (-α/-α, --/αα)
HPLC

Newborn: FA

- F (75%)
- A (25%)

Adult: A, A2

- A (97%)
- A2
Electrophoretic Methods

Citrate agar electrophoresis

Isoelectric focusing

Capillary electrophoresis

<table>
<thead>
<tr>
<th>Fractions</th>
<th>%</th>
<th>Ref. %</th>
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<tbody>
<tr>
<td>Hb F</td>
<td>100.0</td>
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Molecular Methods

Gap PCR

DNA Sequencing

Hb Hekinan: a1 27(B8)Glu->Asp
Codon 27
Molecular testing for deletions (MLPA)

mutation 1
β⁰ thalassemia
(codon 41/42, -TTCT)

mutation 2
Vietnamese 27kb deletion

Interpretation:
β-thalassemia

Multiplex ligation-dependent probe amplification (MLPA)
Next Generation Sequencing

Library Preparation

Clonal Amplification

Sequencing

Sample DNA
Fragmentation
Adapter ligation
Fragment library

Amplify each fragment into a clone
Emulsion PCR  Bridge amplification

Sequence clonal amplicons in flow cell
Image fluorescence or luminescence
Convert to sequence
**Does NGS have a role in NBS?**

### Benefits
- Test for additional conditions
- Increase sensitivity/specificity

### Considerations
- Cost
- Lab: TAT, technical training/qualifications, equipment
- Bioinformatics
- Storage and retention of genetic information
- Report interpretation, provider education
- Genetic counseling
- ELSI issues!

Newborn Screening or Newborn Diagnosis?
<table>
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<tr>
<th>Institution</th>
<th>Number of infant genomes</th>
<th>Objectives include</th>
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<tr>
<td>Brigham and Women’s, Boston Children’s hospitals, Boston</td>
<td>120 sick, 120 healthy</td>
<td>Study how parents and doctors use genomic data</td>
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<tr>
<td>Children’s Mercy Hospital, Kansas City, Missouri</td>
<td>500 sick</td>
<td>Diagnose genetic disorders within 24 hours</td>
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<tr>
<td>University of California, San Francisco</td>
<td>~1250 sick, ~200 healthy</td>
<td>Assess parent interest in drug metabolism genes</td>
</tr>
<tr>
<td>University of North Carolina, Chapel Hill</td>
<td>200 sick, 200 healthy</td>
<td>Study how to share results with multicultural families</td>
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NBS in Limited Resource Countries

• Current testing paradigm in developed countries
  – centralized laboratory
  – specialized equipment, cold-storage, reagents, technical expertise
  – complex workflow: sample collection, transport, data processing, follow up

• Insufficient resources in countries with highest prevalence
  – single national facility covering 20 million population
  – access limited to areas close to central lab
  – delays in turn-around time, tracking and follow up
  – high proportion of births outside of hospital
  – no established primary (well-child) care

Tshilolo, 2009
POC Diagnostics in Low Resource Countries

WHO ASSURED Criteria
- Affordable
- Sensitive (Hb S in presence of Hb F)
- Specific (Hbs S, C, A)
- User-friendly
- Rapid and reliable
  - results available at time of visit
- Equipment-free
- Deliverable (to areas needed)
- .... minimally invasive

Test utility at POC:
1) Identify infants
2) Screen for carriers
Point of Care Technologies for SCD

• Hb solubility
  – Paper-based matrix (Halcyon)

• Lateral Flow Immunoassays
  – SickleScan (Biomedomics)
  – HemoType SC (Silverlake)

• Electrophoresis (HemeChip)

• Sickle cell density
  – Aqueous multiphase polymer system (AMPS)
  – Magnetic levitation (Smartphone)

• Other POC for detection of anemia
  – Redox reaction; color-based assay for severe anemia (Anemocheck)
  – Hemolysis (ETCO)

(Silver Lake Research Corporation, Correlia Biosystems Inc, Halcyon Biomedical*, Daktari Dx*, BioMedomics*)
Paper-based POC for SCA (age > 1 year)

- Detects presence of Hb S (insoluble) vs. soluble Hbs (Hb A, F, C) by mobility through paper substrate
  - Blood mixed with buffer containing
    - Saponin- irreversibly **lyses** red blood cells (RBCs) > release Hb
    - Sodium hydrosulfite - converts Hb to **deoxy-Hb**:
      - deoxy Hb A, Hb E, Hb F, Hb C = soluble
      - deoxy Hb S = insoluble
  - Blood stain pattern
    - **Differential color count** in region polymerized Hb S vs. soluble Hbs
    - Analyzed by scanner for correlation with Hb S concentration

Paper-based POC for SCA (age > 1 year)

- SCD index derived from blood stain pattern
  - fraction soluble Hb (color intensity) at 5 mm
  - Semi-quantitative %Hb S
- Testing in Cabinda, Angola (226 adults)
  - Accurately identified Hb AA, Hb AS and Hb SS
  - 94% sensitivity / 97% specificity for Hb S
- Limitations of assay
  - Cannot distinguish Hb AS (trait) from Hb SC (disease), as similar amount Hb S
  - Not specific for +/- Hb A, C, other Hbs
  - Turnaround time = 35 minutes
  - Limit of detection = 20% Hb S, cannot use to identify newborns
Biomedomics POC Assay (SickleSCAN™)

- Simple technology using antibodies for Hb A, S, C
- LOD
  - HbA > 40%
  - HbS > 1%
  - HbC > 2%
- Test is **QUALITATIVE**
- In lab, 137 WB samples
  - 99% sensitivity, specificity for detection Hb A, S, C disorders
  - Results determined by custom-coded scanner

SickleSCAN POC Assay (Biomedomics)

• POC testing
  – USA (n=71): Accuracy 99% for Hb A, S, C
  – Angola (n=139): Accuracy >98% for Hb SS, 100% for HbSC
  – No interference by high Hb F
  – Hb S and Hb C detected at concentrations as low as 1-2%
• DBS samples—clear positive bands, similar sensitivity and specificity as whole blood
• Storage at 37°C gave reliable results
• Limitations: variable intensity of Hb A band
  – LOD Hb A >40% (S/beta+ thal will be called as AS trait)
  – Polyclonal Abs (other variants E, D, O-Arab cross-react with Hb A)

• ASSURE POC criteria - simple, rapid, robust, high sensitivity/specificity for detection of Hb A, S, C
  – COST?

A rapid, inexpensive and disposable point-of-care blood test for sickle cell disease using novel, highly specific monoclonal antibodies.

Hb S in sample

Gold particle conjugate with Hbs S, C, A

C S A Control

RESULT: A/A A/S A/C S/S S/C C/C
POC Micro-electrophoresis Assay (HemeChip)

89% Sensitivity, 86% Specificity