Genome- & AI-Informed Individualized Care of infants in ICUs

Stephen Kingsmore, MD DSc
I do not have a conflict of interest with regard to this presentation
55% Disease of Unknown Etiology

Search for etiological diagnosis

Interim empirical treatment

Infants in NICUs & PICUs

Improvement or worsening

Treatment Modification

Genetic Testing 6+ weeks

- 15% of 250,000 US NICU and PICU infants have 1 of 14,000 genetic diseases
- 60% go undiagnosed and ∴ are mistreated; leading cause of death

Discharged home undiagnosed

Delayed diagnosis; anxiety, suffering

Unnecessary morbidity and mortality

Delayed palliative care

Unnecessary Cost

NSIGHT2, ClinicalTrials.gov ID NCT03211039, 213 enrollees, 47% of NICU, 15% incidence
<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
<th>Cost</th>
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Two Early Studies of Clinical Utility of rWGS-based Individualized Medicine

35 Received standard tests
2 (6%) Genetic disease diagnosis
0 (0%) Change in management

35 Received rWGS
20 (57%) Genetic disease diagnoses
11 (31%) Changes in management
4 (11%) Favorable change in outcome
1 (3%) Life saved

(before individualized medicine mentoring)

npj Genomic Med (2018 3:6)

Proportion Diagnosed

Time to Diagnosis (days)

rWGS + Standard Testing
Standard Testing
Two Recent Studies of Effectiveness of rWGS-based Individualized Medicine

48 Eligible: Inpatient, <1 year of age, no etiologic diagnosis, possible genetic disease

42 Consented, received standard of care and rWGS

4 (10%) Genetic disease diagnosis by standard test

1 (2%) Change in management

0 (0%) Change in outcome

18 (43%) Genetic disease diagnoses by rWGS

13 (31%) Changes in management

11 (26%) Changes in outcome

Time from blood sample receipt to diagnosis

Ultra-rapid WGS

Rapid WGS

Rapid WES

npj Genomic Medicine (2018) 3:10

NSIGHT2, ClinicalTrials.gov ID NCT03211039
2019 status of rWGS-based individualized medicine for NICU and PICU Infants

55% Disease of Unknown Etiology

Search for etiological diagnosis inc. rWGS

Interim empirical treatment

rWGS 1.5-10 days

35% Genetic Disease Diagnosis

Infants in NICUs & PICUs

25% Individualized Medicine

20% improved outcomes
Less cost & suffering
Barriers to Adoption of rWGS-based individualized medicine for NICU and PICU infants

- Capital & labor intensity of individualized medicine
  - rWGS and rWES methods are too slow to guide NICU and PICU management
  - rWGS and rWES methods not scalable
- Shortage of expert medical geneticists, laboratory directors, genetic counselors
- Lack of clinician familiarity with individualized medicine
  - Shortage of expert medical geneticists, genetic counselors
- Insufficient evidence of efficacy
  - Absence of reimbursement
- Many genetic diseases lack effective Rx
  - Most treatments lack adequate evidence
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3rd generation whole genome sequencing with automated genetic disease diagnosis

Median time to result 20 hours; In Press
17 month old boy with fever ($T_{\text{max}} 103^\circ\text{F}$) x 3 days, vomiting, diarrhea, abdominal pain, labored breathing, skin lesions x 1 day
Rady Emergency Department Work Up

- **Blood tests:**
  - Metabolic panel: metabolic acidosis
  - C-reactive protein: markedly elevated
  - Complete blood count: low white cell count

- **Abdominal ultrasound & computed tomography:** No intussusception, possible mild colitis

- **Lumbar puncture**
  - Cardiovascular decompensation = hypovolemic shock → intravenous fluids
  - SiO$_2$ 88% on FiO$_2$ 21% → Continuous Positive Airway Pressure ventilation
  - Sepsis suspected → intravenous vancomycin + ceftriaxone
  - Admitted to PICU → switched to intravenous meropenem
Hospital Day 2

- Blood culture: Pseudomonas aeruginosa
- Skin rash diagnosed as echthyma gangrenosum
- Rapid genome sequencing ordered
Step 1: Order in Epic Electronic Health Record

- Counseling Provided and Consent Obtained: Yes
- Select Test Type: Proband
- Opt Out of Additional Use: No
- Include Incidental Findings: Yes
- Briefly Provide Clinical Description: Pseudomonal sepsis, leukopenia
- Are Family Member Samples Expected: Yes
- Name and DOB of Expected Family Member Sample: Mother
- Ultra-Rapid Processing: Yes
Step 2: Genome library preparation (2.5 hours)

- 0.5 ml blood, dried blood spot
- Illumina Nextera Flex
Step 3: Genome Sequencing (15.5 hours)

- 2 x 100 nucleotide paired sequences
- Illumina NovaSeq 6000 instrument
- S1 flowcell
- Trio or 2 Probands per flowcell
- 40X proband; 30X parents
Step 4: Identify all disease-causing variants in child’s genome: 45 min

125 billion nucleotides sequenced

2.8 billion genomic nucleotides assigned

4.9 million variants identified & genotyped

Glossary: Nucleotide – a single DNA letter (base); Adenine, Cytosine, Guanine or Thymidine

Variant – a DNA change from the (normal) reference genome sequence
### Step 5: Variant pathogenicity scoring

<table>
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<tr>
<th>Variant Category</th>
<th>Criteria</th>
</tr>
</thead>
</table>
| Pathogenic (P): 99% disease causing | 1 VS + (1S or 2M/Sup)  
1 S + (3M or 2M+2Sup) |
| Likely Pathogenic (LP): 90% disease causing | 1 VS/S + 1 M  
1 S + (1 M or 2 Supp)  
3 M  
2 M + 2 Supp  
1 M + 4 Supp |
| Variant of Uncertain Significance (VUS): 10% disease causing |  |

<table>
<thead>
<tr>
<th>Very Strong (VS)</th>
<th>Null variant (nonsense, frameshift, ±1 or 2 splice site position, initiation codon, exon deletion) in gene where LOF known to cause disease</th>
</tr>
</thead>
</table>
| Strong (S)       | Same amino acid change as previously established pathogenic variant  
De novo in a patient with the disease and no family history  
Functional studies show damaging effect on the gene  
Prevalence in affected individuals significantly greater than controls |
| Moderate (M)     | Located in mutational hot spot/functional domain without benign variation  
Extremely low frequency in Gnomad  
Recessive disorders, detected in trans with a pathogenic variant  
Protein length changed by in-frame indel in nonrepeat region or stop-loss  
Novel missense at amino acid where different missense known to be pathogenic  
Assumed de novo, but without confirmation of paternity and maternity |
| Supporting (Supp) | Cosegregation with disease in multiple affected family members in gene known to cause disease  
Missense variant in gene with low rate of benign missense variants and where missense variants commonly cause disease  
Multiple computational tools call deleterious  
Phenotype highly specific for disease with single genetic etiology  
Reputable source reports as pathogenic, but unpublished |

Step 5: Variant Pathogenicity Scoring: 2 mins

Diploid MOON software with InterVar post-processing

Variants present in <1:100 people
Pathogenic and Likely Pathogenic Variants
Why collect a deep phenotype

- The clinical features of NICU infants do NOT correspond well with classical descriptions of their disease
- The ability to make a diagnosis is critically dependent on a full clinical description

Glossary: Phenotype – the clinical features of a patient with a disease

76 children with genetic diseases; natural language processing of EHR; Text book: Mendelian Inheritance in Man
Step 6: Deep Phenotyping by Natural Language Processing of Epic EMR: 20 sec

CliniThink CLiXENRICH natural language processing software
Step 7: Translate phenotypes to a hierarchical standardized vocabulary

Admitted from ED for poor feeding, ketonuria and metabolic acidosis

HP:0001941 Acidosis
HP:0001942 Metabolic acidosis

HP:0002919 Ketonuria

HP:0011968 Feeding difficulties
Step 8: Pattern Recognition creates a comprehensive differential diagnosis
Step 9: Automated Diagnosis: 2 mins
Manual Diagnosis: 1 – 10 hours

Diploid MOON software with InterVar post-processing
The patient's phenotype might be caused by:

- X-linked recessive
- Inherited from mother
- Loss of splice donor site of intron 11
- Classified as pathogenic
- Confirmed by functional studies
Hospital Course

• Diagnosis after 22 hours
• Individualized medicine
  • Double coverage, double duration antibiotics
  • Intravenous immunoglobulin to maintain IgG level >600mg/dL
  • Magnetic resonance imaging: no additional septic emboli

• Prognosis
  • Normal life
  • 10% have significant infections despite treatment

• Genetic counseling
  • Mother is a carrier
  • Maternal relatives at-risk

• Discharged home on day 13

Smith and Berglof, Gene Reviews, 2016
Diagnostic performance of 3rd generation rWGS-based individualized medicine

- Retrospective, n=84 children with 86 diagnoses
  - Expert manual interpretation: Precision 98%, Recall 98%
  - Automated interpretation: Precision 99%, Recall 95%

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Retrospective Patients</th>
<th>Prospective Patients</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>263 8 days</td>
<td>6194 5 days</td>
</tr>
<tr>
<td></td>
<td>6124 14 years</td>
<td>290 3 days</td>
</tr>
<tr>
<td></td>
<td>3003 1 year</td>
<td>352 7 weeks</td>
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<tr>
<th>Abbreviated Presentation</th>
<th>Method</th>
<th>Total (hours)</th>
<th>Molecular Diagnosis</th>
<th>Gene and Causative Variant(s)</th>
</tr>
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<tr>
<td>Neonatal seizures</td>
<td>Auto.</td>
<td>20:25</td>
<td>Early Infantile Epileptic Encephalo-</td>
<td>KCNQ2 c.727C&gt;G</td>
</tr>
<tr>
<td>Rhabdomyolysis</td>
<td>Auto.</td>
<td>19:56</td>
<td>Glycogen Storage Disease V</td>
<td>PYGM c.2262delA c.1726C&gt;T</td>
</tr>
<tr>
<td>Dystonia, Dev. delay</td>
<td>Auto.</td>
<td>19:20</td>
<td>Dopa-Responsive Dystonia</td>
<td>TH c.785C&gt;G c.541C&gt;T</td>
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<tr>
<td>Hypoglycemia seizures</td>
<td>Auto.</td>
<td>19:14</td>
<td>None</td>
<td>n.a.</td>
</tr>
<tr>
<td>Pulmonary hemorrhage</td>
<td>Std.</td>
<td>40:24*</td>
<td>Permanent neonatal diabetes</td>
<td>INS c.26C&gt;G</td>
</tr>
<tr>
<td>Diabetic ketoacidosis</td>
<td>Auto.</td>
<td>19:11</td>
<td>None</td>
<td>n.a.</td>
</tr>
<tr>
<td>Neonatal seizures</td>
<td>Std.</td>
<td>19:10</td>
<td>None</td>
<td>n.a.</td>
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<tr>
<td>HIE, anemia</td>
<td>Auto.</td>
<td>57:21</td>
<td>X-linked agamma-globulinemia</td>
<td>BTK c.974+2T&gt;C</td>
</tr>
<tr>
<td>Pseudomonal septic shock</td>
<td>Std.</td>
<td>31:02†</td>
<td>Benign familial neonatal seizures 1</td>
<td>KCNQ2 c.1051C&gt;G</td>
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<tr>
<td>Neonatal seizures</td>
<td>Auto.</td>
<td>34:38</td>
<td></td>
<td></td>
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<tr>
<td>Age 17 months</td>
<td>Std.</td>
<td>22:04</td>
<td></td>
<td></td>
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<tr>
<td>Age 3 days</td>
<td>Auto.</td>
<td>38:37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 20:53</td>
<td>Std.</td>
<td>48:23</td>
<td></td>
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Summary: Strategies are being implemented to address barriers to rWGS-based individualized medicine for NICU and PICU infants

- Automated 20-hour diagnosis by rWGS
- Lack of clinician familiarity with individualized medicine
  - APIs for FAQs, counseling
  - Case-based learning via podcast/webinar
- Insufficient evidence of efficacy
  - Cost effectiveness studies
  - Payor collaborations
- Many genetic diseases lack effective treatments
  - APIs for reference individualized management guidance
Summary: By 2021 we predict the early majority of children’s ICUs to implement individualized medicine

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<td>2021</td>
<td>Hybrid Effectiveness-Implementation Studies</td>
<td>$5,000</td>
<td>1 day</td>
<td>Sustain / Evolve</td>
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Outcomes:

- ⅓ diagnosed
- ¼ individualized medicine
- ⅕ improved outcomes
Acknowledgments

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