Newborn Screening for Pompe Disease in New York Identifies a Wide Spectrum of Variants in the GAA Gene
Pompe Disease

- AKA: alpha-1,4-glucosidase deficiency; acid maltase deficiency; glycogen storage disease type II

- Lysosomal storage disorder - accumulation of glycogen in lysosomes due to enzyme deficiency

- Autosomal recessive disease caused by mutations in the GAA gene

- Estimated incidence in the US is 1 in 28,000 - 40,000

- Treatment: Enzyme Replacement Therapy (Lumizyme)
## Pompe Disease

<table>
<thead>
<tr>
<th>Type</th>
<th>Age at onset</th>
<th>Symptoms</th>
<th>Prognosis without treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic Infantile-Onset</td>
<td>Birth to first few months of life</td>
<td>Cardiac defects; poor muscle tone and weakness; enlarged liver</td>
<td>Death by 1 year due to heart failure</td>
</tr>
<tr>
<td>Non-classical (Atypical) Infantile-Onset</td>
<td>Within the 1st year of life</td>
<td>Delayed motor skills; progressive muscle weakness</td>
<td>Death in early childhood due to respiratory problems</td>
</tr>
<tr>
<td>Late-onset</td>
<td>Onset after the 1st year of life</td>
<td>Progressive muscle weakness especially in legs and trunk; breathing difficulties</td>
<td>Variable</td>
</tr>
</tbody>
</table>
NYS Pompe Screening Algorithm

GAA Enzyme Activity determined by MS/MS
< 20% Daily Mean

Retested in duplicate*

GAA Enzyme Activity determined by MS/MS
Average < 15% Daily Mean

DNA Sequencing + Exon 18 Deletion Analysis

*recent modification to include testing on LSD 6-plex assay
Sanger Sequence Analysis of the GAA Gene

- DNA extracted from 3mm blood spot using an in-house developed method
- Amplify exons 2 – 20 and 20bp at the intron/exon boundaries in 14 fragments (amplicons)
- Sequence each amplicon bi-directionally
- Identify variants by comparison to reference sequence
- Perform a gap PCR gel-based assay to identify commonly reported exon 18 deletion
- Classify variants for pathogenicity
Classifying Variants for Pathogenicity

- **Databases**
  - Erasmus MC Pompe Center - 558 variants
    - non-ACMG classifications (i.e. “severe”, “potentially less severe”)
  - *in vitro* data
  - links to publications
  - EmVClass (Emory) – 313 variants
    - classification by Emory Genetics Lab
  - ClinVar – 432 variants
    - classification based on submitter(s)
    - consensus
  - gnomAD and ExAC – allele frequencies

- **Publications**
- Prediction programs – PolyPhen; SIFT
- ACMG criteria for classification of variants
Pompe Screening Algorithm

GAA Enzyme Activity < 15%
Daily Mean

DNA Sequencing + Exon 18 Deletion

2 Pathogenic/Likely Pathogenic/VOUS
Referral

1 Pathogenic/Likely Pathogenic/VOUS
Referral

Pseudo-deficiency Alleles Only
No Referral

Benign or Likely Benign Variants Only
No Referral
Pseudodeficiency alleles

Variants which result in lower GAA enzyme activity but which are NOT associated with development of Pompe disease

<table>
<thead>
<tr>
<th>Variant (aa-3)</th>
<th>Variant (aa-1)</th>
<th>Variant (cDNA)</th>
<th>Allele Frequency (gnomAD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.Gly576Ser</td>
<td>p.G576S</td>
<td>c.1726G&gt;A</td>
<td>0.017 (0.14 in East Asians)</td>
</tr>
<tr>
<td>p.Glu689Lys</td>
<td>p.E689K</td>
<td>c.2065G&gt;A</td>
<td>0.055 (0.24 in East Asians)</td>
</tr>
<tr>
<td>p.Asp91Asn</td>
<td>p.D91N</td>
<td>c.271G&gt;A</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Targeted genotyping of pseudodeficiency alleles to rule out false positives?

- 46.7% of infants referred for diagnostic testing also had at least 1 pseudodeficiency allele
GAA sequence analysis reduces referral rate

<table>
<thead>
<tr>
<th>Screening began</th>
<th>October 1, 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td># Babies screened (thru 8/18/2017)</td>
<td>676,573</td>
</tr>
<tr>
<td># Babies sequenced</td>
<td>149</td>
</tr>
<tr>
<td># Babies with common benign variants only</td>
<td>19</td>
</tr>
<tr>
<td># Babies with common benign variants + pseudodeficiency alleles</td>
<td>23</td>
</tr>
<tr>
<td># Babies referred for diagnostic evaluation</td>
<td>107</td>
</tr>
</tbody>
</table>

Reduction in Referrals using DNA analysis – 28.2%
### Pompe Referrals (676,573 infants tested)

<table>
<thead>
<tr>
<th>Category</th>
<th>Number</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Infants Referred for Diagnostic Testing</td>
<td>107</td>
<td>1 in 6323</td>
</tr>
<tr>
<td># of Infants Diagnosed with Infantile-Onset Pompe Disease</td>
<td>5</td>
<td>1 in 135,315</td>
</tr>
<tr>
<td># of Infants Diagnosed with Infantile-Onset Pompe Disease (non-classical)</td>
<td>1</td>
<td>1 in 135,315</td>
</tr>
<tr>
<td># Infants with 2 Pathogenic variants</td>
<td>18</td>
<td>1 in 37,587</td>
</tr>
<tr>
<td># Infants with 1 Pathogenic variant + 1 VOUS</td>
<td>19</td>
<td>1 in 18,286</td>
</tr>
<tr>
<td># Infants with 2 VOUS</td>
<td>11</td>
<td>1 in 14,095</td>
</tr>
<tr>
<td># Likely Carriers (1 pathogenic, likely pathogenic or VOUS)</td>
<td>54</td>
<td>1 in 12,529</td>
</tr>
</tbody>
</table>
## Variants Identified in Infantile-Onset Pompe Disease

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Variants</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical</td>
<td>p.Pro285Arg (c.854C&gt;G)</td>
<td>Missense; Reported in IOPD</td>
</tr>
<tr>
<td></td>
<td>p.Pro768Leu (c.2303C&gt;T)</td>
<td>Missense; Reported in IOPD</td>
</tr>
<tr>
<td>Classical</td>
<td>p.Cys103Gly (c.307T&gt;G)</td>
<td>Missense; Reported in both IOPD and LOPD</td>
</tr>
<tr>
<td></td>
<td>p.Gly334Cys (c.1000G&gt;T)</td>
<td>Missense; VOUS</td>
</tr>
<tr>
<td>Classical</td>
<td>p.Asp399ValfsX6 (c.1195-19_2190-17del)</td>
<td>Deletion; Reported in IOPD</td>
</tr>
<tr>
<td></td>
<td>p.Asp399ValfsX6 (c.1195-19_2190-17del)</td>
<td></td>
</tr>
<tr>
<td>Classical</td>
<td>p.Val766Ser (c.2297A&gt;C)</td>
<td>Missense; Reported in both IOPD and LOPD</td>
</tr>
<tr>
<td></td>
<td>c.955+5G&gt;C</td>
<td>Splice site; VOUS</td>
</tr>
<tr>
<td>Non-classical</td>
<td>c.-32-13T&gt;G</td>
<td>Splice site; Common in LOPD</td>
</tr>
<tr>
<td></td>
<td>p.Glu730Ter (c.2188G&gt;T)</td>
<td>Nonsense; Reported in IOPD</td>
</tr>
</tbody>
</table>
### Pathogenic/Likely Pathogenic Variants identified in > 2 Referred Infants:

<table>
<thead>
<tr>
<th>Variant (cDNA)</th>
<th>Variant (aa)</th>
<th>Allele Freq. (gnomAD)</th>
<th># Infants homozygous</th>
<th># Infants heterozygous</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.-32-13T&gt;G</td>
<td>-</td>
<td>0.003</td>
<td>5</td>
<td>28</td>
</tr>
<tr>
<td>c.2560C&gt;T</td>
<td>p.Arg854Ter</td>
<td>0.0002</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>c.752C&gt;T_</td>
<td>p.Ser251Leu_</td>
<td>0.0004/</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>c.761C&gt;T</td>
<td>p.Ser254Leu</td>
<td>0.0002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.2238G&gt;C</td>
<td>p.Trp746Cys</td>
<td>0.0003</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>c.2237G&gt;C</td>
<td>p.Trp746Ser</td>
<td>0.00006</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>c.307T&gt;G</td>
<td>p.Cys103Gly</td>
<td>0.00003</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>
The VOUS Headache

- 49/107 Referrals (45.8%) had at least 1 VOUS
- 2/5 (40%) Infantile-onset cases were compound heterozygous for a VOUS and a known pathogenic variant
  - VOUS ≠ Benign
- 30/48 (62.5%) “Possible” Late-onset Pompe referrals had at least 1 VOUS making it difficult to provide clinicians with any prediction regarding phenotype
- 27/49 (55%) Referrals with VOUS also had pseudodeficiency alleles further complicating phenotype prediction
## Variants of Uncertain Significance (VOUS) identified in >1 Referred Infants:

<table>
<thead>
<tr>
<th>Variant (cDNA)</th>
<th>Variant (protein)</th>
<th>Allele Freq. (gnomAD)</th>
<th># Infants homozygous</th>
<th># Infants heterozygous</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.1888+5G&gt;T</td>
<td>-</td>
<td>0.00002</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>c.2069C&gt;T</td>
<td>p.Pro690Leu</td>
<td>0.00006</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>c.2051C&gt;T</td>
<td>p.Pro684Leu</td>
<td>0.00007</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>c.1424C&gt;T</td>
<td>p.Pro475Leu</td>
<td>0.00002</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>c.1320G&gt;T</td>
<td>p.Met440Ile</td>
<td>0.0003</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>c.2509C&gt;T</td>
<td>p.Arg837Cys</td>
<td>0.00002</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>c.1048G&gt;A</td>
<td>p.Val350Met</td>
<td>0.0001</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
The VOUS Migraine

p.Val222Met (c.664G>A)

- 10/107 (9.3%) infants referred
  - 3 homozygous
- Erasmus database: “Non-pathogenic” based on \textit{in vitro} data
- EmVClass database: “Benign” based on allele frequency
- gnomAD database: Allele frequency = 0.0007 overall
  - 0.005 in South Asians (4 homozygotes)
- Hungarian newborn screening program {Wittmann 2012 JIMD}
  - 16/64 infants screen positive were at least heterozygous for p.V222M
  - 5 homozygous
  - 1 compound het
  - 10 carriers
- No reports in affected individuals
- Pseudodeficiency allele?
Summary

- 5 infantile-onset cases Pompe disease
  - All 5 infants are currently on ERT therapy

- DNA sequence analysis reduces referral rate
  - 28% pseudos or benign variants only
  - Prevents unnecessary diagnostic testing and parental stress

- 67 different reportable variants identified
  - 47 (70%) in only a single individual

- > 60% of infants referred with 2 GAA variants had at least 1 VOUS
  - Phenotype?

- Long term follow-up + Data sharing = Genotype-Phenotype Predictions
Acknowledgements

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Beth Vogel, MS, CGC

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NICHD

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Possible Future LSD Screening Algorithm

- CLIR (yes) Second Tier Markers?
- Low IDUA or GAA activity (<20% daily mean/CLIR analysis)
  Retest in duplicate using same assay and also with 6-plex LSDs

- ≤ 8% (IDUA?)
  ≤ 12% (GAA)
  DNA testing
  1 or more variants
  Referral

- ≥ 8-10.0% (IDUA?)
  ≥ 12-15% (GAA)
  Compare to other enzymes
  Adjust if others low
  0 variants
  Screen negative

- >10% (IDUA?) of daily mean
  >15% (GAA)
  Screen negative

Borderlines: Correction for Multi-enzyme Retests

Example (also see SOP):
Average of GAA results from normal testing is 13.5% (a borderline result)
GALC = 50%
ABG = 80%
GLA = 70%
IDUA = 45%
ASM = 120% (we do not care about ASM for purpose of adjustment)

New GAA Result:
13.5% (100/80) = 16.9% (this is above our current cutoff of 15%, so no second tier testing).

- We plan to convert to use of CLIR, but this method reduces second tier testing
- Conservative adjustment, uses highest value and only applied to borderline samples
- Could consider other options... (e.g. average of others)