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

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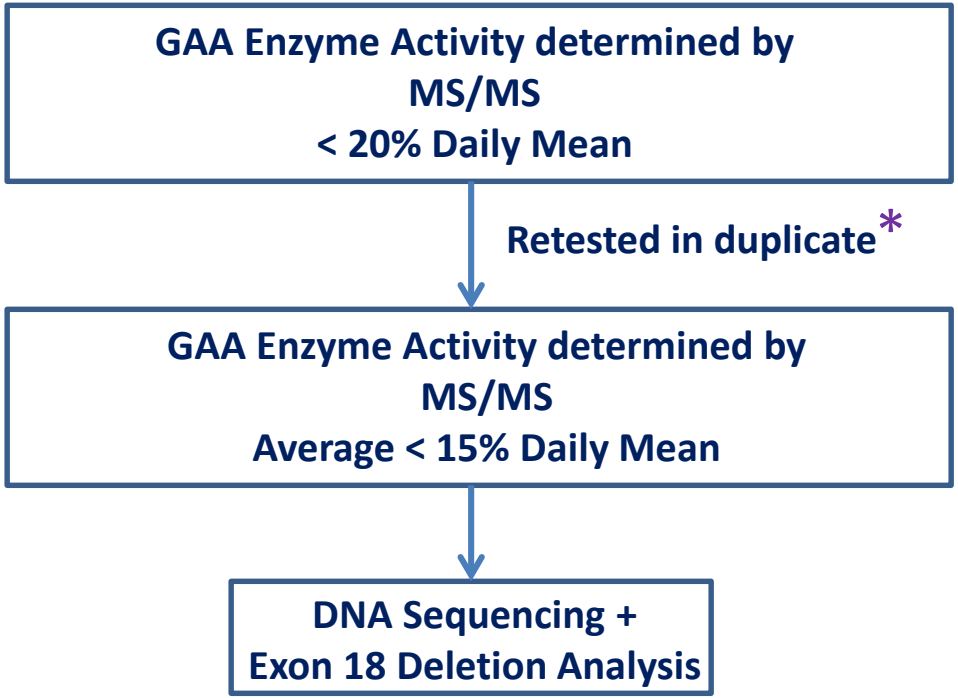
Pompe Disease

- 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

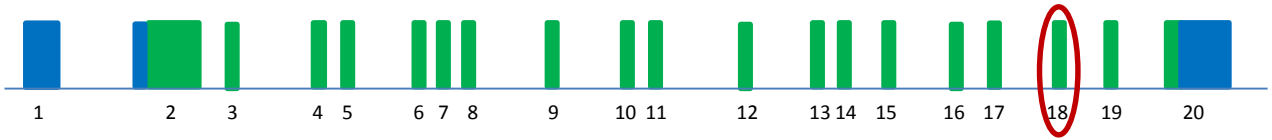
Type	Age at onset	Symptoms	Prognosis without treatment
Classic Infantile-Onset	Birth to first few months of life	Cardiac defects; poor muscle tone and weakness; enlarged liver	Death by 1 year due to heart failure
Non-classical (Atypical) Infantile-Onset	Within the 1st year of life	Delayed motor skills;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

NYS Pompe Screening Algorithm



*recent modification to include testing on LSD 6-plex assay

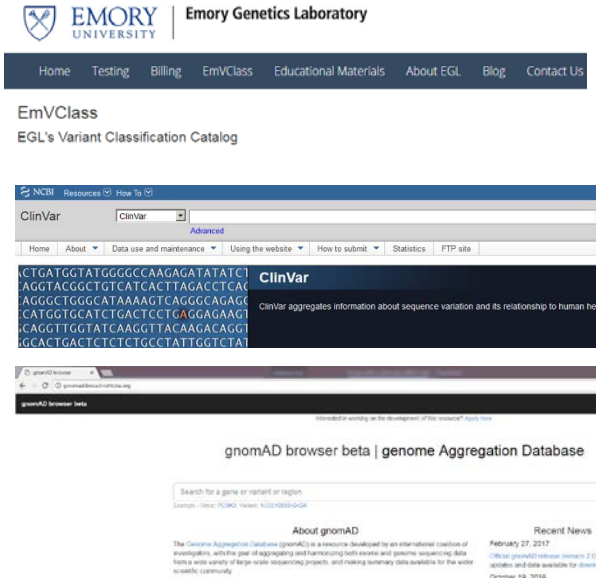
Sanger Sequence Analysis of the GAA Gene



- 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



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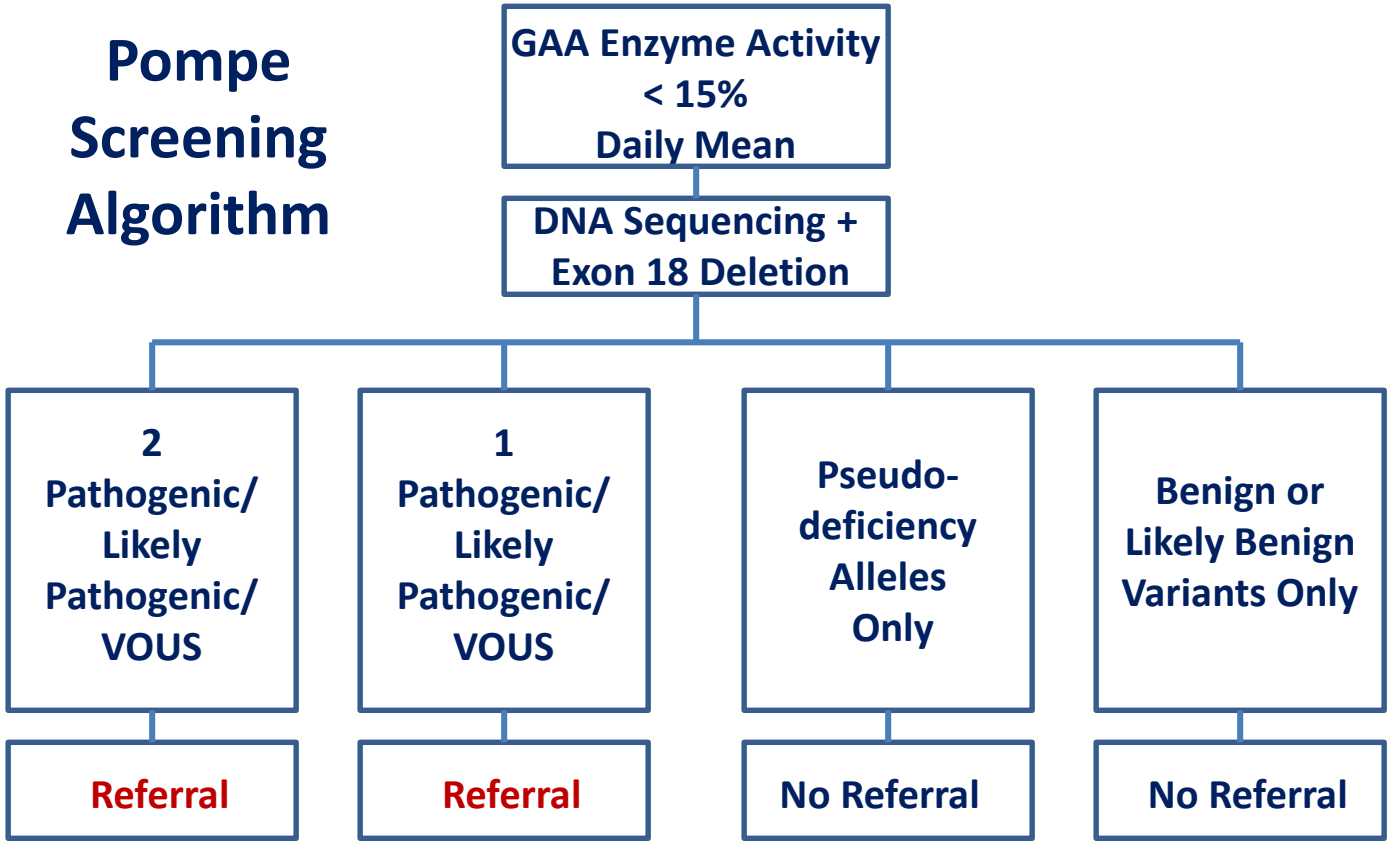
Pompe Screening Algorithm



Pseudodeficiency alleles

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

Variant (aa-3)	Variant (aa-1)	Variant (cDNA)	Allele Frequency (gnomAD)
p.Gly576Ser	p.G576S	c.1726G>A	0.017 (0.14 in East Asians)
p.Glu689Lys	p.E689K	c.2065G>A	0.055 (0.24 in East Asians)
p.Asp91Asn	p.D91N	c.271G>A	0.021

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

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

Reduction in Referrals using DNA analysis – 28.2%

Pompe Referrals (676,573 infants tested)

<table>
<tr><td># of Infants Referred for Diagnostic Testing</td><td colspan="2">107</td><td colspan="3">1 in 6323</td></tr>
<tr><td># of Infants Diagnosed with Infantile-Onset Pompe Disease</td><td colspan="2">5
(1 non-classical)</td><td colspan="3">1 in 135,315</td></tr>
<tr><td># Infants with 2 Pathogenic variants</td><td>18</td><td rowspan="3">48
“Possible” Late-Onset Pompe Disease</td><td>1 in 37,587</td><td rowspan="2">1 in 18,286</td><td rowspan="3">1 in 14,095</td></tr>
<tr><td># Infants with 1 Pathogenic variant + 1 VOUS</td><td>19</td><td>1 in 35,609</td></tr>
<tr><td># Infants with 2 VOUS</td><td>11</td><td>1 in 61,507</td><td></td></tr>
<tr><td># Likely Carriers
(1 pathogenic, likely pathogenic or VOUS)</td><td colspan="2">54</td><td colspan="3">1 in 12,529</td></tr>
</table>

Variants Identified in Infantile-Onset Pompe Disease

Diagnosis	Variants	Notes
Classical	p.Pro285Arg (c.854C>G)	Missense; Reported in IOPD
	p.Pro768Leu (c.2303C>T)	Missense; Reported in IOPD
Classical	p.Cys103Gly (c.307T>G)	Missense; Reported in both IOPD and LOPD
	p.Gly334Cys (c.1000G>T)	Missense; VOUS
Classical	p.Asp399ValfsX6 (c.1195-19_2190-17del)	Deletion; Reported in IOPD
	p.Asp399ValfsX6 (c.1195-19_2190-17del)	
Classical	p.Val766Ser (c.2297A>C)	Missense; Reported in both IOPD and LOPD
	c.955+5G>C	Splice site; VOUS
Non-classical	c.-32-13T>G	Splice site; Common in LOPD
	p.Glu730Ter (c.2188G>T)	Nonsense; Reported in IOPD

Pathogenic/Likely Pathogenic Variants identified in > 2 Referred Infants:

Variant (cDNA)	Variant (aa)	Allele Freq. (gnomAD)	# Infants homozygous	# Infants heterozygous
c.-32-13T>G	-	0.003	5	28
c.2560C>T	p.Arg854Ter	0.0002	0	11
c.752C>T_ c.761C>T	p.Ser251Leu_ p.Ser254Leu	0.0004/ 0.0002	0	6
c.2238G>C	p.Trp746Cys	0.0003	0	6
c.2237G>C	p.Trp746Ser	0.00006	0	3
c.307T>G	p.Cys103Gly	0.00003	0	3

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:

Variant (cDNA)	Variant (protein)	Allele Freq. (gnomAD)	# Infants homozygous	# Infants heterozygous
c.1888+5G>T	-	0.00002	0	5
c.2069C>T	p.Pro690Leu	0.00006	1	3
c.2051C>T	p.Pro684Leu	0.00007	0	3
c.1424C>T	p.Pro475Leu	0.00002	0	2
c.1320G>T	p.Met440Ile	0.0003	0	2
c.2509C>T	p.Arg837Cys	0.00002	0	2
c.1048G>A	p.Val350Met	0.0001	0	2

The VOUS Migraine

p.Val222Met (c.664G>A)

- 10/107 (9.3%) infants referred
  - 3 homozygous
- Erasmus database: “Non-pathogenic” based on *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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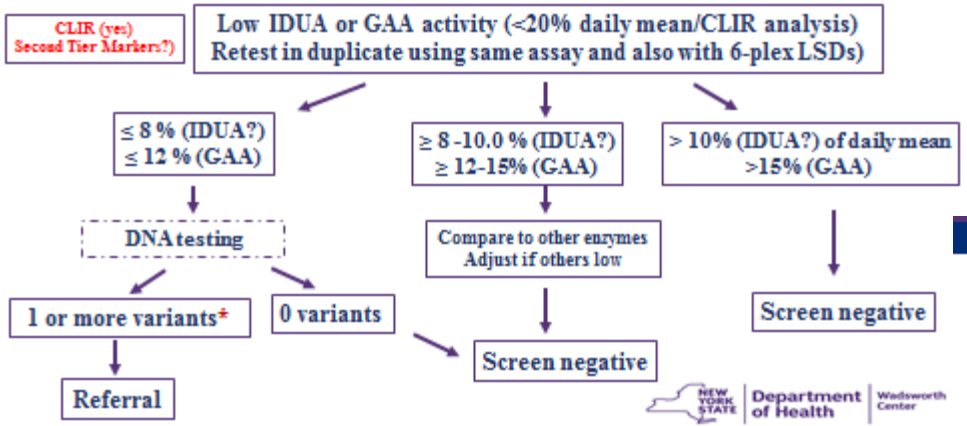
Possible Future LSD Screening Algorithm



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)

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