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

September 11, 2017

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Newborn Screening Program**

Pompe Disease

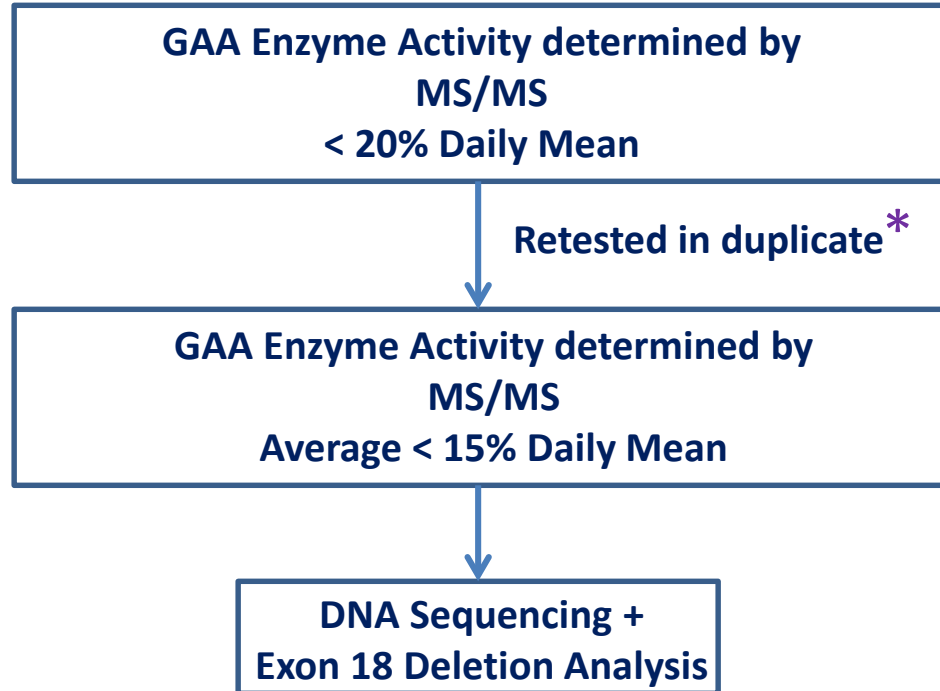
- **AKA: alpha-1,4-glucosidase deficiency; acid maltase deficiency; glycogen storage disease type II**
- **Lysosomal storage disorder - accumulation of glycogen in lysosomes due to enzyme deficiency**
- **Autosomal recessive disease caused by mutations in the GAA gene**
- **Estimated incidence in the 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 1 st year of life	Delayed motor skills; progressive muscle weakness	Death in early childhood due to respiratory problems
Late-onset	Onset after the 1 st year of life	Progressive muscle weakness especially in legs and trunk; breathing difficulties	Variable

NYS Pompe Screening Algorithm



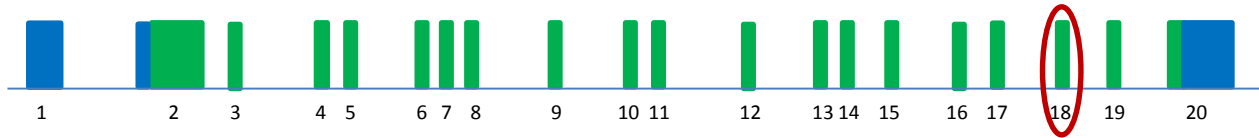
* recent modification to include testing on LSD 6-plex assay



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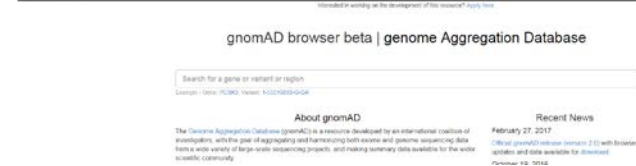
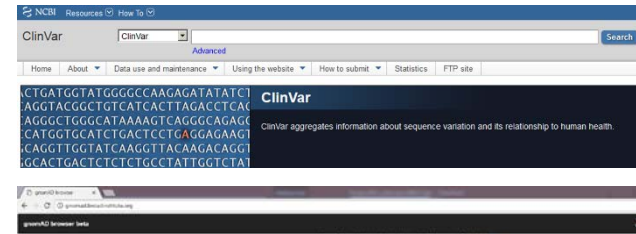
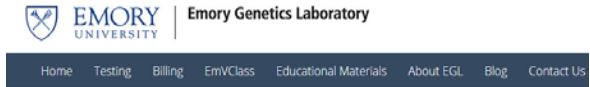
Sanger Sequence Analysis of the GAA Gene



- 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

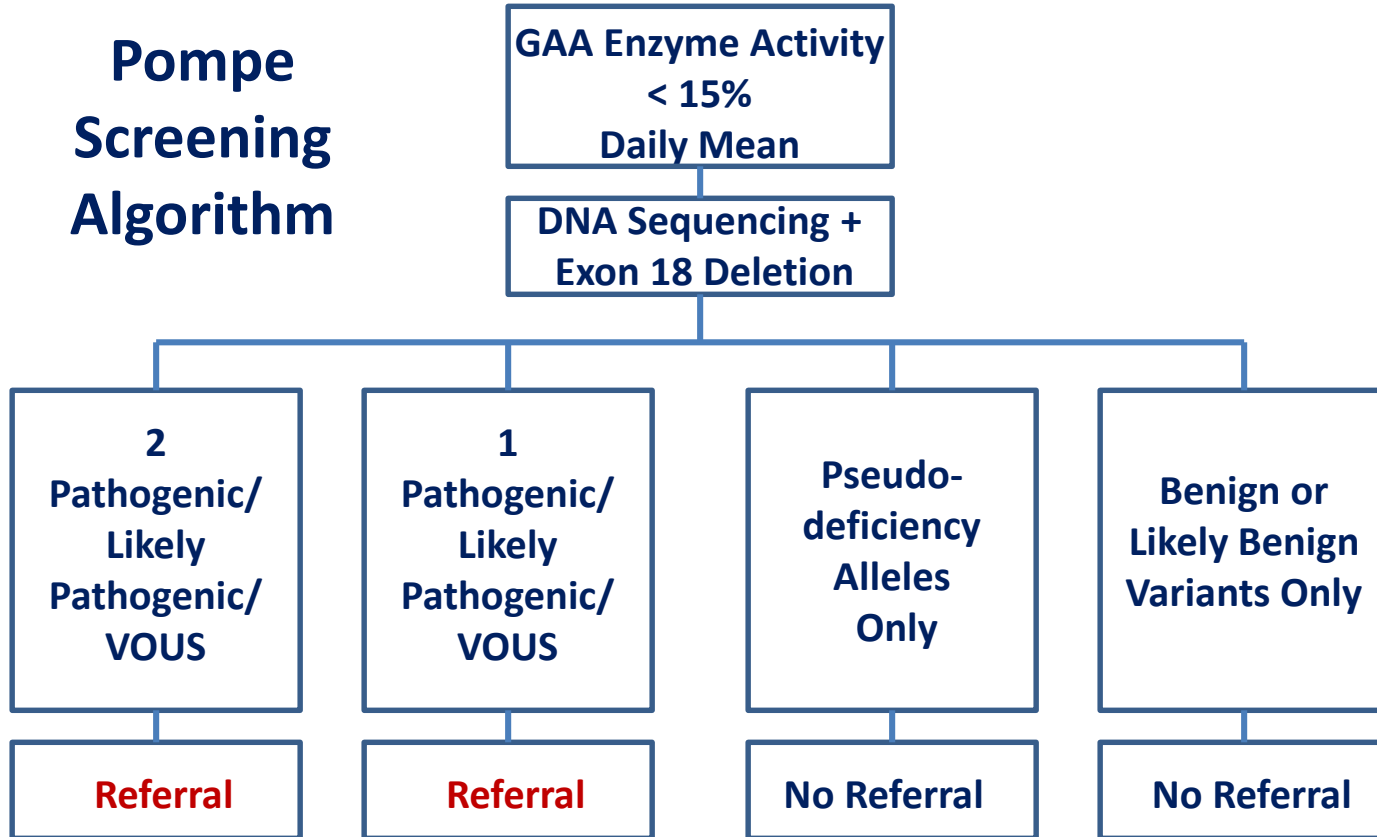
- Databases
 - Erasmus MC Pompe Center -558 variants
 - non-ACMG classifications (i.e. “severe”, “potentially less severe”)
 - *in vitro* data
 - links to publications
 - EmVClass (Emory) – 313 variants
 - classification by Emory Genetics Lab
 - ClinVar – 432 variants
 - classification based on submitter(s)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



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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)

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

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

NYS Newborn Screening Program

Michele Caggana, ScD, FACMG

Erin Hughes, MS

Lisa DiAntonio, MS

Sandra Levin, BS

Carlos Saavedra, MD

Joe Orsini, PhD

Sarah Bradley, MS, CGC

Beth Vogel, MS, CGC

Funding
NICHD



Eunice Kennedy Shriver National Institute
of Child Health and Human Development



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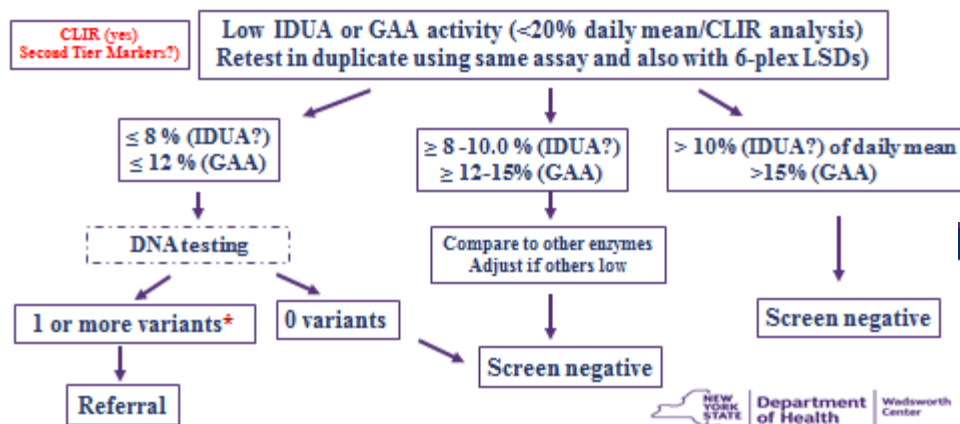
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Possible Future LSD Screening Algorithm



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:

$13.5\% \times (100/80) = 16.9\%$ (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)