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Second-tier Sequencing Analysis for VLCAD Deficiency in Texas: The First 14 Months

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Objectives

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- Brief overview of TX NBS testing
- Brief overview of VLCAD Sanger sequencing methodology
- Brief overview of pathogenicity determination
- Evaluate molecular findings
- Assess utilities of 2nd-tier VLCAD DNA testing
- Lessons learned

Texas NBS Program

Testing Performed for 53 Disorders

- Two screens:
  - 1st screen: 24-48 hours of age
  - 2nd screen: 1-2 weeks of age
- June 2017-July 2018: Received 867,690 specimens (~446,709 newborns)
  - ~2,350 specimens per day (6 days per week)
- 270 reported abnormal/borderline for VLCAD deficiency
  - 265 reflexed to DNA sequencing
  - 15 diagnosed cases with incidence of ~ 1:29,800

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Molecular Testing in TX NBS Program

- SCID qPCR: TREC & RNaseP
- GALT ARMS-PCR: 4 common variant panel
- MCAD RT-PCR (allelic discrimination): 4 common variant panel
- Hb RT-PCR (allelic discrimination) and Sanger Sequencing of the β–globin gene
- VLCAD Sanger Sequencing of the ACADVL gene
- CF Luminex: 60 mutation panel

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VLCAD DNA Testing

- Implemented June 1, 2017
- Testing performed 5 days per week
- Abnormal and Borderline specimens reflexed for DNA testing
- Only one screen DNA tested
- Sanger sequencing
- Expected turnaround time: 2 weeks

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VLCAD Sanger Sequencing Methodology

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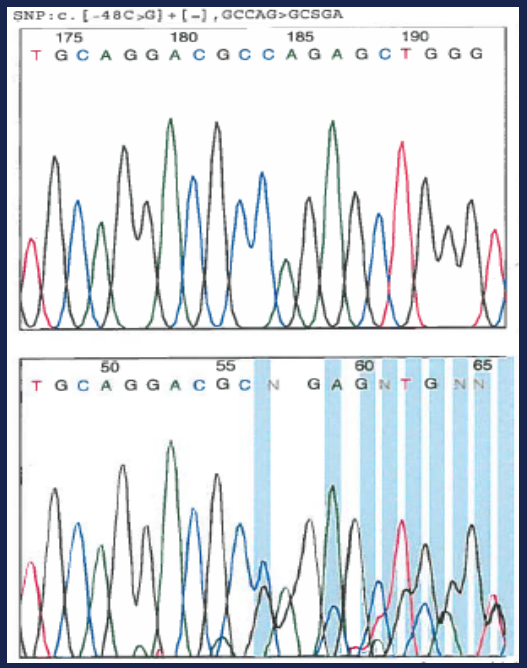
- NBS DNA extraction
  - QIAGEN Generation DNA Purification
- Sequencing PCR
  - ABI BigDye Direct Cycle Sequencing kit
- Post-cycle clean up
  - ZR-96 DNA Sequencing Clean-up kit
- Sequencing instrument
  - ABI 3130 Genetic Analyzer
- Variant analysis
  - Mutation Surveyor V5.1.0



Variant Pathogenicity Determination

- Pathogenicity categories:
  Pathogenic, Likely Pathogenic, Uncertain Significance, Likely Benign, Benign, No variant identified
- Pathogenicity determination:
  ACMG guidelines (PMID 25741868)
- Three reviewers for each variant:
  - Each reviewer will fill out worksheet
  - Discrepancy requires discussion
  - Time requirement: ~4-5 hours/variant

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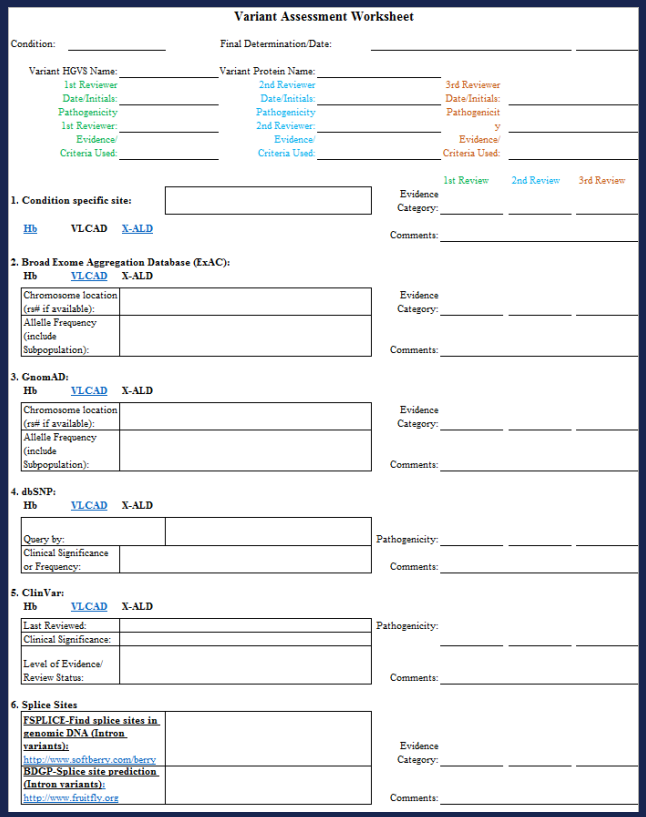
Example Worksheet



Variant Assessment Worksheet

Condition: _____ Final Determination/Date: _____ _____

Variant HGVS Name: _____ Variant Protein Name: _____

1st Reviewer	2nd Reviewer	3rd Reviewer
Date/Initials:	Date/Initials:	Date/Initials:
Pathogenicity	Pathogenicity	Pathogenicit
1st Reviewer:	2nd Reviewer:	y
Evidence/	Evidence/	Evidence/
Criteria Used:	Criteria Used:	Criteria Used:

1st Review 2nd Review 3rd Review

1. Condition specific site:

Hb VLCAD X-ALD

Evidence Category: _____ _____ _____

Comments: _____

2. Broad Exome Aggregation Database (ExAC):

Hb VLCAD X-ALD

Chromosome location (rs# if available):	
Allelle Frequency (include Subpopulation):	

Evidence Category: _____ _____ _____

Comments: _____

3. GnomAD:

Hb VLCAD X-ALD

Chromosome location (rs# if available):	
Allelle Frequency (include Subpopulation):	

Evidence Category: _____ _____ _____

Comments: _____

4. dbSNP:

Hb VLCAD X-ALD

Query by:	
Clinical Significance or Frequency:	

Pathogenicity: _____ _____ _____

Comments: _____

5. ClinVar:

Hb VLCAD X-ALD

Last Reviewed:	
Clinical Significance:	
Level of Evidence/ Review Status:	

Pathogenicity: _____ _____ _____

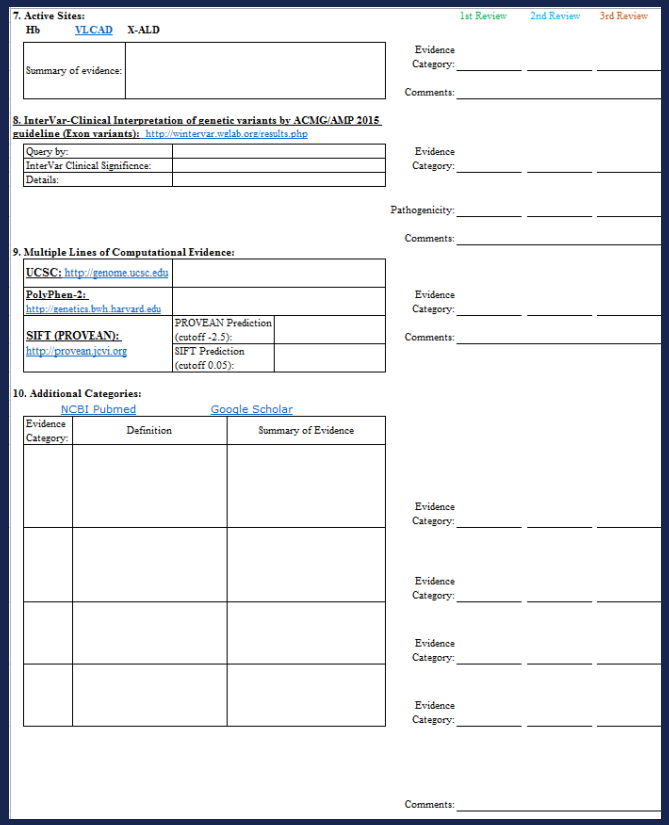
Comments: _____

6. Splice Sites

FSPLICE-Find splice sites in genomic DNA (Intron variants): http://www.softberry.com/berry	
BDGP-Splice site prediction (Intron variants): http://www.fruitfly.org	

Evidence Category: _____ _____ _____

Comments: _____



7. Active Sites: 1st Review 2nd Review 3rd Review

Hb VLCAD X-ALD

Summary of evidence:	

Evidence Category: _____ _____ _____

Comments: _____

8. InterVar-Clinical Interpretation of genetic variants by ACMG/AMP 2015 guideline (Exon variants): http://wintervar.wglab.org/results.php

Query by:	
InterVar Clinical Significence:	
Details:	

Evidence Category: _____ _____ _____

Pathogenicity: _____ _____ _____

Comments: _____

9. Multiple Lines of Computational Evidence:

UCSC: http://genome.ucsc.edu		
PolyPhen-2: http://genetics.bwh.harvard.edu		
SIFT (PROVEAN): http://provean.jcvi.org	PROVEAN Prediction (cutoff -2.5):	
	SIFT Prediction (cutoff 0.05):	

Evidence Category: _____ _____ _____

Comments: _____

10. Additional Categories:

NCBI Pubmed Google Scholar

Evidence Category:	Definition	Summary of Evidence

Evidence Category: _____ _____ _____

Evidence Category: _____ _____ _____

Evidence Category: _____ _____ _____

Comments: _____

Result Reporting

4 Clinical Significance Statements

- The results suggest Very Long Chain Acyl-CoA Dehydrogenase Deficiency. If you have not already done so, consultation with a metabolic specialist within 24 hours is strongly recommended.
- If you have not already done so, consultation with a metabolic specialist within 24 hours is recommended.
- If clinically indicated, consultation with a metabolic specialist within 72 hours is recommended. As appropriate, recommend genetic counseling within 30 days.
- Notify family of test results. As appropriate, recommend genetic counseling within 30 days

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Variant Distribution Among Abnormal and Borderline Specimens

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	Abnormal		Borderline		Total
	1st screen	2nd screen	1st screen	2nd screen	
Two or more Pathogenic/Likely Pathogenic/VOUS	20	2	1	0	23
One Pathogenic/Likely Pathogenic/VOUS	77	10	25	8	120
Likely Benign or Benign variant only	33	28	19	29	109
No variants	3	5	3	2	13

Diagnosed Cases June 2017-July 2018

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	Abnormal		Borderline		Total
	1st screen	2nd screen	1st screen	2nd screen	
Two or more Pathogenic/ Likely Pathogenic/ VOUS	9	1	0	0	10
One Pathogenic/ Likely Pathogenic/ VOUS	4	0	1	0	5

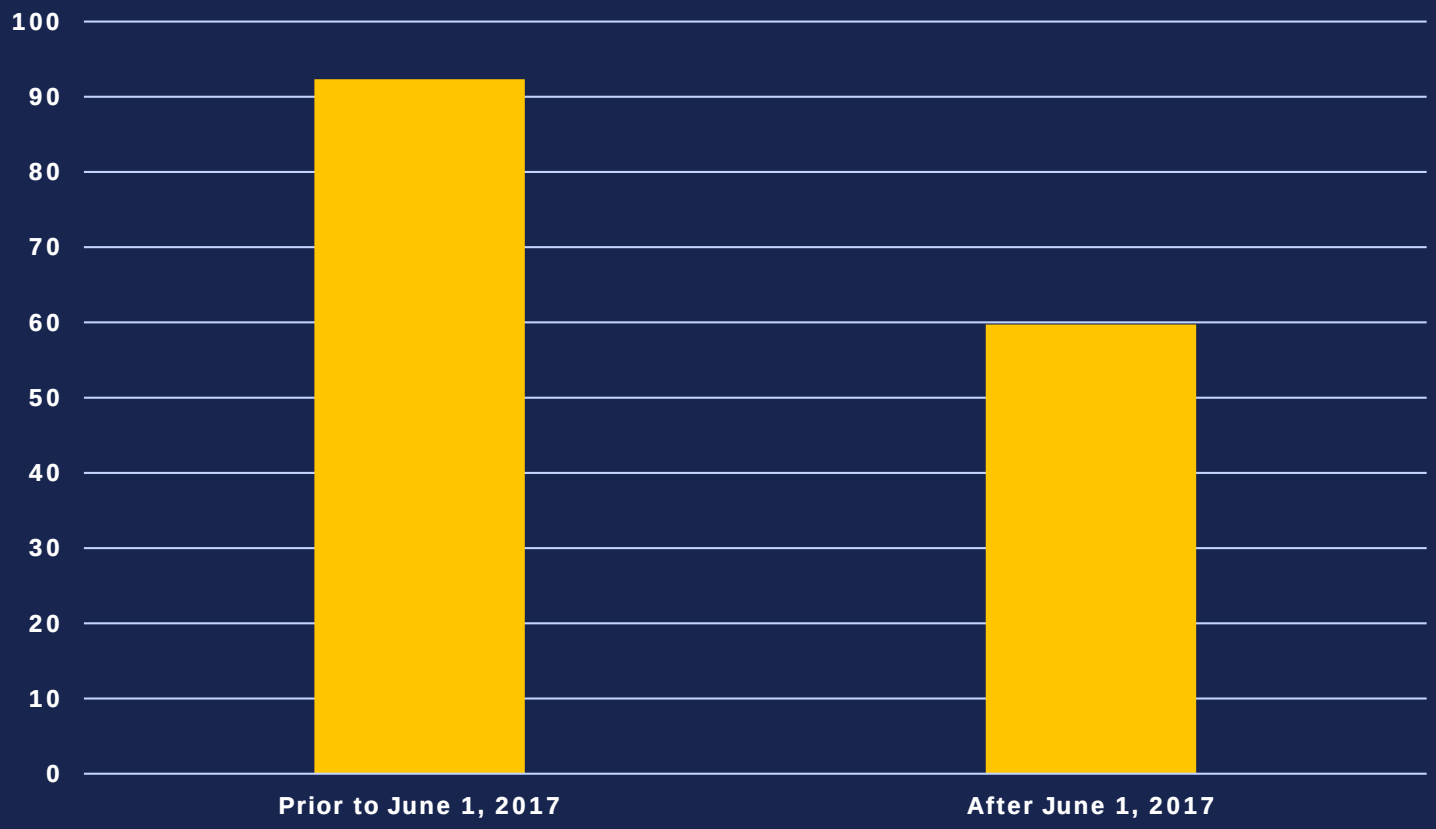
Total Diagnosed Cases: 15

Most Prevalent Variants

Variant	Exon	Reported Pathogenicity	Allele Count
c.848T>C	9	Pathogenic	22
c.1322G>A	13	Pathogenic	6
c.1375C>T	14	Pathogenic	6
c.829_831delGAG	9	Likely Pathogenic	5
c.1066A>G	10	VOUS	5
c.343delG	6	Pathogenic	4
c.1096C>T	11	Pathogenic	3
c.1376G>A	14	Pathogenic	3
c.1531C>T	15	Likely Pathogenic	3
c.950T>C	10	Likely Pathogenic	3
c.751A>G	8	VOUS	3

Total: 143 pathogenic, likely pathogenic, and VOUS variants identified. Most are single alleles.

Average Age at Diagnosis



Lessons Learned

1. Better able to anticipate needs for X-ALD Sanger sequencing
   a. Variant classification
   b. More robust database
   c. Increase capacity of sequencers
   d. Process automation
   e. Expansion of LIMS reporting functionality
2. Universal coverage of sequencing: supplemental information to assist specialists for case prioritization and quicker diagnosis
3. Necessity of reporting Benign/Likely Benign

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Newborn DNA Analysis Team

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Thank you

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