Second Tier Full Gene Sequencing for Follow Up of Positive Newborn Screening for VLCADD and GA1

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INTRODUCTION

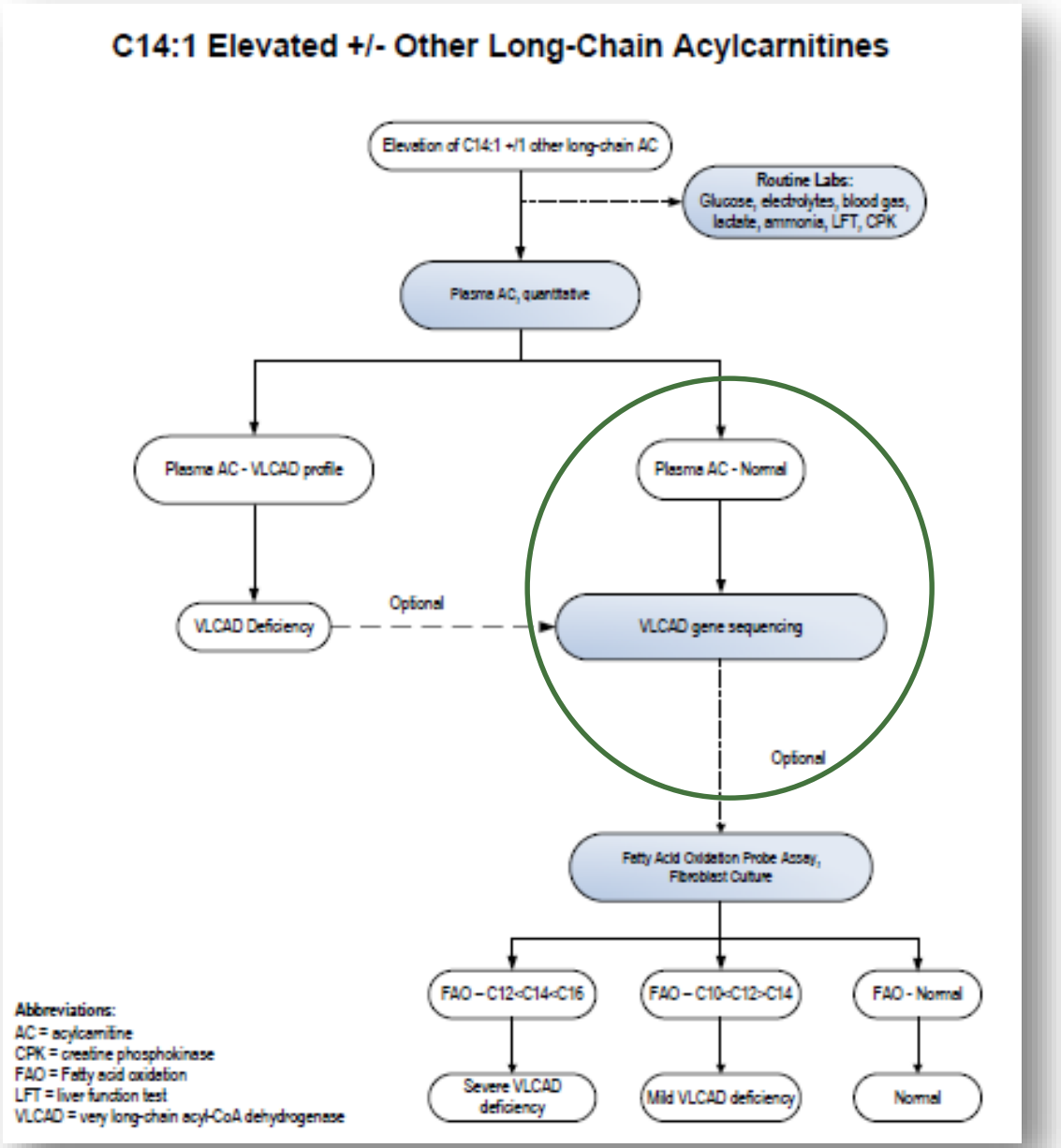
VLCADD deficiency

- Autosomal recessive
- Incidence: 1 in 40-120,000
- Clinical presentation
  - Severe, early-onset form:
    - Cardiomyopathy, hepatomegaly, hypotonia
  - Intermediate form:
    - Hypoketotic hypoglycemia
  - Late-onset form
    - Intermittent rhabdomyolysis, muscle pain and/or exercise intolerance

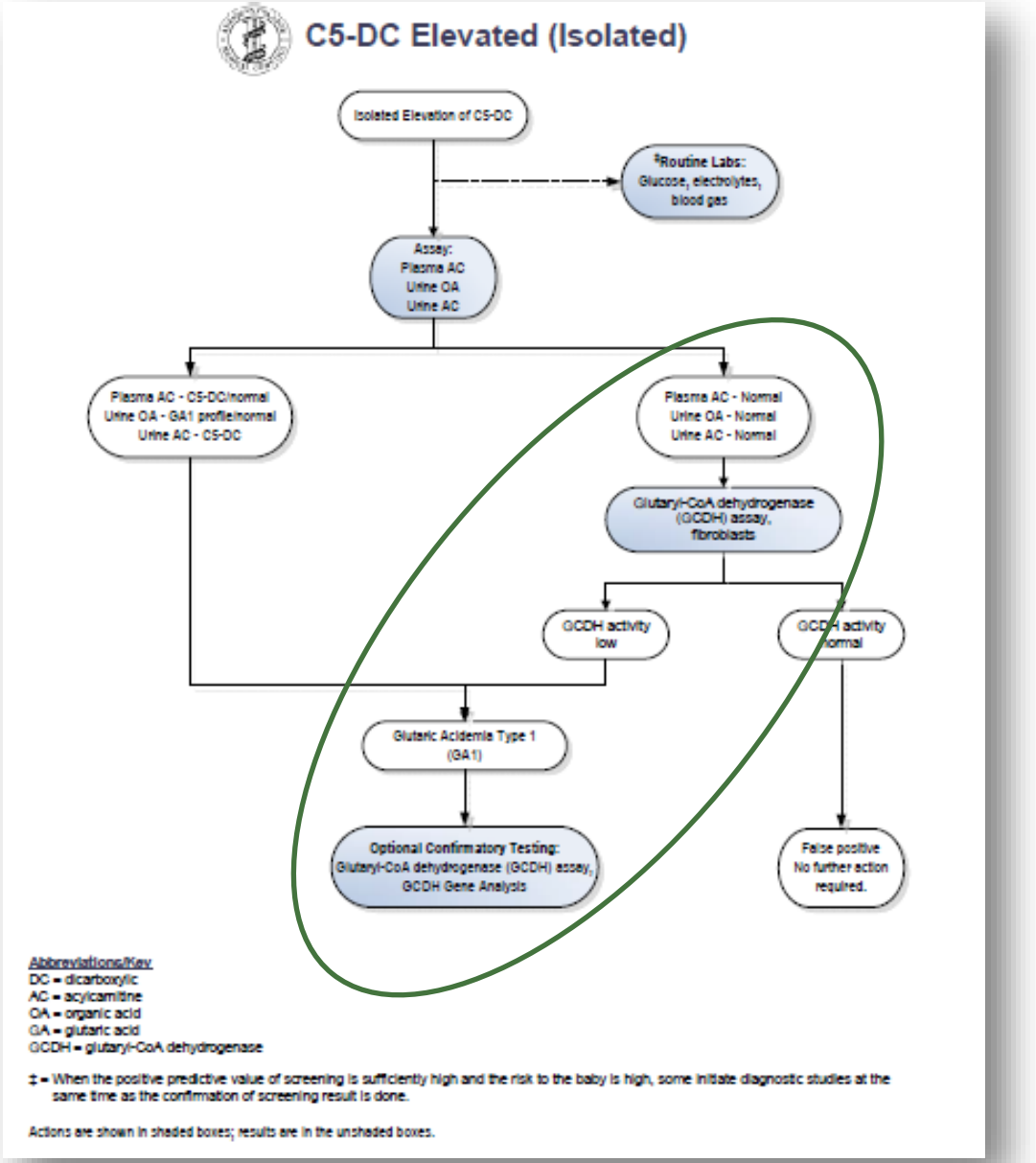
Glutaric aciduria, type I (GA-1)

- Autosomal recessive
- Incidence: 1 in 30-40,000
- Clinical presentation
  - Macrocephaly
  - Hypotonia
  - Acute encephalopathic episode
    - Spasticity
    - Dystonia
    - Dyskinesia
    - Seizures
  - High and LOW excretors

ACMG ACT Sheet VLCADD Algorithm



ACMG ACT Sheet GA-1 Algorithm



INTRODUCTION

Molecular analysis is often need to follow-up screen positives for VLCAD deficiency and Glutaric aciduria, type 1.

South Carolina	2010*	2011	2012
Total Births	55,813	54,898	53,691
Elevated C14:1	51	82	61
Elevated C5DC (D) or C5DC + C6OH (U)	31	49	86

*Derivatized method

INTRODUCTION

Implemented **2nd Tier full gene sequencing** in **April 2013** (in addition to repeat screen):

- VLCAD deficiency (*ACADVL* gene)
  - C14:1 ≥ 0.43 µM
- Glutaric aciduria, Type 1 (*GCDH* gene)
  - C5DC + C6OH ≥ 0.45 µM

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METHOD

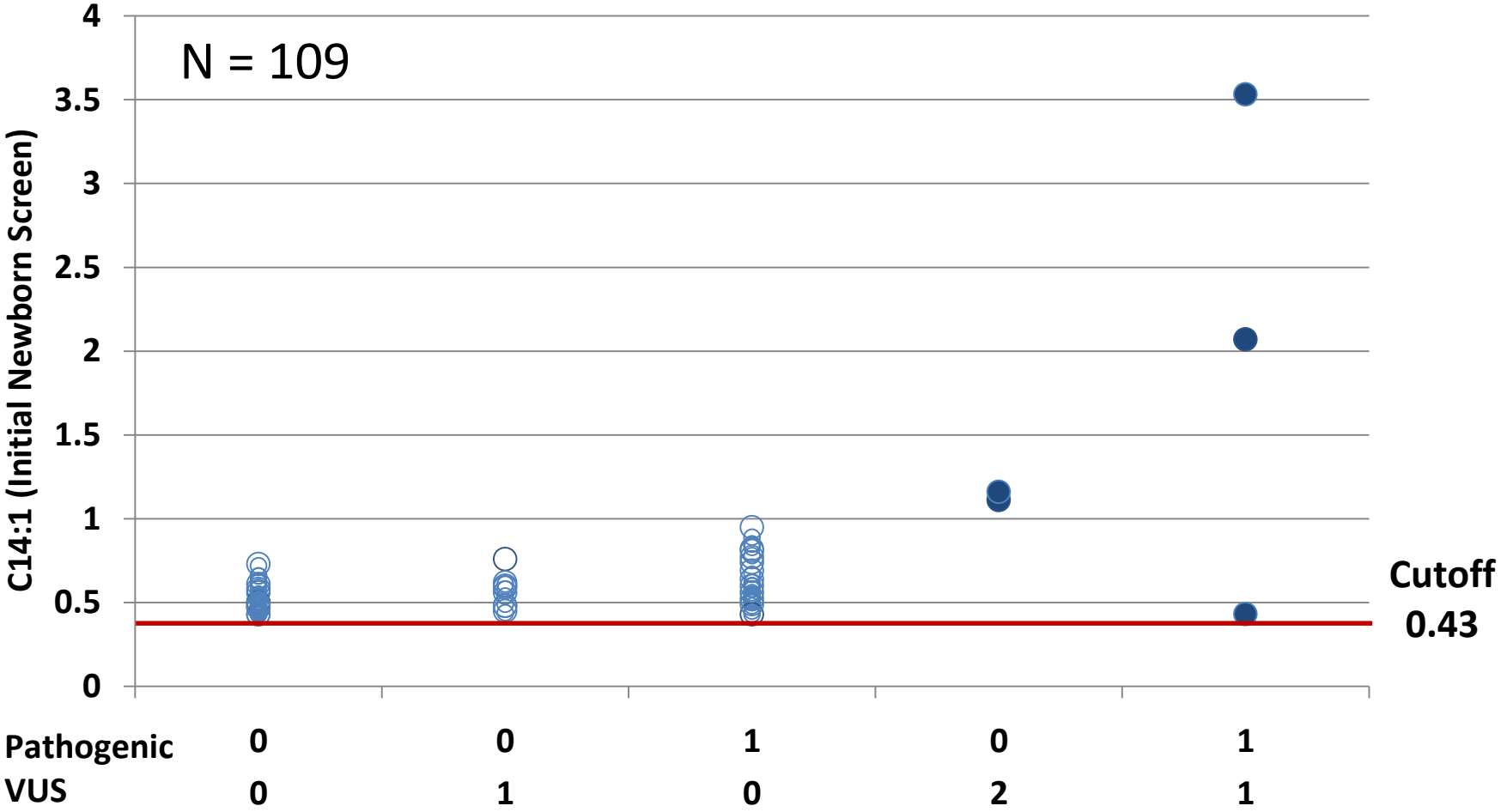
Two – 3 mm punches

Quiagen EZ1 Advanced
DNA Tissue Kit
50 µl of DNA
1-5 ng/ µl

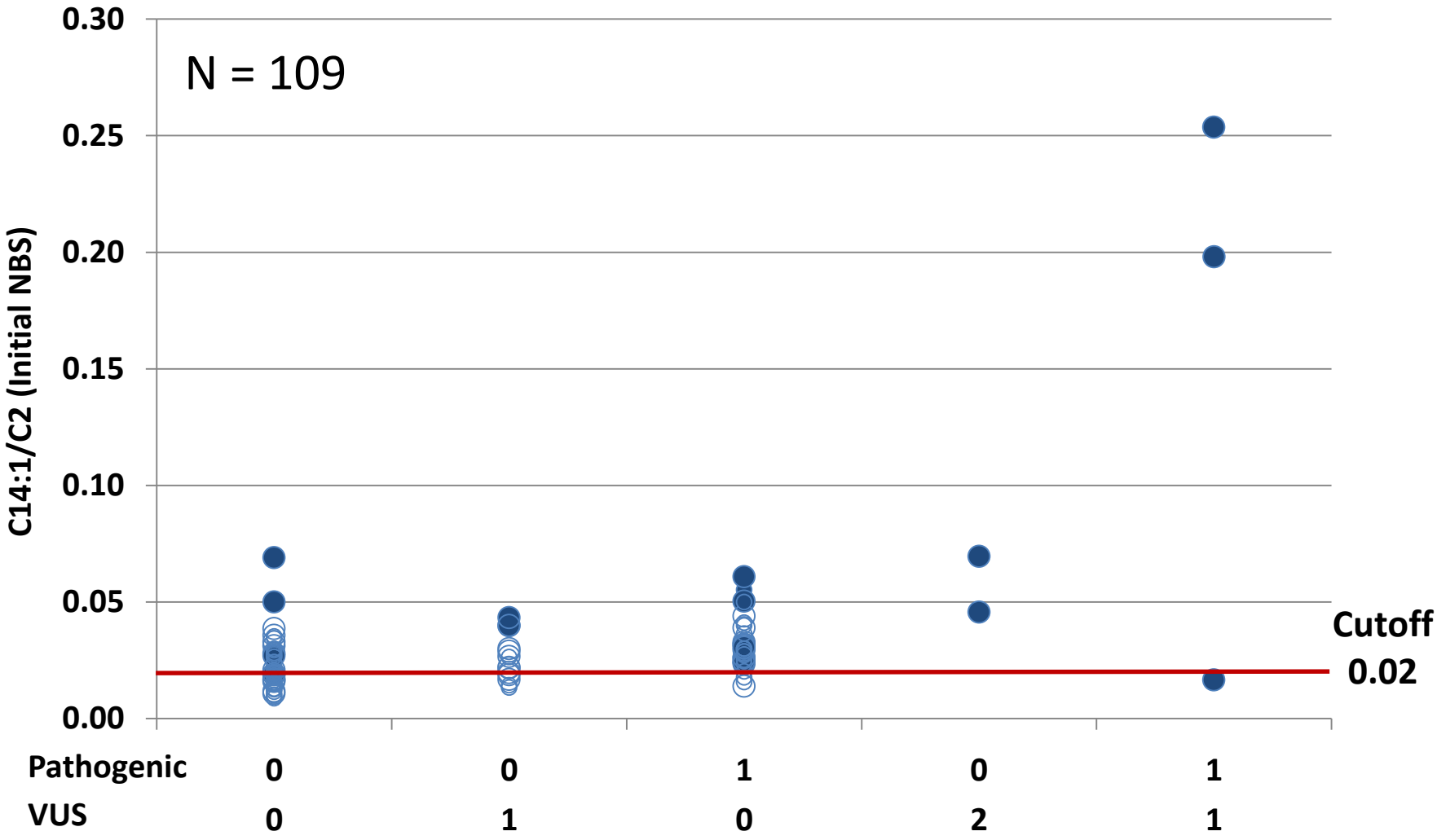
1 µl DNA/PCR Reaction

ACADVL gene: 20 exons
GCDH gene: 12 exons

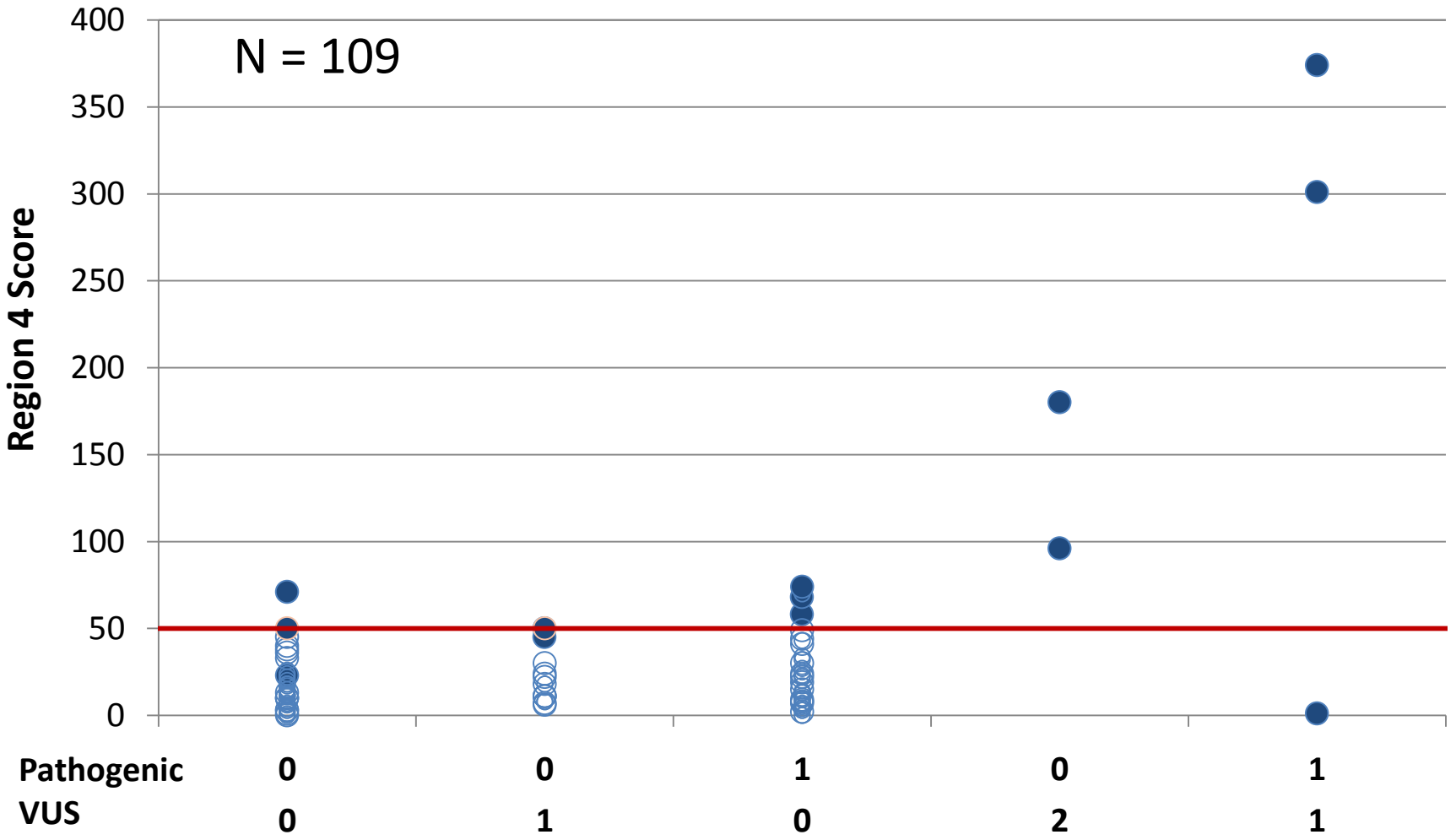
ACADVL Results by C14:1



ACADVL Results by C14:1/C2



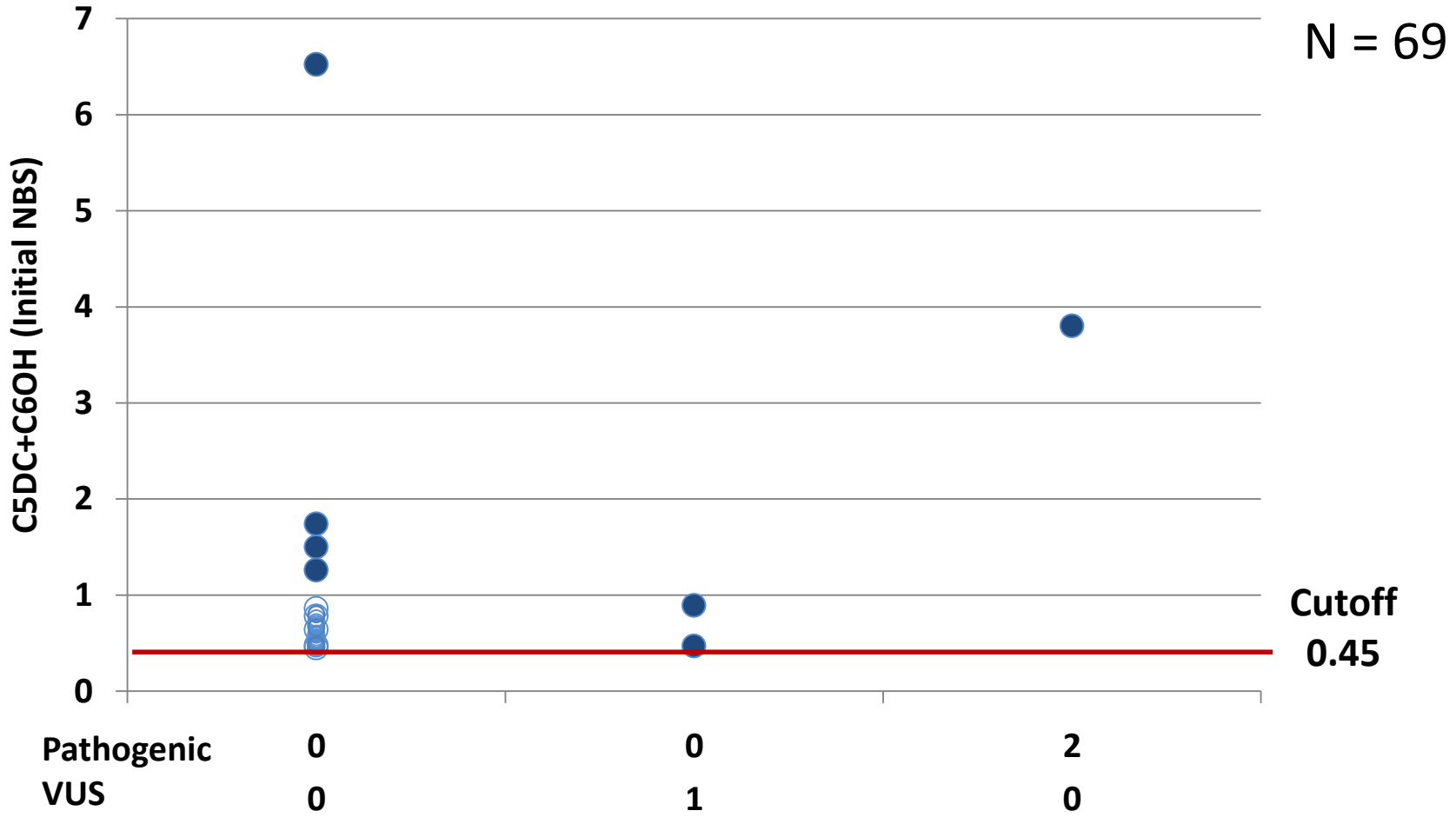
ACADVL Results by Region 4 Score



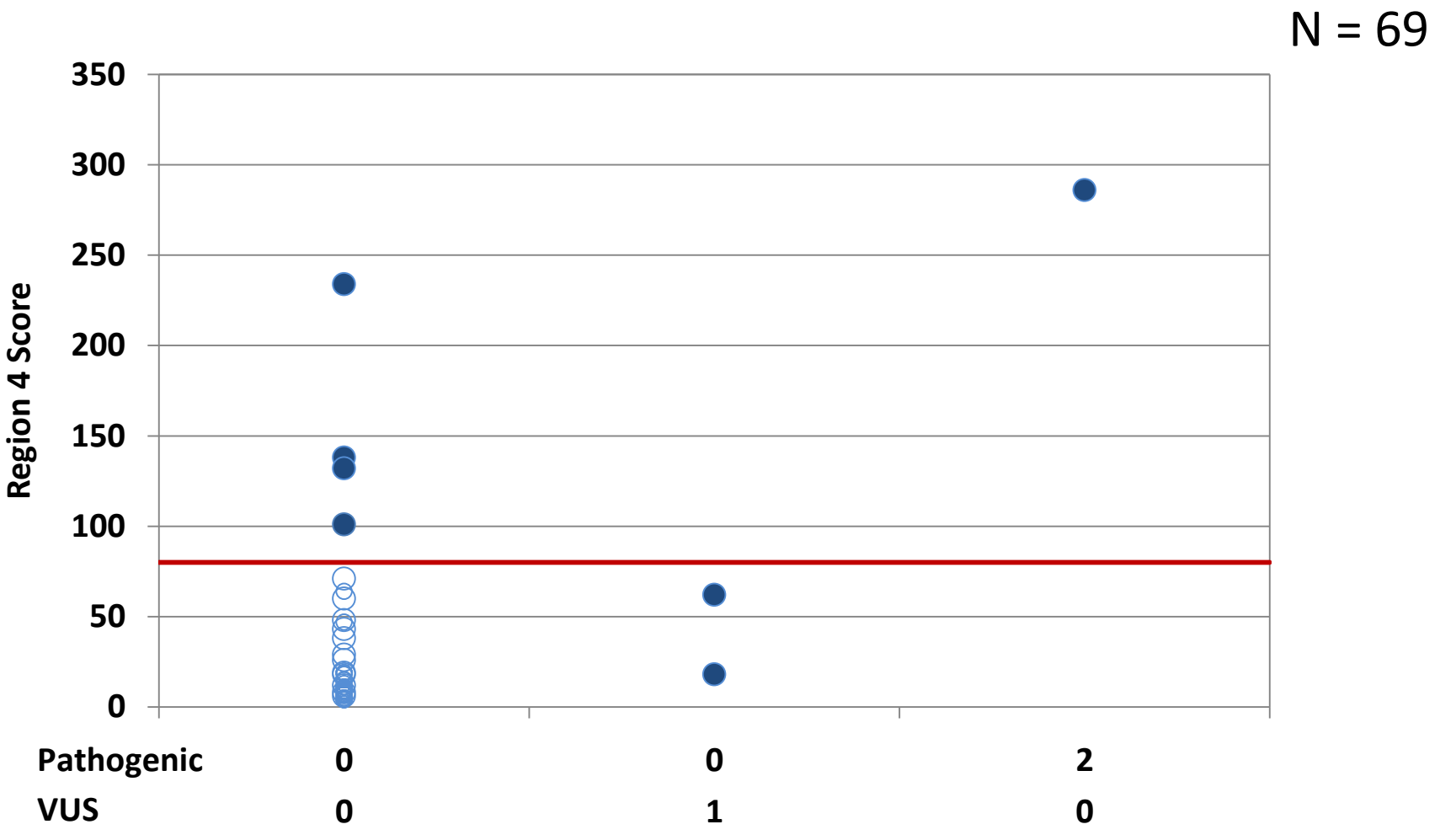
ACADVL Mutations

Exon/ Intron	Nucleotide change	AA change	Interpretation	Exon/ Intron	Nucleotide change	AA change	Interpretation
2	c.128G>A	p.G43D	VUS	**12**	c.1246_1248delGCC		VUS
7	c.497_498delTC		VUS	**13**	c.1284G>A	p.K428K	VUS
7	c.538G>A	p.A180T	VUS	**13**	c.1316G>A	p.G439D	Pathogenic
7	c.535G>T	p.G179W	Pathogenic	**13**	c.1322G>A	p.G441D	Pathogenic
8	c.637G>A	p.A213T	Pathogenic	**13**	c.1273G>A	p.A425T	Pathogenic
8	c.628A>C	p.T210P	VUS	**14**	c.1375C>T	p.R459W	Pathogenic
9	**c.848T>C**	**p.V283A**	**Pathogenic**	**14**	c.1405C>T	p.R469W	Pathogenic
10	c.1066A>G	p.I356V	VUS	**16**	c.343delG		Pathogenic
10	c.1064T>C	p.I355T	VUS	**16**	c.1591C>T	p.R531W	Pathogenic
10	c.1066A>G	p.I356V	VUS	**16 In**	c.1605+3A>G		VUS
10	c.1077_1077+1delGGinsCAC		Pathogenic	**17 In**	c.1678+3_6delAAGT		VUS
10	c.1064T>C	p.I355T	VUS	**18**	c.1748C>G	p.S583W	VUS
11	c.1096C>T	p.R366C	Pathogenic	**20**	c.1839G>A	p.R613R	VUS
11	c.1153C>T	p.R385W	Pathogenic	**20**	c.1844G>A	p.R615Q	Pathogenic
11 In	c.1182+1G>A		Pathogenic	**20**	c.1913C>T	p.S638F	VUS

GCDH Results by C5DC+C6OH



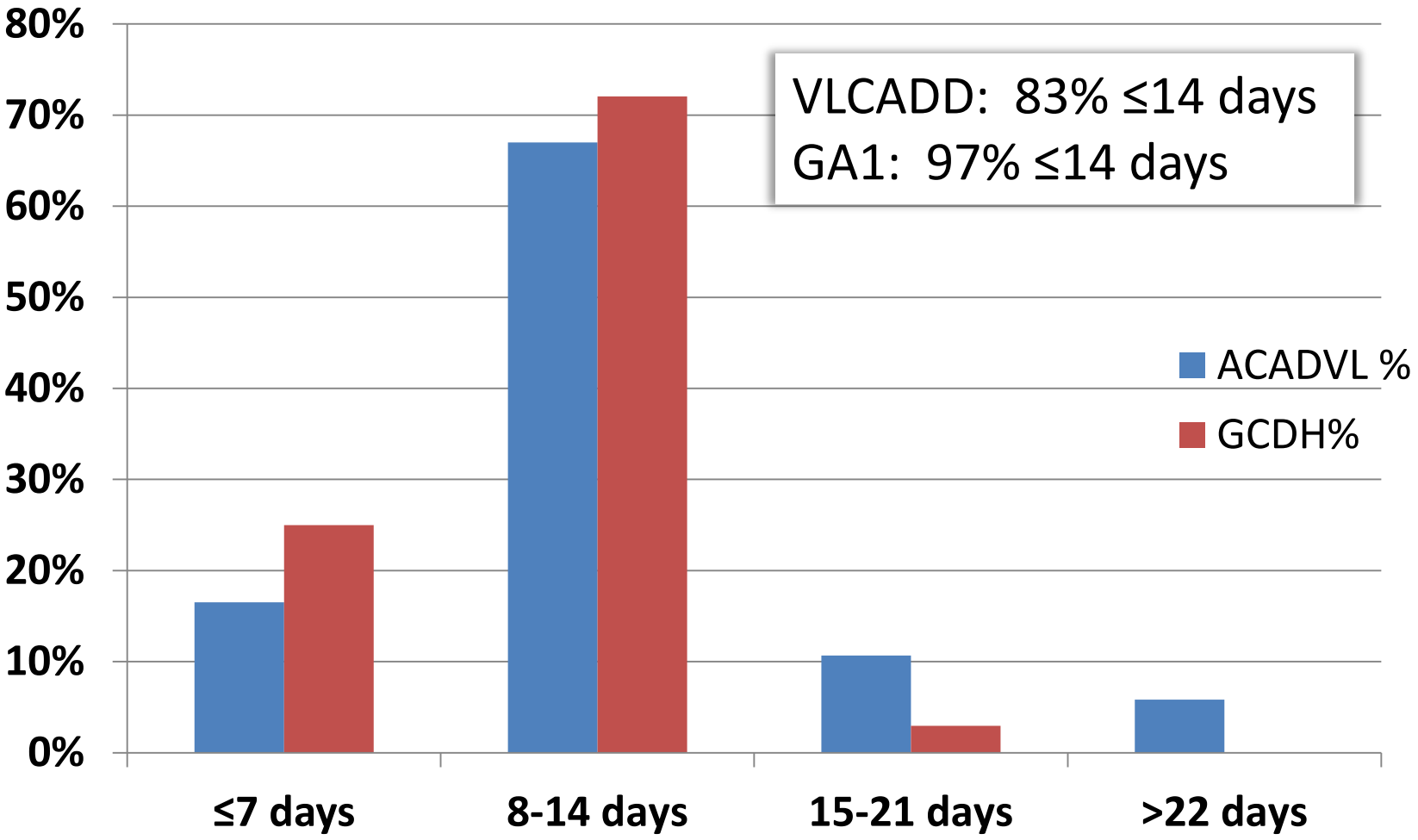
GCDH Results by Region 4 Score



GCDH Mutations

Exon/ Intron	Nucleotide change	AA change	Interpretation
8	c.640A>G	p.T214A	Pathogenic
9	c.862G>A	p.G288S	VUS
9	c.937C>T	p.R313W	Pathogenic
11	c.1085C>A	p.A362D	SNP (<1%)

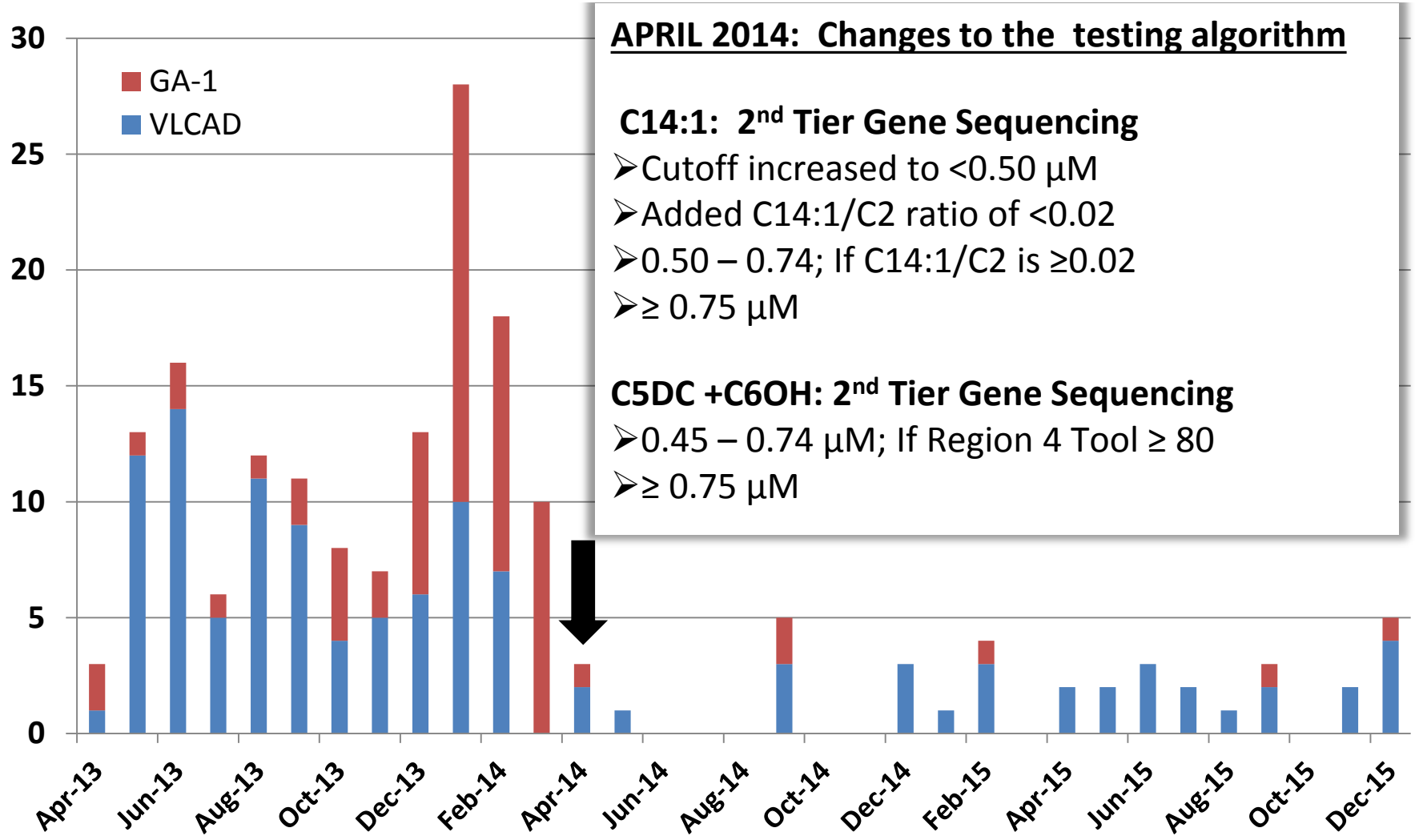
TURN AROUND TIMES



FOLLOW-UP

- **Short-term Follow-up (DHEC):**
  - Communicate results to Primary Care Provider and Metabolic Geneticist:
    - Initial Screen
    - Repeat Screen
    - Molecular Report
- **Metabolic Geneticist (Greenwood Genetic Center):**
  - Reviews all abnormal AC profiles and molecular testing
  - Carriers:
    - Families offered genetic counseling via telephone or in person.
  - Indeterminate/Affected:
    - Seen and followed in Metabolic clinic.

Gene Sequencing Performed for VLCADD and GA-1



APRIL 2014: Changes to the testing algorithm

C14:1: 2nd Tier Gene Sequencing

- Cutoff increased to <0.50 µM
- Added C14:1/C2 ratio of <0.02
- 0.50 – 0.74; If C14:1/C2 is ≥0.02
- ≥ 0.75 µM

C5DC +C6OH: 2nd Tier Gene Sequencing

- 0.45 – 0.74 µM; If Region 4 Tool ≥ 80
- ≥ 0.75 µM

Status

	2010*	2011	2012	2013	2014	2015**
Total Births	55,813	54,898	53,691	53,384	54,559	NA
Elevated C14:1	51	82	61	82	28	23
VLCADD	2 (15 carriers)	1 (14 carriers)	1 (15 carriers)	1 (35 carriers)	0 (12 carriers)	2 (10 carriers)
Elevated C5DC (D) or C5DC + C6OH (U)	31	49	86	69	45	3
GA-1	0 (1 carrier)	0	0	0 (2 carriers)	1 (1 carrier)	0

*Derivatized method

**Preliminary

FUTURE PLANS

2nd Tier Gene Sequencing for Carnitine Uptake Defect, in progress:

SLC22A5 gene sequencing if:

C0 ≤ 8.00 µM

and

C3 + C16 ≤ 2.00

Potential to add full gene sequencing for other disorders:

- Galactosemia
- Biotinidase deficiency
- CPT1A
- MCAD deficiency
- Lysosomal storage disorders (when screened)

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