Homozygosity for a Carnitine Palmitoyltransferase 1A Genetic Variant is Associated with an Increased Risk for Infant Mortality:

Implications for Newborn Screening

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Background

2003: Alaska initiates expanded newborn screening via MS/MS

- All core conditions and secondary targets recommended by the ACMG
- Carnitine palmitoyltransferase 1A (CPT1A) deficiency, a rare disorder of fatty acid oxidation

2003 – 2008: An unusually high number of infants with CPT1A deficiency

- Elevated C0/C16+C18 ratio
- All affected infants were Alaska Natives
- Incidence: 1/130

All infants homozygous for a single sequence variant

- c.1436C→T; p.P479L
- Arctic Variant
- Enzyme activity reduced by 80% in cultured fibroblasts

Evidence suggests low ascertainment

Sensitivity of MS/MS to identify infants homozygous for the CPT1A arctic variant

2,500 consecutive newborn screening cards

- MS/MS and DNA testing

MS/MS: 18 positive screens

- Zero on the first screen
- 16 on a second
- 2 on a third screen

DNA:

- 2063 homozygous WT (83%)
- 248 heterozygous (10%)
- 173 homozygous variant (7%)

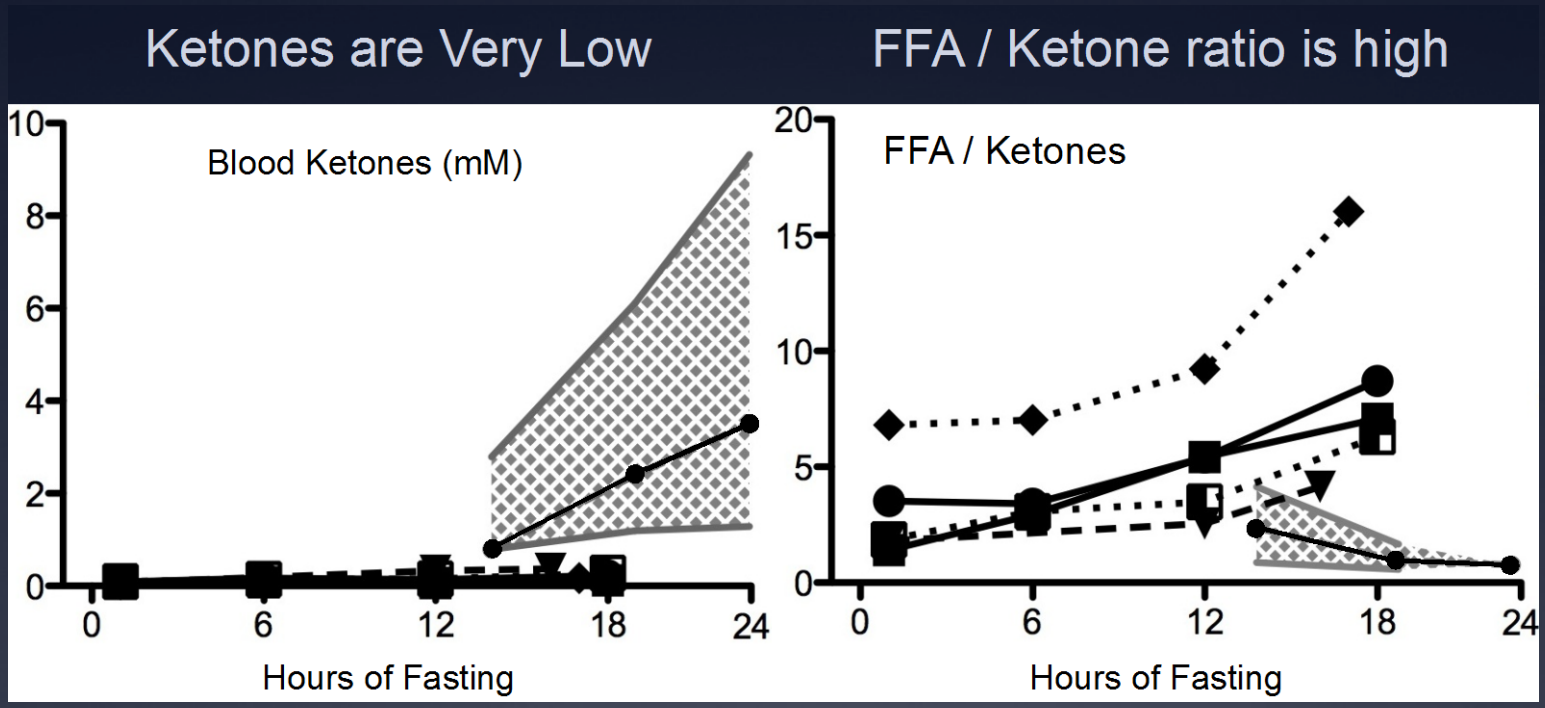
Gessner et. al., *J Pediatrics* 2011;158:124-9

10,500 births per year in Alaska

- 735 homozygous infants annually
- Ascertainment by MS/MS = 10%

Do we need to ascertain all infants?

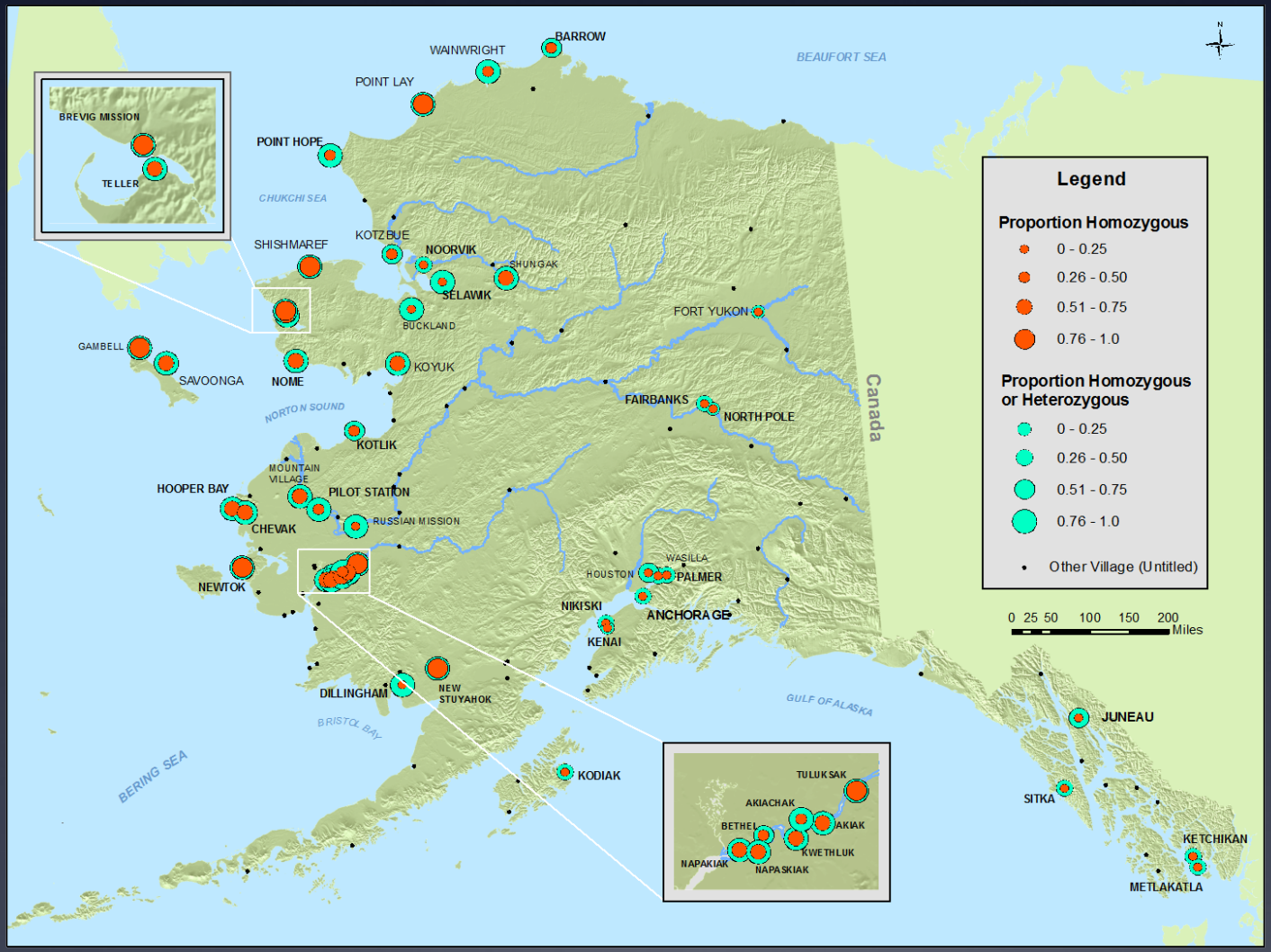
Impact of the CPT1A arctic variant on fasting:



Gillingham et. al., *Molecular Genetics and Metabolism* 104 (2011) 261–264

Epidemiologic Evidence:

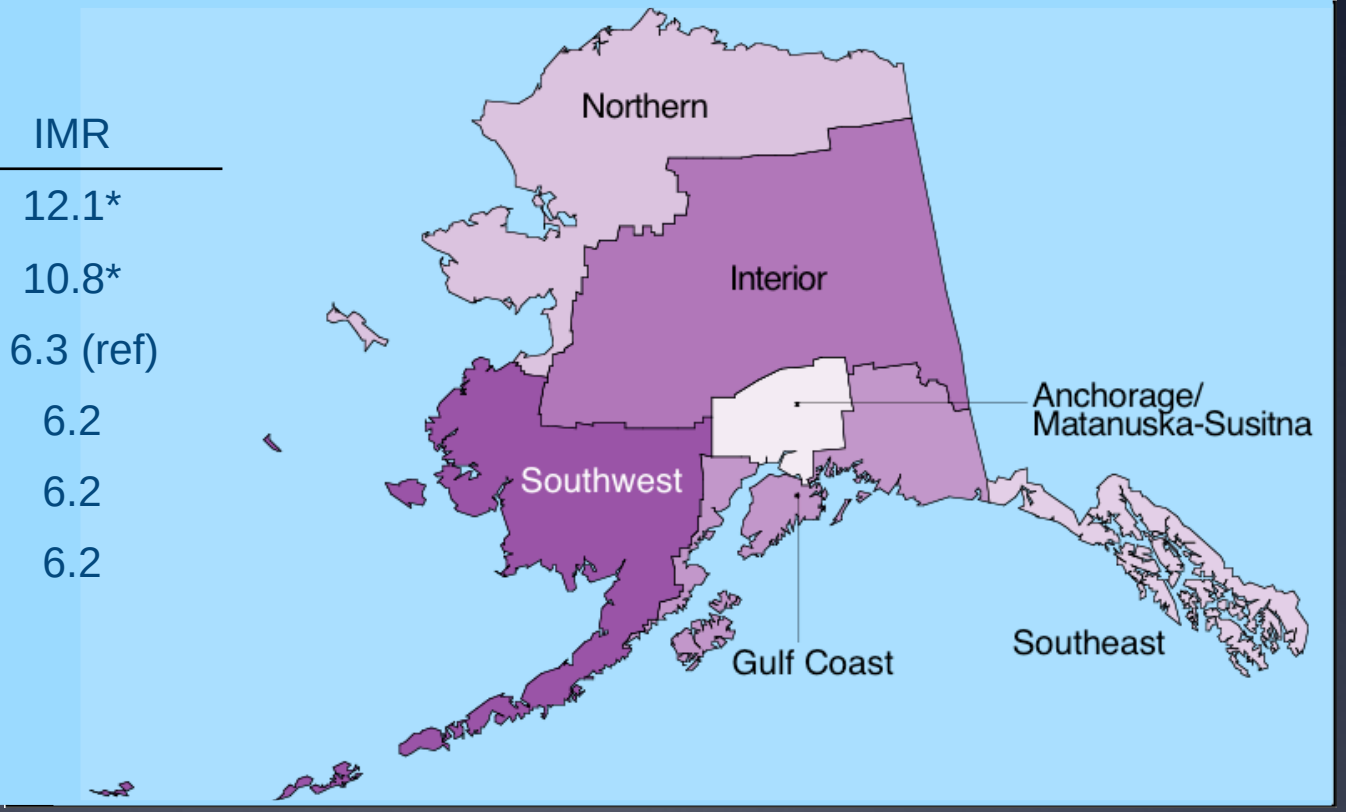
Geographic Distribution of the CPT1A Arctic Variant



Epidemiologic Evidence:

Infant mortality rates by region (1992-2004)

Region	IMR
Northern	12.1*
Southwest	10.8*
Anchorage/Mat-Su	6.3 (ref)
Gulf Coast	6.2
Interior	6.2
Southeast	6.2



Data source: Alaska Bureau of Vital Statistics

Impact of the CPT1A Arctic Variant on Infant Mortality

Unmatched case-control study

- Cases: 110 Alaska Native infant deaths (2006 through 2010)
- Controls: 395 Alaska Native births from the same time period
- 80% power to detect an odds ratio of ~ 2.0 with 95% confidence

Genotyped using DNA from newborn screening cards

Rates of homozygosity for the CPT1A arctic variant in cases and controls

	Cases	Controls
All subjects	46 of 110 (42%)	119 of 395 (30%)
Homozygous and heterozygous	46 of 79 (58%)	119 of 269 (44%)
Residents of Western and Northern Alaska	38 of 58 (66%)	112 of 185 (51%)

Association between homozygosity for the arctic variant and infant mortality

All birth weights		
	Odds ratio (95% CI)	Adjusted* odds ratio (95% CI)
All subjects	1.7 (1.1, 2.6)	1.8 (1.1, 2.9)
Homozygous and heterozygous	1.8 (1.1, 2.9)	2.3 (1.3, 4.0)
Residents of Western and Northern Alaska	1.8 (0.98, 3.3)	2.5 (1.3, 5.0)

* Odds ratio adjusted for maternal education, maternal prenatal alcohol and tobacco use, and a composite variable combining marital status and presence of the father's name on the birth certificate.

Normal birth weights		
	Odds ratio (95% CI)	Adjusted* odds ratio (95% CI)
All subjects	1.7 (1.1, 2.7)	1.8 (1.1, 2.9)
Homozygous and heterozygous	1.7 (0.99, 2.9)	2.2 (1.2, 3.9)
Residents of Western and Northern Alaska	2.2 (1.1, 4.2)	3.0 (1.4, 6.3)

Association between the arctic variant and cause of death

Cause of Death	OR (95%CI)
SIDS or asphyxia of unknown etiology	0.50 (0.22 to 1.1)
Infectious disease	2.9 (1.0, 8.0)
Congenital anomaly	0.91 (0.34, 2.4)
Injury	1.2 (0.38, 3.6)

Illness Preceding Death	OR (95%CI)
Any hospitalization	5.1 (1.7, 16)
Pneumonia*	15 (1.9, 125)
Sepsis or meningitis*	2.9 (0.88, 9.2)

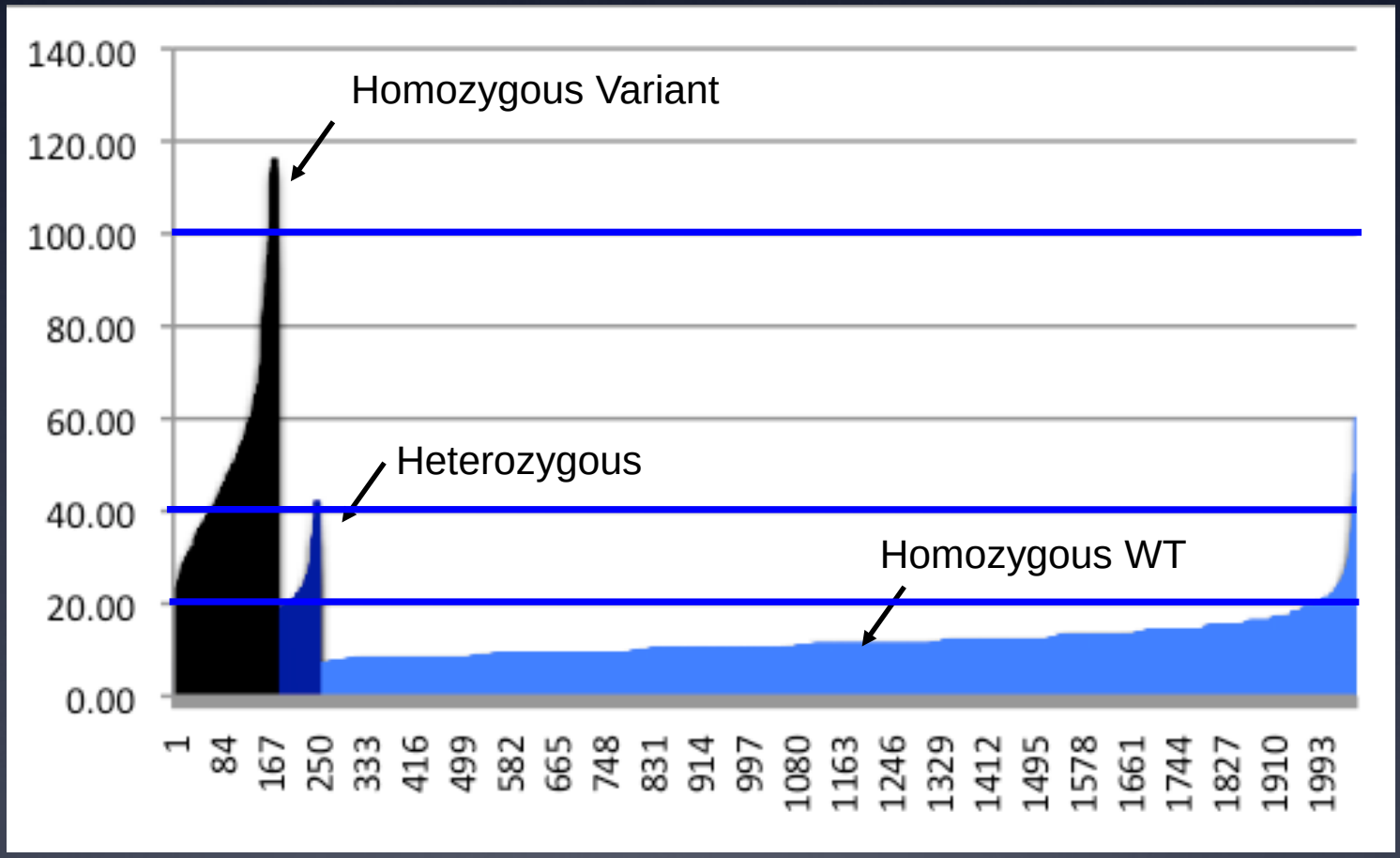
Summary:

- Homozygosity for the arctic variant of CPT1A is associated with an increased risk of infant mortality
- The arctic variant accounts for a significant part of the increased risk observed in Southwestern and Northern Alaska
- Early identification of homozygous infants could potentially reduce infant mortality in Alaska Native infants
- MS/MS based newborn screening only ascertains 10% of homozygous infants

Where Do We Go From Here ?

Newborn screening by MS/MS: C0/C16+C18

C0 / (C16 + C18)



Impact of changes in the C0/C16+C18 cutoff:

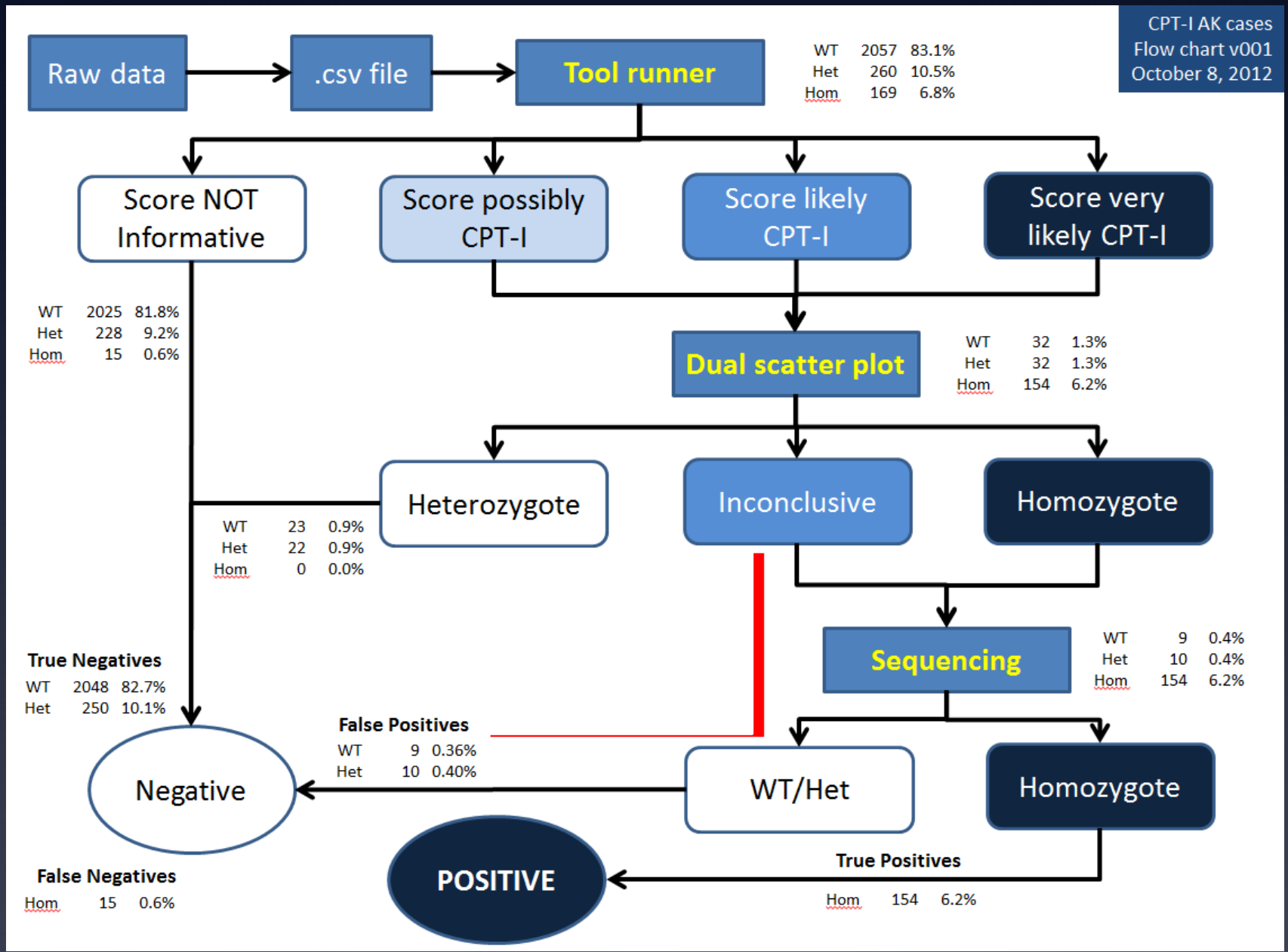
Ratio	Sensitivity	Specificity	Negative predictive value
130	0% (0/166)	100% (2243/2243)	93.1% (2243/2409)
100	3.0% (5/166)	100% (2243/2243)	93.3% (2243/2404)
40	61.4% (102/166)	99.9% (2240/2243)	97.2% (2240/2304)
20	99.4% (165/166)	95.1% (2132/2243)	100% (2132/2133)

Newborn screening by MS/MS: Region 4 Stork Tools

Barcode	Test	Conc
20080020088	Ala	325
20080020088	Arg	20.8
20080020088	ASA	0.7
20080020088	C0	46.1
20080020088	C10	0.19
20080020088	C10:1	0.13
20080020088	C12	0.26
20080020088	C12:1	0.2
20080020088	C14	0.41
20080020088	C14-OH	0.07
20080020088	C14:1	0.36
20080020088	C14:2	0.03
20080020088	C16	4.93
20080020088	C16-OH	0.05
20080020088	C16:1	0.47
20080020088	C16:1-OH	0.23
20080020088	C18	1.1
20080020088	C18-OH	0.03
20080020088	C18:1	1.82
20080020088	C18:1-OH	0.03
20080020088	C2	48.8
20080020088	C3	2.42
20080020088	C3DC	0.14
20080020088	C4	0.14
20080020088	C5	0.19
20080020088	C5-OH	0.44
20080020088	C5:1	0.13
20080020088	C5DC	0.18
20080020088	C6	0
20080020088	C8	0.05
20080020088	Cit	15.9
20080020088	Leu	118
20080020088	Met	38.6
20080020088	Phe	84
20080020088	Tyr	108
20080020088	Val	135

Number of Screening samples	2476
Analytes per screen	36
Rows of data	89136

Barcodes (n = 2476)	Genotype (Hom = homozygous for the c.1436C>T variant)
20080020088	WT
20080020089	WT
20080020093	WT
20080020094	WT
20080020095	WT
20080020106	Hom
20080020107	Hom
20080020108	WT
20080020109	Het
20080020110	Het
20080020111	WT
20080020112	WT
20080020113	WT


CPT-I AK cases
Flow chart v001
October 8, 2012
Raw data
.csv file
Tool runner
WT 2057 83.1%
Het 260 10.5%
Hom 169 6.8%
Score NOT Informative
Score possibly CPT-I
Score likely CPT-I
Score very likely CPT-I
WT 2025 81.8%
Het 228 9.2%
Hom 15 0.6%
Dual scatter plot
WT 32 1.3%
Het 32 1.3%
Hom 154 6.2%
Heterozygote
Inconclusive
Homozygote
WT 23 0.9%
Het 22 0.9%
Hom 0 0.0%
Sequencing
WT 9 0.4%
Het 10 0.4%
Hom 154 6.2%
True Negatives
WT 2048 82.7%
Het 250 10.1%
False Positives
WT 9 0.36%
Het 10 0.40%
Negative
WT/Het
Homozygote
False Negatives
Hom 15 0.6%
POSITIVE
True Positives
Hom 154 6.2%

Newborn screening by MS/MS: Region 4 Stork Tools

FIRST SCREEN	
Sensitivity	**91.12%**
Specificity	**99.18%**
PPV	**89.00%**
NPV	**89.00%**
FPR	**0.76%**

SECOND SCREEN	
Sensitivity	**84.96%**
Specificity	**96.23%**
PPV	**61.00%**
NPV	**99.00%**
FPR	**3.53%**

Summary and Current Plans

- Homozygosity for the arctic variant of CPT1A is associated with an increased risk of infant mortality
- Early identification of homozygous infants could potentially reduce infant mortality in Alaska Native infants
- MS/MS based newborn screening will not achieve 100% ascertainment
- A new screening algorithm based on universal DNA testing is being adopted
- Educational efforts are being expanded (DVD, Health Aide training, etc.)

Summary and Current Plans

Developing a comprehensive long term follow up study

- Track health outcomes on all Alaska Native infants (2,500/yr) by genotype
- Assess possible dietary impact on health outcomes
  - Prenatal
  - Neonatal / Maternal
- Evaluate the impact of full ascertainment on infant mortality in Alaska Natives

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Questions?