Diet and the CPT1A Arctic Variant: Impact on the Health of Alaska Native Children

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The *CPT1A* Arctic Variant:

**Background**

- Discovery and Prevalence
- Physiologic Effects
- Epidemiologic findings
- Newborn Screening

**Work in Progress**

- Diet and the Health Effects of the Arctic Variant
Discovery and Prevalence

In late 2003 Alaska initiated MS/MS based newborn screening

• Panel included all primary and secondary targets

• During the first 3 years more than 50 infants with evidence of reduced Carnitine Palmitoyltransferase 1A (CPT1A) activity were identified
  ▪ Based on an elevated C0/C16+C18

• First seven infants were evaluated by enzyme assay
  ▪ Significantly reduced CPT1A activity (80%) and Malonyl-CoA sensitivity
  ▪ All 7 infants were homozygous for a c.1436C→T variant in CPT1A
    ▪ The Arctic Variant
  ▪ All 7 infants were Alaska Native

• A significant number were not being identified by routine newborn screening
All Alaska newborn screening samples from the first 3 months of 2008 were evaluated by MS/MS and DNA analysis (n=2,484)

- 18 positive MS/MS screens (C0/C16+C18 > 100)
- 173 DNA positive
- Ascertainment by MS/MS = 10%

Statewide prevalence among Alaska Native Infants

- 26% homozygous
- 35% heterozygous

Prevalence in Northern and Western Alaska

- 51% homozygous
- 47% heterozygous
- Gene frequency = 0.7
Physiologic Effects of the *CPT1A* Arctic Variant

*CPT1A* is critical for ketone generation during fasting
Impact of the Arctic Variant on fasting ketogenesis

Inpatient fasting study:

- Five homozygous 3-4 year old Alaska Native children
- Ability to generate ketones from fatty acids
- Ability to maintain blood glucose

**Ketones are Very Low**

**FFA / Ketone ratio is high**

*Blood Ketones (mM)*

*FFA / Ketones*

*Hours of Fasting*

*Molecular Genetics and Metabolism 2011;104: 261–264  Funding: Oregon Clinical and Translational Research Institute*
Epidemiologic Findings:

<table>
<thead>
<tr>
<th>Region</th>
<th>IMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>12.1*</td>
</tr>
<tr>
<td>Southwest</td>
<td>10.8*</td>
</tr>
<tr>
<td>Anchorage/Mat-Su</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>(ref)</td>
</tr>
<tr>
<td>Gulf Coast</td>
<td>6.2</td>
</tr>
<tr>
<td>Interior</td>
<td>6.2</td>
</tr>
<tr>
<td>Southeast</td>
<td>6.2</td>
</tr>
</tbody>
</table>
Impact of the \textit{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
- Genotyping from newborn blood spots
Association between the Arctic Variant and infant mortality

<table>
<thead>
<tr>
<th>All birth weights</th>
<th>Odds ratio (95% CI)</th>
<th>Adjusted* odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>1.7 (1.1, 2.6)</td>
<td>1.8 (1.1, 2.9)</td>
</tr>
<tr>
<td>Homozygous and heterozygous</td>
<td>1.8 (1.1, 2.9)</td>
<td>2.3 (1.3, 4.0)</td>
</tr>
<tr>
<td>Residents of Western and Northern Alaska</td>
<td>1.8 (0.98, 3.3)</td>
<td>2.5 (1.3, 5.0)</td>
</tr>
</tbody>
</table>

* 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.

<table>
<thead>
<tr>
<th>Normal birth weights ( &gt; 2,500 gms)</th>
<th>Odds ratio (95% CI)</th>
<th>Adjusted* odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>1.7 (1.1, 2.7)</td>
<td>1.8 (1.1, 2.9)</td>
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<tr>
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<td>1.7 (0.99, 2.9)</td>
<td>2.2 (1.2, 3.9)</td>
</tr>
<tr>
<td>Residents of Western and Northern Alaska</td>
<td>2.2 (1.1, 4.2)</td>
<td>3.0 (1.4, 6.3)</td>
</tr>
</tbody>
</table>
Association between the Arctic Variant and cause of death

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIDS or asphyxia of unknown etiology</td>
<td>0.50 (0.22 to 1.1)</td>
</tr>
<tr>
<td>Infectious disease</td>
<td>2.9 (1.0, 8.0)</td>
</tr>
<tr>
<td>Congenital anomaly</td>
<td>0.91 (0.34, 2.4)</td>
</tr>
<tr>
<td>Injury</td>
<td>1.2 (0.38, 3.6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Illness Preceding Death</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any hospitalization</td>
<td>5.1 (1.7, 16)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>15 (1.9, 125)</td>
</tr>
<tr>
<td>Sepsis or meningitis</td>
<td>2.9 (0.88, 9.2)</td>
</tr>
</tbody>
</table>

Funding provided by the NICHD: R03 HD060728

*Genetics in Medicine* 2016; 18 (9): 933-939

*Journal of Pediatrics* 2013; 163:1716-1721

*Pediatrics* 2010; 126 (5): 945-951
Newborn Screening for the *CPT1A* Arctic Variant:

Ascertainment by MS/MS using the C0/C16+C18 ratio = 10%

Algorithm development with Dr. Piero Rinaldo using the R4S tools
### Results

<table>
<thead>
<tr>
<th></th>
<th>FIRST SCREEN</th>
<th>SECOND SCREEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>91.12%</td>
<td>84.96%</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.18%</td>
<td>96.23%</td>
</tr>
<tr>
<td>PPV</td>
<td>89.00%</td>
<td>61.00%</td>
</tr>
<tr>
<td>NPV</td>
<td>89.00%</td>
<td>99.00%</td>
</tr>
<tr>
<td>FPR</td>
<td>0.76%</td>
<td>3.53%</td>
</tr>
</tbody>
</table>

Alaska initiated universal DNA testing for the Arctic Variant in July 2016.
Summary:

- Approximately 50% of Alaska Native infants born in Western and Northern Alaska are homozygous for the *CPT1A* Arctic Variant
  - Approximately 700 every year

- Homozygosity is associated with:
  - An impaired ability to generate ketones during fasting
  - An increased risk of hypoglycemia during fasting
  - An increased risk of infant mortality, particularly associated with lower respiratory tract infections

- Based on experience with other disorders of fatty acid oxidation, early identification of homozygous infants could potentially reduce infant mortality in Alaska Native infants

- Due to the low ascertainment of MS/MS based newborn screening universal DNA testing has begun
Work in Progress

Diet and the Health Effects of the Arctic Variant
Diet and the Health Effects of the Arctic Variant

Rationale:

- The $CPT1A$ Arctic Variant arose ~ 6 - 23,000 years ago and was under very strong positive selection in circum-arctic populations
  - The Arctic Variant is the most common allele of $CPT1A$ among Yupik and Inupiat Alaska Native people, Canadian and Greenland Inuit, and indigenous people of Eastern Siberia
  - Diet includes seal, whale, salmon, and other foods that contain very high levels of omega-3 polyunsaturated fatty acids (n-3 PUFAs)
- High n-3 PUFA intake will increase expression of $CPT1A$ via activation of peroxisome proliferator-activated receptor-alpha (PPAR-α)
  - Overexpression mitigates the impact of reduced catalytic activity
  - Reduced Malonyl-CoA sensitivity allows for continuous fatty acid oxidation
Hypothesis:

- n-3 PUFA status modifies the health effects of the Arctic Variant

- Risks associated with homozygosity for the variant will be lowest in infants with the highest n-3 PUFA levels
Study Goals

1. To work with the Alaska Native people from the Norton Sound and Yukon-Kuskokwim regions to learn whether the types of foods eaten by pregnant mothers and their infants can modify the health effects of the arctic variant.

2. To learn whether the home environment or other factors identified by community members have an impact on the health effects of the arctic variant.
Study Overview

- Recruit ~ 1,000 expectant mothers near the end of pregnancy (~36 weeks)
  - Collect health and diet information
  - Collect blood via finger-stick to measure n-3 PUFAs
- Newborn infants will be followed until 2 years of age
  - Collect blood via heel-stick for PUFA analysis at 0, 6, 12, and 24 months of age
  - Obtain information on infant feeding practices, health, and other concerns from caregivers
  - Collect health information from the EHR, including CPT1A genotype from newborn screening report
- Hire local personnel to assist with recruitment and data collection
- Work with local community members, health care providers, tribal leadership and other stakeholders in all aspects of the study
  Case report forms will be built from existing CPT1A deficiency forms
Study Overview

• The Longitudinal Pediatric Data Resource (LPDR) will be used for all data management
  • Case report forms will be built from existing CPT1A deficiency forms
Study Team

Oregon Health & Science University
- David Koeller, MD (Co-PI)
  - Metabolic physician
  - Oregon and Alaska
- Joyanna Hansen, PhD, RD
  - Metabolic dietitian
  - Nutritional epidemiologist

Southcentral Foundation
- Denise Dillard, PhD (Co-PI)
  - Director of Research, SCF
- Matt Hirschfeld, MD/PhD
  - Pediatrician
  - Medical Director, Maternal Child Health Services, ANMC

Funding: Pending
Questions