The Arctic Variant of CPT-1A

Matthew Hirschfeld, MD/PhD

Department of Pediatric Hospital Medicine
Alaska Native Medical Center
Anchorage, AK
Background

- **CPT-1** = carnitine palmitoyltransferase type 1
  - Expressed in fibroblasts, liver, brain, skin, skeletal muscle, kidney
- **CPT-1A** = liver isoform of CPT-1
  - All reported cases of human deficiency of CPT-1 are due to defect in the CPT-1A isoform
CPT-1A Function

- Responsible for the 1\textsuperscript{st} and rate-limiting step in mitochondrial fatty acid oxidation
- Located in the outer (cytosolic) membrane of the mitochondrion
A Closer Look

Diagram showing the transport of Acyl-CoA from the cytosol to the mitochondrial matrix, involving Carnitine and transport enzymes CPTI and CPTII.
Symptoms of “Classic” CPT-1A Deficiency

- Occur after prolonged fast, when glucose and glycogen stores become depleted
- Presents with hypoketotic hypoglycemia, fatigue, vomiting, liver dysfunction, and seizures
History of “Classic”
CPT-1A Deficiency

• Deficiency is rare
  • 2004 review reported 30 cases worldwide
  • First cases in Alaska diagnosed in 2004—confirmed by skin biopsy (only method of confirming CPT-1A deficiency at the time)
  • Found when Alaska changed to MS/MS to perform their newborn metabolic screens (NBMS)
“Classic” CPT-1A Deficiency And Expanded NBMS

• Expanded screening utilizes tandem mass spectroscopy (MS/MS) to evaluate the levels of free carnitine and acylcarnitines.

• CPT1A deficiency is identified by a high ratio of free carnitine ($C_0$) to the sum of palmitoylcarnitine ($C_{16}$) plus stearoylcarnitine ($C_{18}$): $\frac{C_0}{C_{16}+C_{18}}$

• A ratio $> 130$ suggests CPT-1A deficiency.

• Due to a relative inability to make acylcarnitines because of CPT-1A deficiency.
A Closer Look

![Diagram showing the transport of Acyl-CoA from cytosol to the mitochondrial matrix via interactions with Carnitine, CPT (Carnitine PalmitoylTransferase I), and Translocase.]

- CPTI (Carnitine PalmitoylTransferase I)
- CPTII (Carnitine PalmitoylTransferase II)
The Arctic Variant

- A missense mutation (P479L) found in all affected Alaska Native people
- NBMS confirmed by PCR—Gold Standard
- Same mutation found in Canadian and Greenland Inuit populations and in British Columbia First Nations populations
- Skin biopsy results showed that this mutation gives 10-25% enzyme activity
The Arctic Variant Regulation

- P479L occurs in a region of CPT-I responsible for regulation of activity
  - CPT-1A is inhibited by malonyl CoA
    - Malonyl CoA increases when carbohydrates are ingested
  - Mutation causes protein to always be “on” by not allowing malonyl CoA to bind
Newborn Screening

• NBMS has now detected about 300 cases in Alaska since 2004

• However, we know we’re detecting the minority of cases

• There are actually 750 infants with the P479L variant born per year in Alaska

• Can’t set the $C_0/C_16+C_{18}$ ratio to detect all cases without large number of false positives
Distribution

Percent of Native Alaskan Newborns who are CPT1A c.1436C>T Homozygotes:
- 60-70%
- 50-59%
- 30-39%
- 20-29%
Questions Surrounding the Arctic Variant of CPT-1A

• All of the infants who had a skin biopsy had 10-25% residual activity—is this enough activity to eliminate symptoms?

• Could the Arctic variant be a contributing factor to the higher rates of SIDS or severe illness in the rural villages in Northern/Southwest Alaska?

• Why does this variant have such high prevalence in Arctic populations?
1st Project

- 5 families with a child between the ages of 3-5 years with the Arctic variant of CPT-1A flown to Doernbecher Children’s Hospital for an 18 hour fasting study

- Labs at 6, 12, 18 hours drawn: Chem7, insulin, acylcarnitines, free fatty acids, lactate, pyruvate, ketones (acetoacetate and 3-hydroxybutyrate)

- Hourly serum glucose starting at 6 hours of fasting

- MRS done to determine if fatty deposits occur in liver
Results

- All kids had an abnormal fasting response
- 2/5 became symptomatic with glucose below 50 before the fast was finished
- None produced ketones
- Parents described lifelong symptoms of hypoglycemia with fasting
Questions Surrounding the Arctic Variant of CPT-1A

• All of the infants who had a skin biopsy had 10-25% residual activity—is this enough activity to eliminate symptoms?
• Could the Arctic variant be a contributing factor to the higher rates of SIDS or severe illness in rural villages in Northern/Southwest Alaska?
• Why does this variant have such high prevalence in Arctic populations?
2nd Study

- Perform PCR on last 3 years of NBMS cards from patients who marked “Alaska Native” race and link the results to State of Alaska data looking at infant mortality and vital statistics
# CPT-1 Deficiency and SIDS

## Infant mortality rates (IMR) 1992-2004

<table>
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<th>Region</th>
<th># Deaths</th>
<th>IMR</th>
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<tbody>
<tr>
<td>Northern</td>
<td>83</td>
<td>12.1*</td>
</tr>
<tr>
<td>Southwest</td>
<td>123</td>
<td>10.8*</td>
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<td>Anchorage Bowl</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>Southeast</td>
<td>79</td>
<td>6.2</td>
</tr>
</tbody>
</table>
Distribution

Percent of Native Alaskan Newborns who are CPT1A c.1436C>T Homozygotes:
- North Slope: 60-70%
- North West Arctic: 50-59%
- Nome: 30-39%
- Bethel: 20-29%

Map showing distribution of the percent of Native Alaskan Newborns who are CPT1A c.1436C>T Homozygotes across different regions in Alaska, with varying shades indicating the percentage ranges.
Preliminary Data

• When we looked at 2500 sequential births in Alaska
• Infant mortality was associated with the Arctic variant
• Children were more likely to be hospitalized in the first 3 years of life if they were homozygous for the Arctic variant
Questions Surrounding the Arctic Variant of CPT-1A

• All of the infants who had a skin biopsy had 10-25% residual activity—is this enough activity to eliminate symptoms?

• Could the Arctic variant be a contributing factor to the higher rates of SIDS or severe illness in the rural villages in Northern/Southwest Alaska?

• Why does this variant have such high prevalence in Arctic populations?
Traditional Diet
Permanent Ketosis

- Arctic populations *traditionally* eat a diet of 80% fat, 15% protein, and less than 5% carbohydrate *(mostly from muscle glycogen)*

- Essentially a ketogenic diet, and their bodies get used to functioning without glucose

- Fatty-acid oxidation generates ketone bodies to be used for energy
Ketogenic Diet When Ketogenesis Is Not Working Well?

- The Arctic variant of CPT-1A might be advantageous to people observing a traditional diet because of its insensitivity to malonyl-CoA.
- If a ketogenic diet has to be interrupted due to lack of fatty foods, the sudden disruption causes severe weakness, nausea, and headaches.
- Overcome by eating more carbohydrates, which are not readily available in the Arctic.
Ketogenic Diet When Ketogenesis Is Not Working Well?

- Non ketogenic diet most common at the end of winter, when low-fat meat is consumed—more muscle glycogen.

- If the Arctic variant of CPT-1A is not inhibited by malonyl CoA with carbohydrate ingestion, then ketogenesis would tend to continue, and the malaise would occur less frequently and less severely.

- Could allow for increased survival in a unforgiving environment.
Also

• Marine animals have high levels of n-3 polyenoic fatty acids, which increase activity of CPT-1A

• These fatty acids are passed into breast milk

• May partially off-set the 80% drop in activity
Other Effects

• In Greenland Inuit people, homozygotes for the Arctic variant have higher levels of HDL

• Unknown if this affects cardiovascular disease
Unknown

- Does the Arctic variant of CPT-1A play a role in other adult disease?
- Diabetes
Thanks

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Fasting Project Continued

• Their livers were imaged using magnetic resonance spectroscopy. The hypothesis was that these kids will have fatty livers compared to controls because of their inability to metabolize fats efficiently—like classic CPT-1A deficiency

• Actually, completely normal livers
Fasting Project Continued

• 2 of the 5 kids became symptomatic with plasma glucose below 51
• One went below 40
Fatty Acid Oxidation