The Arctic Variant of CPT-1A

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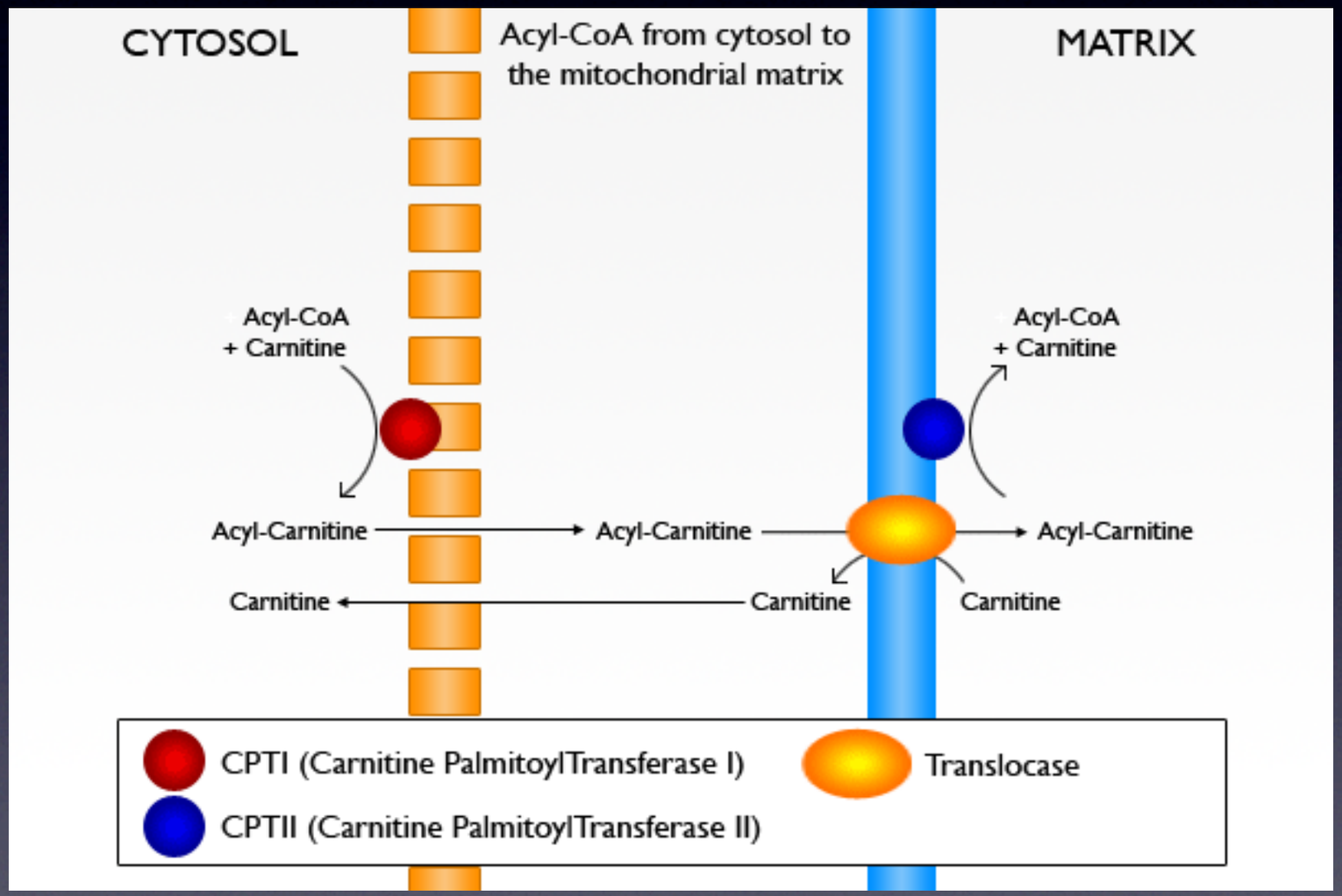
Background

- 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 1st and rate-limiting step in mitochondrial fatty acid oxidation
- Located in the outer (cytosolic) membrane of the mitochondrion

A Closer Look



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

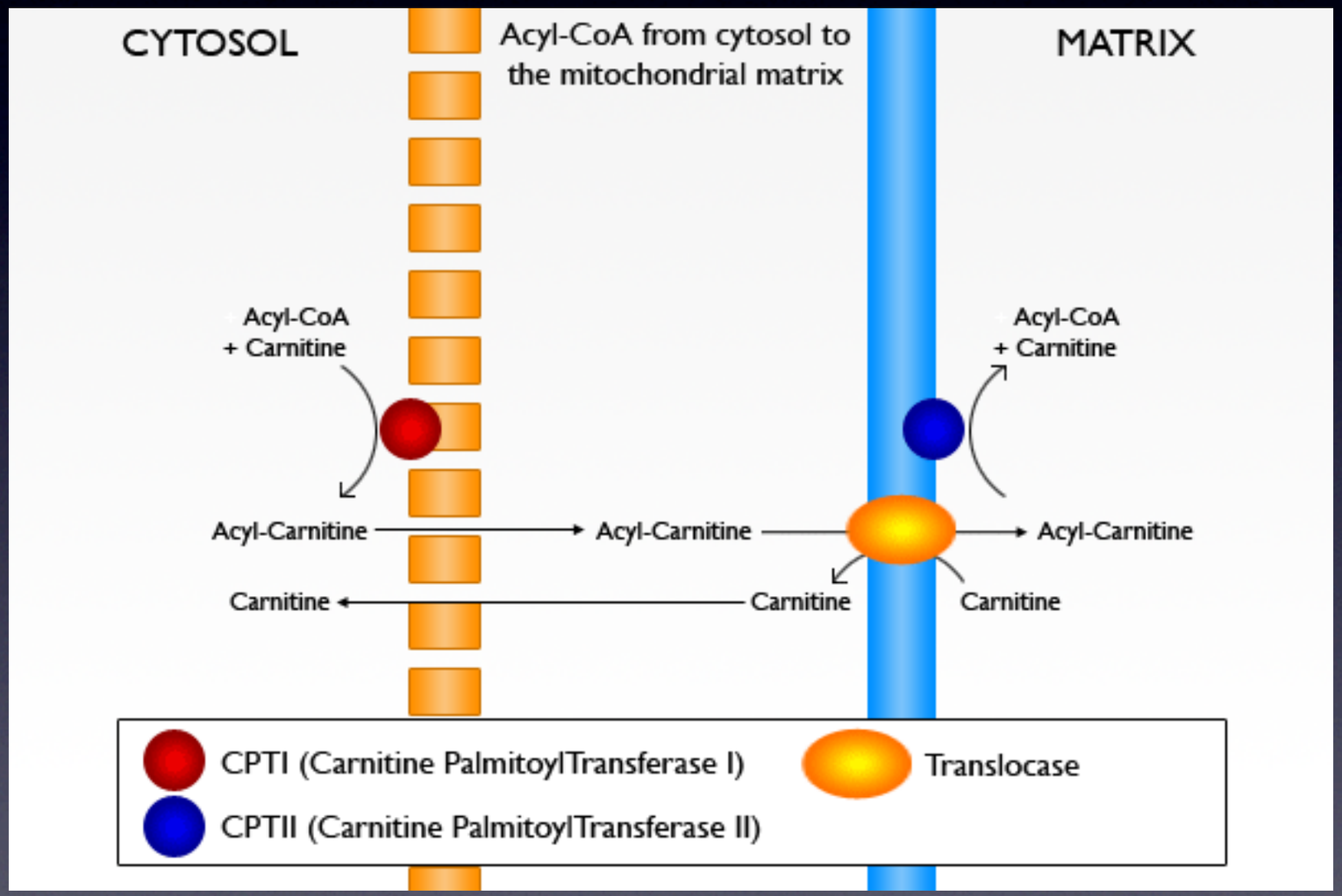
History of "Classic" CPT-1A Deficiency

- 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 (C0) to the sum of palmitoylcarnitine (C16) plus stearoylcarnitine (C18)=C0/C16+C18
  - A ratio > 130 suggests CPT-1A deficiency
    - Due to a relative inability to make acylcarnitines because of CPT-1A deficiency

A Closer Look



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

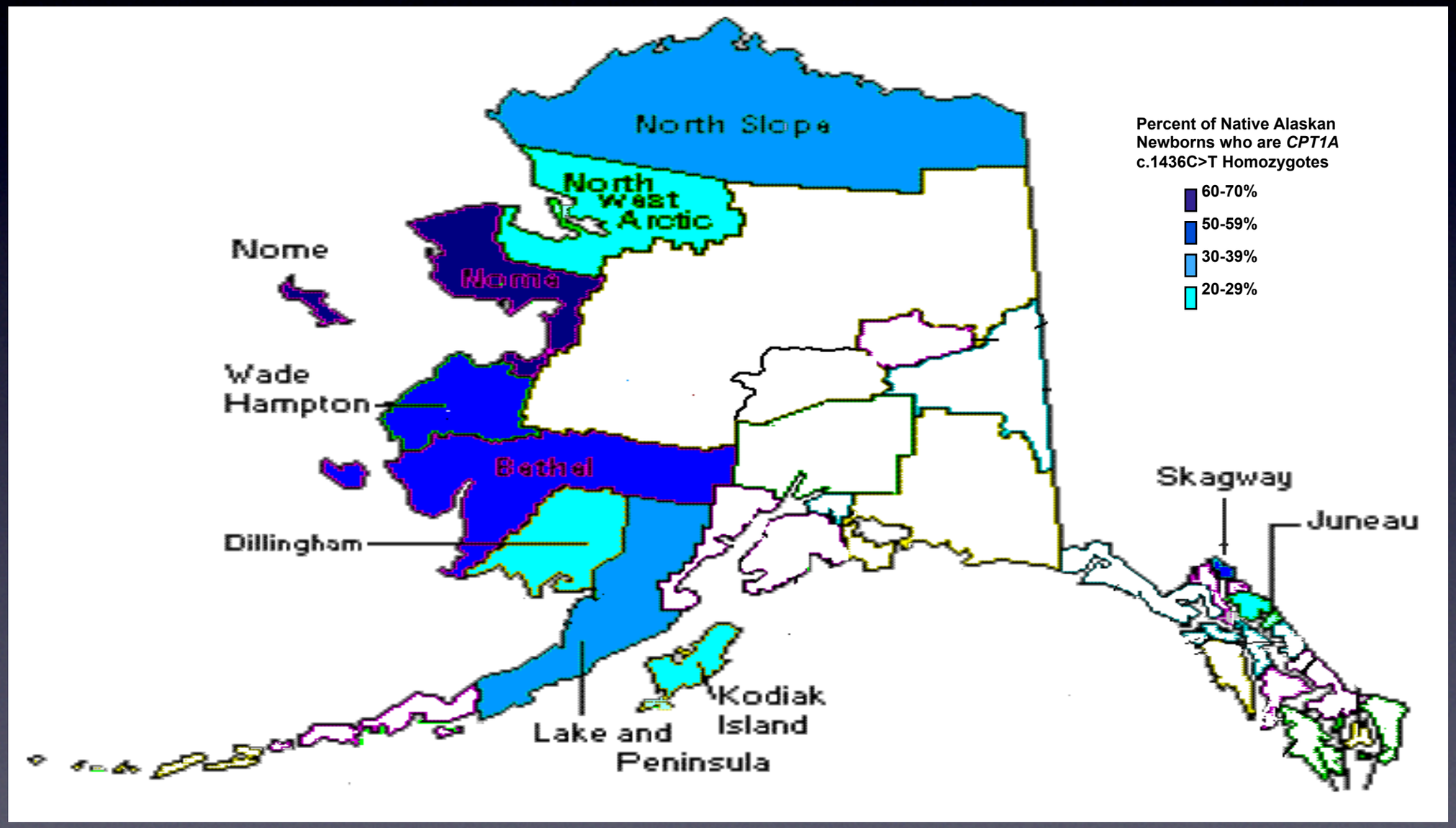
The Arctic Variant Regulation

- P479L occurs in a region of CPT-1 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 C0/C16+C18 ratio to detect all cases without large number of false positives

Distribution



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 glucoses below 50 before the fast was finished
  - None produced ketones
  - Parents described lifelong symptoms of hypoglycemia with fasting

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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

Region	# Deaths	IMR
Northern	83	12.1*
Southwest	123	10.8*
Anchorage Bowl	425	6.3
Gulf Coast	84	6.2
Interior	140	6.2
Southeast	79	6.2

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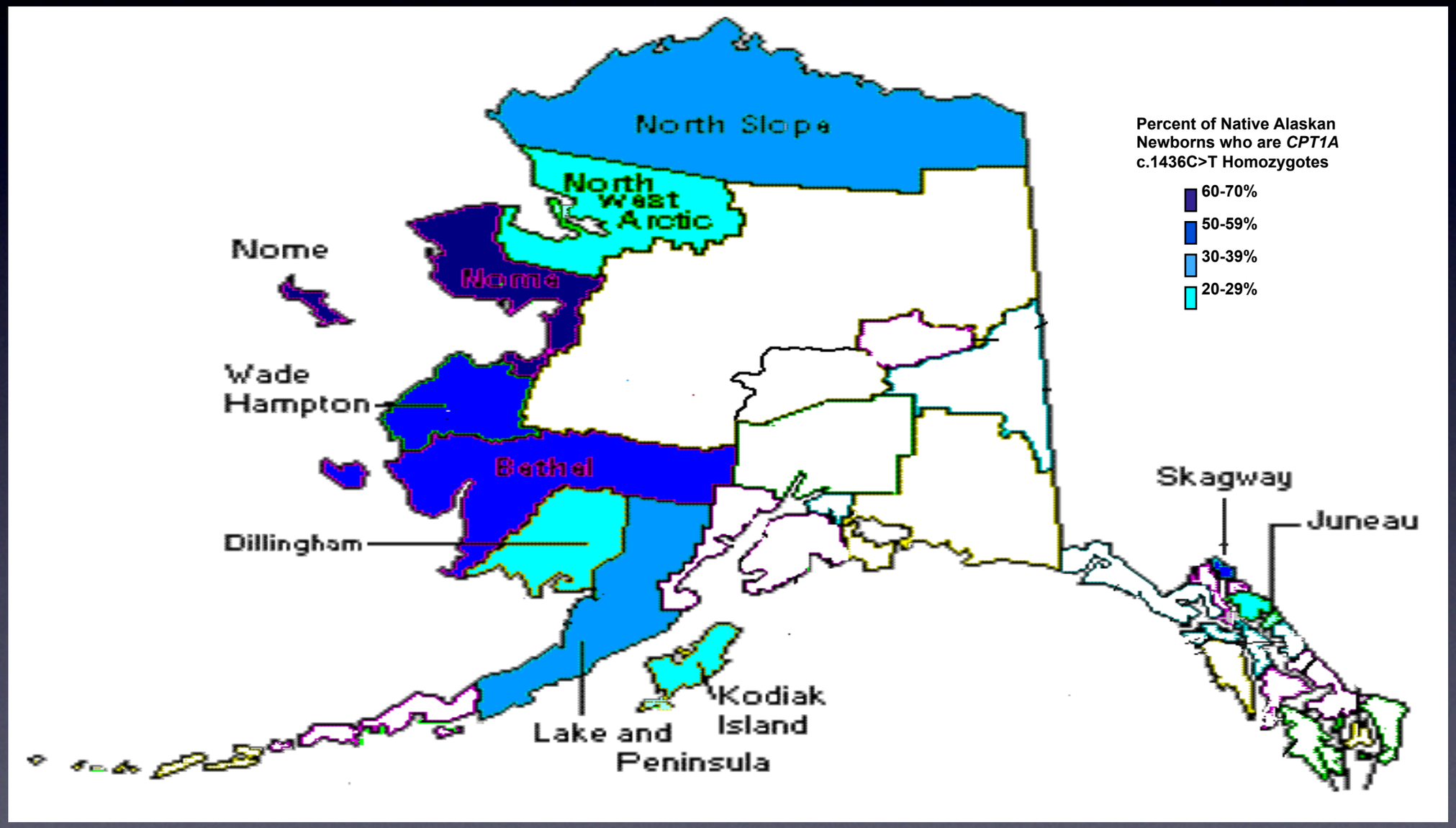

Preliminary Data

- 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

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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