Prevalence and Distribution of the c.1436C→T Sequence Variant of Carnitine Palmitoyltransferase 1A among Alaska Native Infants

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Objectives To use genotype analysis to determine the prevalence of the c.1436C→T sequence variant in carnitine palmitoyltransferase 1A (CPT1A) among Alaskan infants, and evaluate the sensitivity of newborn screening by tandem mass spectrometry (MS/MS) to identify homozygous infants.

Study design We compared MS/MS and DNA analyses of 2409 newborn blood spots collected over 3 consecutive months.

Results Of 2409 infants, 166 (6.9%) were homozygous for the variant, all but one of whom were of Alaska Native race. None of the homozygous infants was identified by MS/MS on the first newborn screen using a C0/C16 + C18 cutoff of 130. Among 633 Alaska Native infants, 165 (26.1%) were homozygous and 218 (34.4%) were heterozygous for the variant. The prevalence was highest in Alaska's northern/western regions (51.2% of 255 infants homozygous; allele frequency, 0.7).

Conclusions The CPT1A c.1436C→T variant is prevalent among some Alaska Native peoples, but newborn screening using current MS/MS cutoffs is not an effective means to identify homozygous infants. The clinical consequences of the partial CPT1A deficiency associated with this variant are unknown. If effects are substantial, revision of newborn screening, including Alaska-specific MS/MS cutoffs and confirmatory genotyping, may be needed. *(J Pediatr 2011;158:124-9)*.

Tandem mass spectrometry (MS/MS) has significantly increased the number of inborn errors of metabolism that can be identified presymptomatically[1] and is now being used in all 50 US states. The Alaska Newborn Metabolic Screening Program began using MS/MS in October 2003, with a panel that included all of the recommended core and secondary disorders,[2] including carnitine palmitoyltransferase 1A (CPT1A) deficiency.

CPT1A deficiency is a rare autosomal recessive disorder of fatty acid oxidation that impairs fasting ketogenesis and gluconeogenesis by the liver.[3] Symptoms are triggered by fasting and exacerbated by stress, such as fever and infection. In patients with only partial CPT1A deficiency, lethargy with or without hypoglycemia may be the sole symptom. More severe deficiency can result in sudden unexplained infant death or hypoketotic hypoglycemia with liver failure.[3,4] Early identification and intervention can prevent these symptoms, underscoring the importance of newborn screening.

During the first 5 years of newborn screening using MS/MS in Alaska (January 2004 through December 2008), an unexpectedly high number of infants (n = 176; birth prevalence, 0.33%) had a positive newborn screen for CPT1A deficiency. DNA analysis performed for diagnostic confirmation found that all 176 infants were homozygous for a c.1436C→T sequence variant in the *CPT1A* gene, which results in a partial loss of catalytic activity (~80%), predicting a relatively mild clinical phenotype.[5,6] Consistent with previous reports of a high prevalence of this sequence variant among some Canadian Aboriginal populations,[6] most of the infants were Alaska Natives. Of the 176 infants homozygous for the c.1436C→T variant, 32 (18%) were identified on the first newborn screen (mean age, 80 hours), 132 (75%) were identified on a second screen (mean age, 28.6 days), and 12 (7%) were identified on a third screen (mean age, 40 days) conducted to follow up on an unrelated abnormality. Although CPT1A deficiency due to homozygosity for this variant is predicted to have a mild phenotype, specific data are lacking. Determining the clinical consequences of homozygosity for this variant will require a high rate of ascertainment and longitudinal tracking of the health outcomes of identified infants. We undertook the current study out of concern that newborn screening by MS/MS was not

CPT1A	Carnitine palmitoyltransferase 1A
HWE	Hardy-Weinberg equilibrium
MS/MS	Tandem mass spectroscopy
NWRNSP	Northwest Regional Newborn Screening Program
OHSU	Oregon Health & Science University
SIDS	Sudden infant death syndrome

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identifying all affected infants. To determine the sensitivity of MS/MS, we evaluated newborn bloodspots collected from infants born over a 3-month period by both MS/MS and DNA sequence analysis. We then used these data to determine the true birth prevalence of the c.1436C→T variant, as well as its racial and geographic distribution.

Methods

Newborn screening blood spots from infants born in Alaska are sent to the Northwest Regional Newborn Screening Program (NWRNSP), Hillsboro, Oregon for analysis.[7] In accordance with Alaska and NWRNSP recommendations, approximately 90% of Alaskan infants undergo two screens, the first at 24-48 hours of age, and the second at around 2 weeks of age. For this study, an extra punch sample of dried blood was collected for DNA analysis from 2499 consecutive newborn screening cards that were submitted to the NWRNSP for a first screen.

Routine newborn screening, including MS/MS analysis, was done on all samples following the NWRNSP's standard testing procedures. CPT1A catalyzes the formation of long-chain acylcarnitines from long-chain acyl-CoAs and free carnitine. Consequently, infants with reduced CPT1A activity have diminished levels of long-chain acylcarnitines and increased free carnitine levels. The diagnosis of CPT1A deficiency via MS/MS is based on the presence of an elevated ratio of free carnitine (C0) to the sum of C16 and C18 acylcarnitines (C0/C16 + C18), which has been shown to effectively discriminate between affected and unaffected infants.[8] During the period of this analysis, the NWRNSP considered a C0/C16 + C18 ratio of >130 as positive for CPT1A deficiency. Genotyping for the presence of the c.1436C→T sequence variant in *CPT1A* was done in the Molecular Diagnostic Center at the Oregon Health & Science University (OHSU) through an allelic discrimination assay developed for clinical testing, using DNA extracted from newborn blood spot samples.[9] Samples were coded with a unique identifier and tested anonymously. We defined the wild-type allele (c.1436C) as that corresponding to the sequence of *CPT1A* in the National Center for Biotechnology Information reference sequence database (NM_001876.3:http://www.ncbi.nlm.nih.gov/nuccore/188595713), although we recognize that within some populations of Alaska Natives, the variant allele (c.1436T) is the most prevalent and also could be considered wild type.

Data Analysis

The MS/MS and genotype results for tested infants were linked to birth certificate data obtained from the Alaska Bureau of Vital Statistics, allowing us to determine the geographic and racial distribution. Of the originally selected 2499 newborn screening cards, 90 infants were excluded because they had some combination of missing data for MS/MS (n = 9), DNA analysis (n = 15), race (n = 58), or residence community (n = 52), leaving 2409 evaluated infants. Based on our previous observation that virtually all of the infants homozygous for the c.1436C→T sequence variant were of Alaska Native race, we used only two racial categories: Alaska Native and non-Native. Infants were considered Alaska Native if either paternal or maternal race, as self-reported on the birth certificate, was Alaska Native. The birth certificate information does not distinguish among the 3 primary racial subgroups of Alaska Natives, including the Yupik and Inuit people, who are the primary residents of western and northern Alaska; Athabascan and other Indian people, concentrated in central, south-central, and southeastern Alaska; and Aleut people, the primary residents of the Aleutian Islands. Almost half of Alaska's population lives in Anchorage, which also has the state's largest Alaska Native population, comprising all 3 racial subgroups. All analyses were performed with SPSS version 13 (SPSS Inc, Chicago, Illinois).

Ethics

This study was undertaken as a quality control exercise to evaluate the sensitivity of MS/MS-based newborn screening for CPT1A deficiency due to the c.1436C→T variant and, secondarily, to provide a more accurate determination of prevalence and racial and geographic distributions. Newborn blood spots, birth certificates, and other sources of public health data used in this study are under the legal jurisdiction of the Alaska Division of Public Health, which is legally mandated to conduct newborn metabolic screening for Alaska. In Alaska, routine screening includes evaluation for CPT deficiency, and the Alaska Division of Public Health's responsibility regarding this condition is the same as for all other screened conditions. Consequently, the analysis of these data for public health purposes by employees of the Alaska Division of Public Health is considered exempt from institutional review board (IRB) review. Nevertheless, the Alaska Newborn Screening program has an Advisory Panel, which includes representation from the Alaska Native community, that reviews policies and procedures, including the current evaluation. In addition, all work associated with evaluation and other work on CPT1A in Alaska is done in full collaboration with the Alaska Native community, including the Alaska Native Tribal Health Consortium, which supports and in some cases actively participates in the evaluation and research program surrounding this issue.

Testing for the c.1436C→T sequence variant in DNA extracted from newborn screening cards at OHSU was conducted using an anonymous identifier. The protocol was reviewed by OHSU's IRB and was granted an exemption (IRB #3561).

Results

MS/MS Screening Test Performance for Homozygosity of the c.1436C→T Variant

Among the 2409 evaluated infants, 6.9%, or 1 in 15, were homozygous for the c.1436C→T sequence variant (**Table I**). The mean C0/C16 + C18 ratio was significantly higher for