PREVALENCE OF CARNITINE PALMITOYLTRANSFERASE 1A (CPT1A) VARIANT p.P479L AND RISK OF INFANT MORTALITY IN NUNAVUT, NORTHWEST TERRITORIES, AND YUKON

by

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ABSTRACT

Introduction: The p.P479L (c.1436C>T) variant of hepatic CPT1A is frequent in Inuit and British Columbia First Nations populations of Canada. CPT1A is a major regulatory point in long chain fatty acid oxidation in the liver. CPT1A deficiency is an autosomal recessive disorder that causes metabolic decompensation triggered by fasting, which can progress to seizures and sudden death, if not treated. This study assesses prevalence and clinical impact of the P479L variant in the Canadian territories and reviews modifiable risk factors associated with infant mortality (IM) in Nunavut.

Methods: Ethics approval was obtained from university REBs and local research institutes, with consultation with territorial Aboriginal groups. Newborn screening blood spots from all infants born in 2006 (n=1584) and sudden death in infancy cases (n=31; 1999-2008) in the territories were genotyped for the P479L variant.

Results: P479L homozygosity in each territory was 64%, 3%, and 1% for Nunavut, NWT, and Yukon, respectively. Within NWT, homozygosity was highest in Inuvialuit (21%) and very low in First Nations (1%). Homozygosity in sudden death cases was highest in Nunavut (18/20) and associated with an increased risk (OR: 5.15; 95% CI: 1.19-22.38). Homozygosity was 29% for NWT cases (2/7), 67% in NWT Inuvialuit (2/3), and was not present in Yukon cases (0/4).

Review of Nunavut IM cases (n=78; 1999-2008) identified Sudden Infant Death Syndrome (SIDS) and Sudden Unexpected Death in Infancy (SUDI) as the leading causes of infant death
(47%), followed by death due to infectious disease (28%). At least 23% of IM cases were premature.

Discussion: The P479L variant is very frequent in the Inuit/Inuvialuit of Canada. Although the sample size was small, there was an associated risk for sudden death in infants homozygous for the variant in Nunavut. SIDS and SUDI are the leading causes of infant death in Nunavut, followed by death due to infectious disease. Since deaths in these two categories are largely preventable, prevention strategies and further exploration into the P479L variant and other determinants are indicated. Management strategies, including newborn screening for the P479L variant, need to be developed in consultation with health authorities, local medical professionals, and local communities.
This research was conducted with ethics approval and regulatory approval from UBC Research ethics board (Appendix A) Aurora Research Institute (NWT), Stanton Territorial Health Authority (NWT), Nunavut Research Institute, and the University of Manitoba. Territorial Aboriginal organisation consultation included: Nunavut Tunngavik Inc. (NTI), the Inuvialuit Regional Corporation (NWT), the Dene Nation (NWT), and the Yukon First Nations Health Commission.

Dr. Laura Arbour (LA) identified the overall CPT1 project, developed the concept of the project through consultation with stakeholders and offered guidance, structure and overview during the project. I planned the details of data collection, methods of analysis and carried out the analysis for the project. Sarah McIntosh submitted ethics applications and assisted in the maintenance of ethics approvals throughout the project.

A version of chapter 2 has been published. Collins SA, Sinclair G, McIntosh S, Bamforth F, Thompson R, Sobol I, Osborne G, Corriveau A, Santos M, Hanley B, Greenberg CR, Vallance H, Arbour L. Carnitine palmitoyltransferase 1A (CPT1A) P479L prevalence in live newborns in Yukon, Northwest Territories, and Nunavut. Mol. Genet. Metab. 2010 Nov;101(2-3):200-204. I conducted genotyping of dried blood spot samples for the Yukon, Northwest Territories and the Qikiqtani/Baffin Island and Kitikmeot regions of Nunavut in the BC newborn screening lab, Vancouver BC under the supervision of LA, Dr. Hilary Vallance, and Dr. Graham Sinclair. I analyzed the genotyping results and wrote the manuscript. LA assisted with editing and structure of the manuscript. The Newborn Screening Program, Cadham Provincial Laboratory, Manitoba genotyped samples from the Kivalliq region, Nunavut.

A version of chapter 3 will be submitted for publication. I conducted the chart review of infant mortality cases in Nunavut under the supervision of LA, Geraldine Osborne, Deputy Chief Medical Officer of Health, Nunavut, and Tim Neily, Chief Coroner, Nunavut. I assisted in review of infant mortality cases for NWT with Maria Santos, Epidemiologist for NWT under advisement of Andre Corriveau, then Chief Medical Officer of Health, NWT. I conducted genotyping of cases for NWT and Kitikmeot, Nunavut. Other cases were genotyped at time of birth or death. Yukon samples were genotyped by Dr. Graham Sinclair. Dr. Sinclair and I genotyped BC First Nations 2004 DBS samples under the supervision of Dr. Hilary Vallance and with the support of LA.

A version of chapter 4 will be submitted for publication. I conducted the chart review as described above for chapter 3. I analysed the data and wrote the manuscript. LA offered guidance and assisted with editing of the manuscript. Sam Lauson worked with Nunavut Vital Statistics to cross-reference coroner infant mortality case data with the vital statistics database.
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LIST OF ABBREVIATIONS

AFLP – acute fatty liver of pregnancy
CPT1 – carnitine palmitoyltransferase 1
DBS – dried blood spot card
FAO – fatty acid oxidation
FAOD – fatty acid oxidation disorder
IM – infant mortality
IMR – infant mortality rate
NBS – newborn screening
SIDS – sudden infant death syndrome
SUDI – sudden unexpected death in infancy
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The project could not have been completed without the support of the Chief Medical Officers of Health for each territory, Isaac Sobol (Nunavut), Geraldine Osborne (Deputy CMOH, Nunavut), Andre Corriveau (former CMOH, NWT), and Brendan Hanley (Yukon). I would also like to acknowledge the assistance of Tim Neily (former Chief Coroner, Nunavut), Cathy Menard (Coroner, NWT), Percy Kinney (Coroner, NWT), María Santos (Epidemiologist, NWT), and Sharon Hanley (Chief Coroner, Yukon).

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Throughout my program, I have been privileged to have the encouragement and support of many friends and family members, for which I am deeply grateful.
DEDICATION

To my family, both the human and four-legged members.
CHAPTER 1. INTRODUCTION

1.1 PURPOSE OF STUDY

To determine the prevalence of the p.P479L variant of carnitine palmitoyltransferase 1A (CPT1A) in Canadian northern populations and in sudden unexpected infant death cases of Nunavut, Northwest Territories (NWT), and Yukon, to determine if the variant plays a role in excess infant mortality in these populations and to assess risk factors associated with infant mortality in Nunavut.

1.2 STUDY RATIONALE

Canada’s northern Aboriginal populations have demonstrably higher infant mortality rates than those found in the Canadian non-Aboriginal population [1-5]. Studies of Canadian and Greenland Inuit, British Columbia (BC) First Nations, and Alaska Natives have found a high prevalence of the P479L variant of CPT1A in these populations [6-9]. To date, more than 40 infants homozygous for the P479L variant have presented clinically with features of CPT1A deficiency, including non-ketotic hypoglycemia, seizures, and, in rare cases, sudden death, often occurring during fasting and/or intercurrent illness [6,10,11]. All affected infants have been of First Nations, Inuit, or Alaska Native ancestry. Therefore, it is important to determine whether P479L homozygous infants are at increased risk for impaired fatty acid oxidation and sudden death. If the variant does confer risk, management strategies will need to be implemented, which may include newborn screening and/or public and medical professional education programs.
1.3 LITERATURE REVIEW

1.3.1 INFANT MORTALITY IN CANADA’S NORTH

1.3.1.1 The Canadian Territories

The three Canadian territories comprise the entire northern landmass of Canada above 60° north and comprises 40% of Canada’s landmass (Figure 1.1). Approximately 50% of the inhabitants of the territories are Aboriginal; 25% in Yukon, 50% in Northwest Territories (NWT), and 86% in Nunavut [12]. The northern Canadian Aboriginal groups are the Inuit, Inuvialuit, First Nations, Métis, and Dene [12].

The northern Canadian territory of Nunavut has the highest infant mortality rate in Canada at 14.3/1,000 live births (1999-2007) which is 3 times the Canadian national average [1]. Nunavut is divided into 3 regions, Qikiqtani, which contains the territorial general hospital (QGH), Kitikmeot, and Kivalliq. The health care system in Nunavut depends on a series of community-based health centres, which are supported by regional hospitals and partnerships with southern tertiary care hospitals in the neighbouring provinces (British Columbia, Saskatchewan, Manitoba, Ontario, and Quebec) [13]. Patients requiring intensive care are evacuated to regional hospitals or out of territory to tertiary care centres in these other jurisdictions [14].
1.3.1.2 The Inuit and Inuvialuit of Canada

The Inuit population of Canada live throughout Canada’s arctic and the lands they inhabit are collective called the Inuit Nunaat (“Inuit Homeland”; Figure 1.1) [12]. The term ‘Inuit’ is used to describe a number of closely related northern populations, which are divided into three linguistic branches; Inuit/Inupiaq, Yupik, and Aleut, all which belong to the Eskimo-Aleut family. The Inuit/Inupiaq inhabit Northern Alaska, Canada, and Greenland. The Yupik
inhabit central and southern Alaska and the Chukotka peninsula of Russia. The Aleut inhabit the Aleutian Islands of Alaska and the Commander Island of Russia [16]. The Inuit of Canada are descended from the Thule, who arrived in the Canadian arctic 1000 to 1600 AD [17]. The Inuvialuit inhabit the coastal north-western areas of the Canadian arctic in NWT and are more closely related to the northern coastal Alaska Native populations than other Inuit populations in the rest of Canada [17]. Nunavut is home to the largest Inuit population in Canada (24,635; 49%) and approximately 85% of the residents are Inuit [12]. An estimated 700 infants are born in Nunavut each year, with at least 90%-95% of those infants being born to Inuit women [12,18,19].

1.3.1.3 Inuit Traditional Diet

Most Inuit populations of Canada traditionally subsisted on a diet consisting mainly of marine mammals (beluga and seal), fish, and caribou, which were eaten cooked or raw and including skin, blubber, and internal organs like liver [17,20]. This traditional diet was high in omega 3 fatty acids, moderate in protein, and very low in carbohydrate [20]. Due to considerable changes to both lifestyle and diet, traditional foods are quickly being replaced with western market foods that are high in carbohydrates and fats and low in nutrients. In Greenland Inuit, traditional food source contribution to daily energy has dropped from 83% in 1901 to 18% in 2006; a drop that was more marked in young Inuit (<35 years of age) [21]. Study of current dietary practices of 2 communities in Nunavut found that the most commonly consumed foods were simple carbohydrate rich foods and that ~20% of dietary energy came from sweetened drinks and sugar, with traditional foods comprising ~12% of
dietary intake [20]. Traditional foods are not only important for physical health, but also social and cultural health of individuals and communities. Factors influencing the consumption of traditional foods include availability and accessibility of sources, knowledge, and skills on procurement and use, environmental contaminants, and availability of time to devote to hunting and harvesting [22]. These factors have resulted in the reliance on non-perishable processed foods as many remote northern communities have very limited access to fresh foods (fruits, vegetables and dairy) [23,24].

1.3.1.4 Infant Mortality in the Territories

Infant mortality is a key indicator of child health in a population and can reflect health disparities between populations [25,26]. Infant mortality rates are calculated using the number of infants less than 1 year of age that die for every 1,000 live births in that jurisdiction. The leading causes of infant death in Canada are listed in Table 1.1 [27]. Most infant deaths in Canada occur during the neonatal period (less the 28 days), which are commonly related to perinatal complications, prematurity, congenital anomalies, obstetric care, and neonatal health [27]. However, the fourth leading cause of death in all of Canada is Sudden Infant Death Syndrome (SIDS), which, along with other causes like infectious diseases, commonly occurs during the post-neonatal period (28 days to 1 year after birth) [28]. Post-neonatal mortality rates for Canadian Aboriginal populations are consistently higher than for their non-Aboriginal counterparts [5].
Table 1.1  Leading causes of infant death for all of Canada, 2000-2005

<table>
<thead>
<tr>
<th>CAUSE OF DEATH</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital malformations, deformations and chromosomal abnormalities</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Disorders related to short gestation and low birth weight, not elsewhere classified</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Newborn affected by maternal complications of pregnancy</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sudden infant death syndrome</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Newborn affected by complications of placenta, cord and membranes</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Intrauterine hypoxia and birth asphyxia</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Neonatal haemorrhages</td>
<td>8</td>
<td>9</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Newborn affected by other complications of labour and delivery</td>
<td>11</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Respiratory distress of newborn</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Bacterial sepsis of newborn</td>
<td>9</td>
<td>7</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Accidents (unintentional injuries)</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>13</td>
<td>14</td>
<td>12</td>
</tr>
</tbody>
</table>

Adapted from Statistics Canada [27]

The three Canadian territories, Nunavut, NWT, and Yukon, experience some of Canada’s highest infant mortality rates in Canada (Table 1.2), which exceed the national average by 3, 1.3, and 1.6 times, respectively [1]. Nunavut has the highest infant and post-neonatal mortality rates in Canada, which have remained consistently high since 1999 (14.3 and 7.9/1,000 live births, respectively; 1999-2007); values twice that of NWT, which borders Nunavut [1]. The leading causes of infant and post-neonatal mortality in Inuit inhabited areas of Canada are Sudden Infant Death Syndrome/Sudden Unexpected Death in Infancy
(SIDS/SUDI) and deaths due to infectious disease [2]. SIDS and SUDI deaths comprise 19% of child deaths < 5 years of age in NWT (1997-2006) [29].

Table 1.2  Infant mortality rates, birth rates, and Aboriginal population of the Canadian Territories.

<table>
<thead>
<tr>
<th>Region</th>
<th>IMR¹</th>
<th>Births/year²</th>
<th>Aboriginal Population³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nunavut</td>
<td>14</td>
<td>746</td>
<td>86% (Inuit 85%)</td>
</tr>
<tr>
<td>NWT</td>
<td>7</td>
<td>698</td>
<td>50% (Dene 31%, Inuvialuit 10%, Métis 9%)</td>
</tr>
<tr>
<td>Yukon</td>
<td>6</td>
<td>355</td>
<td>25% (First Nations 21%, Métis 3%, Inuit 1%)</td>
</tr>
<tr>
<td>Canada</td>
<td>5</td>
<td>348,898</td>
<td>4%</td>
</tr>
</tbody>
</table>

¹Averaged infant mortality rates per 1000 live births (1999-2007) [1]
²Total number of births averaged over 5 years (2003-2008) [30]
³Statistics Canada 2006 Census [31]

1.3.2 SUDDEN INFANT DEATH SYNDROME AND SUDDEN UNEXPECTED DEATH IN INFANCY

The risk of Sudden Infant Death Syndrome (SIDS) and Sudden Unexpected Death in Infancy (SUDI) is 3 to 4 times greater for Aboriginal Canadians than for non-Aboriginal Canadians [32] and accounts for a larger proportion of the Inuit infant mortality rates than in other regions of Canada [2,3]. SIDS is defined as the sudden death of an infant less than one year of age that cannot be explained after a thorough investigation is conducted, including a complete autopsy, examination of the death scene, and review of the clinical history [33]. SUDI, sometimes abbreviated as SUID (Sudden, Unexpected Infant Death), is a broader category defined as the sudden and unexpected death of an infant, which may be accompanied by an illness not normally expected to cause death, or may have risk factors
present for overlay or asphyxia [34]. Due to diagnostic overlap, SIDS and SUDI are combined in this study to allow for comparison of rates across jurisdictions and periods [34]. SIDS and SUDI together comprise approximately 9% of infant deaths in Canada [35].

1.3.2.1 SIDS/SUDI Risk Factors

SIDS and SUDI are complex, multi-factorial events and likely due to a combination of environmental, medical, developmental, and genetic factors [36-40]. The ‘triple risk hypothesis’, which has undergone refinement since its first use in 1972, states that SIDS is due to the combination of three risk factors, a vulnerable infant with predisposing factors, a critical development period, and an exogenous stressor [41]. Reducing or eliminating the risk from any one of these factors may decrease the risk of SIDS/SUDI [42], indicating a need to explore a variety of risk factors for SIDS/SUDI, including environmental and genetic factors, which may be contributing to the higher rates of SIDS and SUDI observed in NWT and Nunavut [2,3,29,43].

Medical and environmental risk factors for SIDS and SUDI include sleeping in any position other than supine (i.e. sleeping on stomach or side), prematurity, young maternal age, age of infant less than 6 months, maternal (pre and post-natal) smoking, exposure to environmental smoke, male sex, not being breast-fed, bed-sharing, overheating, the presence of loose bedding, and soft sleep surface [34,41,44-51]. Traditionally, there were seasonal trends in SIDS incidence, with a peak during the winter months. This increased risk may have been due to exposure to viral infections or overheating due to bundling [52,53]. However, studies conducted since the introduction of the ‘Back to Sleep’ campaign, which
advocated placing infants to sleep on the backs (supine position), have demonstrated a
decrease in this seasonality [52,54]. It is possible that the combined risk factors of
respiratory illness, cold climate, cramped housing, environmental smoking, and prone
sleeping account for the majority of the excess post-neonatal mortality and SIDS and SUDI
rates in northern communities [34,55-57].

Genetic and/or biological factors, including cardiac conduction abnormalities (i.e. Long QT
syndrome; LQTS) and fatty acid oxidation disorders (FAOD), may also increase risk for SIDS
and SUDI [37,44,58,59]. Undiagnosed metabolic disorders are considered to account for 3-
6% of SIDS and SUDI cases in all populations [60,61].

1.3.2.2 SUDI and Fatty Acid Oxidation Disorders

Retrospective screening of sudden unexplained infant deaths has found that FAODs
contribute to 3-6% of these deaths [60,61]. Infants with inherited fatty acid oxidation
disorders (FAOD) are normally asymptomatic at birth and may present with symptoms on
the second day of life onwards or when exposed to secondary exogenous stressors, like
intercurrent illness and fasting [37,62]. The initial clinical features of non-ketotic
hypoglycemia may progress to neurologic deterioration and liver damage if the infant is not
treated. Metabolic disorders may also increase risk for SUDI due to high metabolic demands
during early development [60]. FAODs reported to contribute to SUDI include medium-
chain acyl-CoA dehydrogenase, very long-chain acyl-CoA dehydrogenase (VLCAD), long-
chain 3-hydroxy-acyl-CoA dehydrogenase, infantile-type carnitine palmitoyltransferase 2, and carnitine palmitoyltransferase 1A deficiencies [60,61,63].

Although carnitine palmitoyltransferase 1A (CPT1A) deficiency is a very rare FAOD, gene variants in CPT1A that are associated with the disorder are common in certain populations, including Hutterite, Inuit, Alaska Native, and BC First Nations populations [6-9,64-69]. CPT1A deficiency commonly presents as hypoketotic hypoglycemia after prolonged fasting or during intercurrent illness and may rapidly progress to seizures, liver damage, and sudden death, if not treated [63].

1.3.3 CARNITINE PALMITOYLTRANSFERASE 1A DEFICIENCY

1.3.3.1 Carnitine Palmitoyltransferase 1 (CPT1) and the CPT Pathway

The carnitine palmitoyltransferase (CPT) pathway is a critical pathway for flux of long chain fatty acids into the mitochondrion for use in fatty acid oxidation (FAO) and is critical for providing ketone bodies for use as energy during periods of fasting and prolonged exercise [70]. CPT1 is the first protein in the CPT pathway and a key regulatory point for flux through to FAO.

The oxidation of fatty acids represents the major source of energy in heart and muscle; however, oxidation of fatty acids in the liver is usually active only during prolonged fasting, illness, or prolonged muscle activity [71]. The primary substrates for FAO are long chain fatty acids (LCFAs), which need to be transported into the mitochondrion for oxidation by the CPT pathway (Figure 1.2) [70]. LCFAs are released from triglycerol in adipose tissue by
lipases and enter the cell passively or by FA transporters like CD36 [72]. Once inside the cell, LCFAs are activated by acyl-CoA synthease (ACS). The CPT1 enzyme, in the outer mitochondrial membrane, catalyzes the first step of LCFA transport into the mitochondria by transferring the fatty acyl group from acyl-CoA to carnitine. Carnitine translocase transports the resulting acylcarnitine across the mitochondrial matrix to CPT2, which replaces the carnitine on the fatty acyl with CoA (reversing the CPT1 reaction). This transfer allows the fatty acyl group to be transported into the mitochondrion for subsequent FAO. CPT1 activity, and the CPT pathway, is regulated by malonyl-CoA [63,70,73,74].
Figure 1.2 Carnitine palmitoyltransferase pathway. During the fed state, ACC is active and converts the glucose product acetyl-CoA into malonyl-CoA. Accumulation of malonyl-CoA inhibits CPT1 activity. During fasting, glucagon signals activation of AMPKK, which triggers the deactivation of ACC via phosphorylation. Malonyl-CoA levels drop and CPT1 is released from inhibition. CPT1 exchanges the CoA molecule for carnitine on long chain fatty acyl-CoA, which is then shuttled across the mitochondrial intermembrane space to the inner membrane by CACT. CPT2 reverses the CPT1 reaction. Free carnitine returns to the cellular cytoplasm and fatty acyl-CoA is transported into the mitochondria for fatty acid oxidation (FAO) [63,70,73,74]. ACC, acetyl-CoA carboxylase: ACS, acyl-CoA synthetase: AMPK, AMP-activated protein kinase: AMPKK, AMP-activated protein kinase kinase: CPT1, carnitine palmitoyltransferase 1: CPT2, carnitine palmitoyltransferase 2: CACT, carnitine translocase: MCD, malonyl-CoA decarboxylase.
There are three tissue-specific isoforms of CPT1, A (liver, kidneys, brain), B (muscle and heart), and C (brain, testis), encoded by separate genes (11q13.1, 22q13.31, and 19q13.3 respectively) [75,76]. CPT1A and B are localised in the mitochondrial outer membrane with active sites exposed to the cytosolic side of the mitochondrion [77]. CPT1A is the major hepatic isoform, but is also found in the spleen, lung, kidney, adipose tissue, hypothalamus, and heart [73,78]. Although CPT1B is the major form expressed in the adult heart, CPT1A is also present in fetal and neonatal heart tissue. Cook et al. [79] found that CPT1A is expressed in fetal and neonatal rat heart and that this expression switches to CPT1B during development [79]. The CPT1A is also expressed in the hypothalamus, where it plays an important role in appetite control and glucose production [78].

The catalytic function of CPT1C is controversial and has not been well defined. Although studies have found that CPT1C may not be active in the mitochondria, it is known to bind malonyl-CoA and may have a role in the endoplasmic reticulum [75,80,81]. Researchers hypothesize that its function may be in satiety and body weight regulation due to its high expression in the hypothalamus [75]. CPT1C knock out mice had decreased food intake and weight, but become obese when fed a high fat diet [82]. All three CPT1 isoforms bind malonyl-CoA; however, CPT1B is very sensitive to inhibition from malonyl-CoA and has an IC$_{50}$ ~100-fold lower then CPT1A [73]. In molecular and genetic characterisation of CPT1, mutations that cause the CPT1 deficiency have only been found in the CPT1A, the liver isoform [83].
1.3.3.2 Malonyl-CoA

Malonyl-CoA is both a precursor of fatty acid biosynthesis and a critical signalling molecule for maintaining the energetic flux between fatty acid biosynthesis and FAO. When a dietary source of energy is available (i.e. glucose, ‘fed state’), malonyl-CoA levels accumulate and CPT1 activity is inhibited [70]. In the absence of dietary glucose (‘fasted state’), and once glycogen stores have been depleted, the body becomes dependent on FAO for energy. During fasting, hepatic FAO produces ketones for tissues to use in lieu of glucose. Hormones like glucagon activate FAO by signalling adipose tissue to release fatty acyls into the bloodstream [72]. Glucagon also stimulates FAO in the liver by inhibiting malonyl-CoA synthesis through the phosphorylation of acetyl-CoA carboxylase (ACC) [72]. As malonyl-CoA levels drop, CPT1 is released from inhibition and fatty-acyls are transported into the mitochondrion for FAO for use as energy or to be used in ketogenesis in the liver [70].

1.3.3.3 Hormones and CPT1A Expression

Both insulin and the thyroid hormone play a role in regulating CPT1A activity and/or expression. Insulin signalling via the insulin growth factor receptor decreases CPT1A activity by increasing CPT1A sensitivity to malonyl-CoA and decreasing CPT1A mRNA levels [84,85]. This relationship may be disrupted in diabetic patients. Park et al. [84] found that CPT1A expressed in diabetic rats had reduced malonyl-CoA sensitivity, causing increased hepatic CPT1A activity and FAO. Thyroid hormone decreases hepatic CPT1A sensitivity to malonyl-CoA and increases expression of CPT1A mRNA five fold in rats [79].
1.3.3.4 Classical CPT1A Deficiency

First reported in 1981, classical CPT1A deficiency is a rare autosomal recessive disorder, with only ~40 cases reported in the literature worldwide. CPT1A deficient individuals present clinically in early life with metabolic decompensation, including hypoketotic hypoglycaemia that may also be accompanied by hepatic encephalopathy, heart dysfunction (cardiomegaly, fatty infiltration of the heart, bradycardia), liver enlargement and fatty infiltration, increased carnitine and liver enzymes, and decreased long-chain acylcarnitines [63,64,70,73,74,86]. CPT1A deficiency is normally detected during newborn screening using tandem mass spectrometry to measure the ratio of free carnitine to long chain acylcarnitine (C0/(C16+C18) >130) [9,87]. Mutations that cause classical CPT1A deficiency affect enzyme activity directly through functional mutations or indirectly by structural changes [88].

Infants have limited glycogen stores and are highly dependent on long chain FAO for energy [89]. Subsequently, CPT1A deficient individuals are more susceptible during their first two years of life [64]. Symptoms of CPT1A deficiency are precipitated by high-energy demands (intercurrent illness), prolonged fasting, and are exacerbated by fever, infection, and dehydration. Untreated acute metabolic decompensation can progress to seizures, hepatic encephalopathy, seizures secondary to recurrent hypoglycaemia, coma, and, in rare cases, sudden unexpected death [74]. Parents of infants diagnosed with CPT1A deficiency are advised to prevent the onset of metabolic decompensation by avoidance of fasting, oral
administering glucose (i.e. juice), medium-chain triglyceride (MCT) oil supplementation, and seeking medical aid if the child becomes ill with an accompanying fever [62].

1.3.3.5 The p.P479L CPT1A Variant and CPT1A Deficiency

In 2000, Innes et al. [90] described an Inuit family in which two infants were determined to have CPT1A deficiency. The mother of the infants initially presented in pregnancy with features of acute fatty liver of pregnancy (AFLP) and with hyperemesis gravidarum during the subsequent pregnancy, complications which are not normally associated with CPT1A deficiency. Although both infants were born healthy, the second infant presented with bronchopneumonia and low normal blood glucose levels (3.3mmol/L) at 6 weeks of age. Biochemical investigation of mother and children found that both children had markedly decreased CPT1A enzyme activity (2% of normal) and the mother had moderately reduced CPT1A activity (36% of normal). Both children were diagnosed with CPT1A deficiency [6,90].

In a separate study in 2001, Brown et al. [65] reported a BC First Nations adult male patient presenting with features more commonly associated with CPT2 deficiency (adult on-set, muscle cramping, and pain). The patient was investigated for mutations in both CPT1A and CPT2 and was found to have a c.1436C→T transition mutation in CPT1A, causing a proline to leucine substitution (P479L) in the CPT1A enzyme [65]. The proline found at 479 is highly conserved and lies within the binding site for malonyl-CoA, the regulator of CPT1A activity [75,91]. Fibroblasts studies of the P479L variant found the protein had diminished enzymatic activity (2-54% of normal) and reduced response to inhibition by malonyl-CoA (68% of normal), indicating that the protein may be constitutively active, even in the fed
state [6,65]. Fibroblasts studies also determined that the reduced activity of the P479L variant did not greatly diminish FAO at normal body temperatures (37°C); however, there was a marked reduction in FAO at high temperatures (41°C), suggesting instability of the variant [6,65]. The Inuit family described by Innes et al. [90] were subsequently genotyped for the P479L variant, which determined that the mother was heterozygous and her children were homozygous for the P479L variant of CPT1A [6].

Since its discovery, the P479L variant has been found to be very common in Inuit populations of Kivalliq (Nunavut) and Greenland (70% and 54% homozygous, respectively) and the coastal regions of Alaska (51% homozygous) [6,8-10]. As well, 9.8% of BC First Nations are homozygous for the variant (Sinclair and Vallance, personal communication).

1.3.4 P479L IMPACT ON HEALTH

1.3.4.1 P479L Advantage?

Fats from marine mammals are rich in omega 3 fatty acids and high intake of these fats has an inverse relation to circulating plasma triacylglycerol concentrations and decreased risk factors for ischemic heart disease and diabetes in obese individuals [92]. Therefore, the traditional Inuit diet may have had protective effects on Inuit health, which may include P479L homozygous individuals [6,8]. Research into the impact of the P479L variant on modern Aboriginal adult health and risk for diabetes and obesity is another important avenue of research. Studies of CPT1A expression in rats found that neonates who breastfed from dams eating a high fat diet through gestation and lactation had higher level of hepatic
CPT1A expression than neonates whose dams who ate a carbohydrate rich diet [93]. P479L homozygous infants breastfed by mothers eating a traditional high fat diet may have been protected from any adverse effects of the variant by an increased expression of the CPT1A protein; however, research is needed to support this possibility.

1.3.4.2 P479L and Infant Morbidity and Mortality

Does P479L confer risk for sudden unexpected infant death? Many infants homozygous for the variant have presented with symptoms of CPT1A deficiency, including sudden unexpected death [6,7,90,10]. To date, all reported affected individuals have been of Inuit, Alaska Native, or First Nations ancestry. This variant is of particular concern as Inuit and First Nations populations of Canada experience infant mortality rates that far exceed their non-aboriginal counterparts [5], raising questions as to whether the P479L CPT1A variant could be playing a role in the excess infant mortality cases in these populations.

The P479L variant CPT1A protein may cause CPT1A deficiency and confer risk for metabolic decompensation during periods of fever and other illness. The P479L variant is thermolabile (42°C; in vitro) [65,6], so may have a reduced ability to participate in FAO during intercurrent illness and fever. For P479L homozygous infants and children, the protein’s proposed suboptimal function under circumstances of fever and infection might result in symptoms consistent with CPT1A deficiency, namely, non-ketotic hypoglycemia, seizures, and even sudden death. Seven of 10 Inuit infants that died in Kivalliq from 2004-2006 with causes of febrile illness or no known cause were homozygous for P479L variant [6]. However, as the Kivalliq population homozygosity for the study period was 70%, this is not
evidence of a statistically significant risk. Similar sudden unexpected deaths have been reported in P479L homozygous infants in Alaska and BC [7,10,94]. In Gessner et al.’s [94] prospective review of infant mortality in Alaska Native infants, they report higher infant mortality rates for infants homozygous for variant (5/152) over heterozygous (2/219) and wild type (0/245) infants; however their study was very small (cases=7). The P479L variant of CPT1A has been identified as a concern by the BC Coroner’s Office in its five years review of infant death in the province [95]. Retrospective genotyping of First Nations infants who died suddenly in BC since 1999 has found that of the 48 cases, 19 (~40%) were homozygous for the P479L variant. The P479L genotype frequency in the healthy FN population was compared to the P479L frequency in sudden death cases. For mid-Vancouver Island the odds ratio is 3.87 (95% CI: 1.4-10.9) p< 0.006 (Sinclair and Vallance, personal communication). When we combine this information with the high infant mortality rates in Nunavut, NWT, and Yukon, we hypothesize that P479L homozygous infants may be at increased risk of metabolic decompensation and sudden unexpected death during periods of high fever and fasting.

This study will determine the prevalence of P479L homozygosity in all infants born in these regions in 2006 and in infants that died unexpectedly from 1999 to 2008 in all three territories. Comparison of the two groups will allow assessment of whether there is an increased risk of sudden unexpected death for infants homozygous for the P479L variant. This is a retrospective anonymous review requiring ethics approval from the university, each territory, and consultation with Aboriginal organisations within the three territories.
1.3.5 NEWBORN SCREENING

The goal of newborn screening (NBS) is to diagnose diseases prior to the onset of symptoms. Disorders that are amenable to newborn screening should have an effective treatment that increases health outcomes when initiated in a pre-symptomatic stage. In 1968, Wilson and Jungner outlined a series of criteria for consideration before a condition could be included into newborn screening programs (Figure 1.3) [96].

**Figure 1.3 Wilson and Jungner classic screening criteria [96] (adapted from [97])**

<table>
<thead>
<tr>
<th></th>
<th>1. The condition sought should be an important health problem.</th>
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<tr>
<td></td>
<td>2. There should be an accepted treatment for patients with recognized disease.</td>
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<tr>
<td></td>
<td>3. Facilities for diagnosis and treatment should be available.</td>
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<tr>
<td></td>
<td>4. There should be a recognizable latent or early symptomatic stage.</td>
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<tr>
<td></td>
<td>5. There should be a suitable test or examination.</td>
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<td></td>
<td>6. The test should be acceptable to the population.</td>
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<td></td>
<td>7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.</td>
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<td></td>
<td>8. There should be an agreed policy on whom to treat as patients.</td>
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<tr>
<td></td>
<td>9. The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.</td>
</tr>
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<td></td>
<td>10. Case-finding should be a continuing process and not a “once and for all” project.</td>
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</table>

Although these criteria have since been amended or clarified, they still reflect the importance of reviewing the impact of newborn screening on infants and their families (Figure 1.4) [97].
**Figure 1.4  World Health Organization amendments to Wilson and Jungner criteria for screening [97]**

- The screening programme should respond to a recognized need.
- The objectives of screening should be defined at the outset.
- There should be a defined target population.
- There should be scientific evidence of screening programme effectiveness.
- The programme should integrate education, testing, clinical services and programme management.
- There should be quality assurance, with mechanisms to minimize potential risks of screening.
- The programme should ensure informed choice, confidentiality and respect for autonomy.
- The programme should promote equity and access to screening for the entire target population.
- Programme evaluation should be planned from the outset.
- The overall benefits of screening should outweigh the harm.

In Canada, each province determines which tests to include in their provincial newborn screening, subsequently the disorders Canadian infants are tested for varies from province to province (and territory). NBS for infants born in the three Canadian territories is conducted by adjoining provinces. Yukon samples are tested in Vancouver, Kivalliq (Nunavut) samples are tested in Winnipeg, and NWT, Kitikmeot (Nunavut), and Qikiqtani/Baffin Island (Nunavut) samples are tested in Edmonton. In 2006, the American College of Medical Genetics released a review of tests available for newborn screening and recommended 29 conditions which should be core or primary tests in all NBS programs, as well as outlining secondary target tests that would be picked up by tandem mass spectrometry, which included CPT1A deficiency [98].
Newborn screening programs are responsible for primary screening as well as patient follow-up, secondary testing, diagnosing of disorders, and evaluating patients undergoing treatment [98]. Expansion of newborn screening has become contentious as many disorders now included, or in consideration to be included, do not adhere to NBS criteria (Figures 1.3 and 1.4) [97]. Any new test to be added the newborn screening should be able to demonstrate that it adheres to the criteria for newborn screening, especially disorders with variable penetrance or when there is uncertainty as to whether individuals will present clinically [99]. In these situations, it is critical that medical professionals, parents, and communities understand the implications of a positive screen test. A positive screen test for such disorders can impact families in a variety of ways, including invasive follow-up testing, hospitalisation of otherwise healthy infants, expensive dietary requirements, complicated care requirements, and psychosocial impacts like medicalisation of healthy infants and disruption of the parent-infant bond [100].

There is currently no P479L screening available to parents of infants born in any of Canada’s three territories. Before health programs targeting the variant, which may include newborn screening, can be initiated, an evidenced based process is needed to determine if there is an increase risk for morbidity and mortality associated with the variant. If there is an associated risk with homozygosity for the variant, then it may be appropriate to offer newborn screening for the P479L variant in Inuit and BC First Nations populations. Screening for P479L in early infancy during the disease-free interval between birth and
onset of symptoms would allow an early diagnosis and appropriate clinical management of CPT1A deficient infants, avoiding CPT1A deficiency clinical manifestations [101].
2.1 INTRODUCTION

First reported in 1981, classic carnitine palmitoyltransferase 1A (CPT1A) deficiency is a rare autosomal recessive disorder that confers risk for non-ketotic hypoglycaemia, hepatic encephalopathy, seizures, and Sudden Unexpected Death in Infancy (SUDI) [63,64,70,74]. The CPT1 enzyme is located on the outer mitochondrial membrane and is required for the import of long chain fats into the mitochondria for use in fatty acid oxidation (FAO; Figure 1.2) [63,70,73,74]. CPT1A encodes the liver isoform of CPT1; the other two isoforms are CPT1B (heart and muscle) and CPT1C (brain) [73,75]. Those homozygous for a thermolabile variant of CPT1A, p.P479L (c.1436C>T), have decreased CPT1A activity (2%-54% of normal with a mean of 22%); and may be at risk of decompensation during times of fever and intercurrent illness [6,65,102]. Several Nunavut Inuit and British Columbia (BC) First Nations infants and children have presented symptomatically with features consistent with CPT1A deficiency or with sudden unexpected death and were subsequently found to be homozygous for the P479L variant [6,7,10,66]. However, population studies of the P479L

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variant have not yet confirmed whether the variant is contributing to the adverse outcomes observed, or if it is simply observed due to the high P479L frequency in these populations. All those presenting with apparent clinical features to date have been reported in First Nations and Inuit children [6,7,10,66].

Classic CPT1A deficiency is normally detectable through newborn screening by measuring the ratio of free carnitine to 2 long chain acylcarnitines (C16 + C18) using tandem mass spectrometry [87]. Although this standard method has been used to identify a number of Alaskan infants with abnormal acylcarnitine profiles, not all infants homozygous for the P479L variant are identified using the standard cut off values. Furthermore, many infants homozygous for the variant are asymptomatic [6,66,90]. Targeted genotyping of CPT1A has been a routine component of expanded newborn screening in Manitoba targeted only to Hutterite newborns with classical CPT1A deficiency, where the disease causing mutation (c.2129G→A; p.G710E) is prevalent (homozygosity rate of ~1/400) and is associated with severe disease [64]. Whether a similar DNA-based expanded newborn screening should be instituted for those newborns at risk for adverse outcomes due to the CPT1A P479L variant remains to be determined. Previous reports have suggested that the P479L variant is frequent in the Inuit of the Kivalliq region of Nunavut and the Inuit of Greenland (81% and 73%, respectively) [6,8]. To date, screening for the variant in the three territories has not yet been implemented since there is controversy as to whether P479L homozygosity confers risk for infant morbidity and mortality. For that reason, an evidence-based process was initiated to determine the population implications of the variant in the three northern
territories where 50% of the inhabitants are Aboriginal (25% in Yukon, 50% in Northwest Territories (NWT), and 86% in Nunavut) [12].

We present the results of our background study to determine the allele frequency across Canada’s North. These results of this study will be combined with results from study of P479L frequency in infant mortality cases in the three territories to provide an assessment as to whether newborn screening or other public health measures should be considered.

2.2 METHODS

2.2.1 ETHICS

Ethics and regulatory approval was obtained from UBC Research Ethics Board, Aurora Research Institute (NWT), Stanton Territorial Health Authority (NWT), Nunavut Research Institute, and the University of Manitoba. Territorial Aboriginal organisations consultation included: Nunavut Tunngavik Inc. (NTI), the Inuvialuit Regional Corporation (NWT), the Dene Nation (NWT), and the Yukon First Nations Health Commission.

2.2.2 SAMPLE COLLECTION

In collaboration with the Newborn Screening program at the BC Children’s Hospital, the Alberta Newborn Screening Program, and the Newborn Screening program at the Cadham Provincial Laboratory in Manitoba, newborn dried blood spots (DBS) were collected for infants born in 2006 in the Yukon, NWT, and Nunavut and were genotyped for the p.P479L variant of CPT1A. Due to samples not being available prior to April 2006 from the Qikiqtani
region, spots from a full calendar year from April 6, 2006 to March 30, 2007 were tested. The DBS cards were identified and accessed from storage based on patient health identification number, location of birth, or mother’s place of residence. All samples were anonymized and provided with a unique identifier. Individual Aboriginal identity according to genotype could be determined for samples from NWT, where the maternal health number is informative for First Nations, Inuvialuit, Métis, and non-Aboriginal ancestry. This method should identify all Aboriginal residents receiving benefits allocated to Aboriginal groups in NWT; however, there may be some Aboriginal individuals not identified as such by their health care number and, in some rare cases, individuals identified as Aboriginal by their health care numbers who are not ethnically Aboriginal.

2.2.3 GENOTYPE ANALYSIS

DNA was extracted from 3mm bloodspot punches using the Gentra Generation Capture Kit following the manufacture’s protocol (Qiagen, Mississauga, Ont.). Genotyping of the Kivalliq region samples was conducted using the PCR-RFLP technique, as previously described [6]. All other samples were genotyped using TaqMan allelic discrimination RT-PCR assay. DNA was amplified by PCR using a 25 µl reaction mixture containing: 2.5µl of purified DNA, 12.5µl TaqMan Universal PCR Master Mix (Applied Biosystems, Mississauga, ON), 9.375µl of dH2O, and 0.625µl CPT1A Probe and Primer Mix “CPT1a-CPT1, SNP AbD” (containing primers: GGCCTCAACGCTGAACACT (5’); GTGAAAACTCACCCTACCCAAAGGT (3’); normal reporter: CPT1A-CPT1V2, CACGATCGCGGCATC, VIC; mutant reporter: CPT1A-CPT1M2, CACGATCGCGCATC). PCR amplification was conducted using a PRISM 7000 sequence
detection system (Applied Biosystems). Reaction conditions were 2 min at 50°C, 10 min at 95°C, followed by 40 thermal cycles of 15s at 95°C and 1 min at 60°C. Sample genotype was determined using the ABI Prism 7000 SDS software by analysing the allelic specific fluorescence data.

2.2.4 STATISTICAL ANALYSIS

Genotype frequencies were calculated and statistically analyzed using the $X^2$ test to analyse deviation from predicted frequencies from the Hardy-Weinberg equation with $p<0.05$ significance level using STATA 10 (StataCorp. 2007. *Stata Statistical Software: Release 10.* College Station, TX: StataCorp LP). Hardy-Weinberg equilibrium (HWE) analysis was carried out in Aboriginal specific populations when possible.

2.3 RESULTS

The P479L variant genotype frequencies, shown in Table 2.1, varied throughout Canada’s North. The highest prevalence was in Nunavut. The allele frequency varied in the three Nunavut regions, with the Kitikmeot region having the highest (0.85; 95%CI: 0.81-0.89), followed by Kivalliq (0.83; 95%CI: 0.80-0.86), and Qikiqtani (0.68; 95%CI: 0.64-0.72). The P479L variant deviated from HWE ($p<0.001$) in the Qikiqtani region. Aboriginal status was not available for the Nunavut DBS samples; however, ~90% of births are to Inuit women, or approximately 272 of the 302 infants born in 2006 in Qikiqtani [2]. If all heterozygotes (n=89) and P479L homozygotes (n=162) in the Qikiqtani region were of Inuit ancestry, the allele frequency would be 0.76 in this reduced sample and would be within HWE ($p=0.083$).
Table 2.1  Distribution of CPT1A P479L genotypes with estimated allele frequencies in infants born in 2006 in the Northern territories of Canada.

<table>
<thead>
<tr>
<th></th>
<th>wt/wt&lt;sup&gt;a&lt;/sup&gt;</th>
<th>wt/P479L</th>
<th>P479L/P479L</th>
<th>P479L allele</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>f</td>
<td>n</td>
<td>f</td>
<td>n</td>
</tr>
<tr>
<td>Nunavut</td>
<td>695</td>
<td>67 0.10</td>
<td>186</td>
<td>0.27</td>
<td>442</td>
</tr>
<tr>
<td>Qikiqtani</td>
<td>302</td>
<td>51 0.17</td>
<td>89</td>
<td>0.30</td>
<td>162</td>
</tr>
<tr>
<td>Kivalliq</td>
<td>243</td>
<td>11 0.05</td>
<td>62</td>
<td>0.26</td>
<td>170</td>
</tr>
<tr>
<td>Kitikmeot</td>
<td>150</td>
<td>5 0.03</td>
<td>35</td>
<td>0.23</td>
<td>110</td>
</tr>
<tr>
<td>NWT</td>
<td>564</td>
<td>494 0.88</td>
<td>52</td>
<td>0.09</td>
<td>18</td>
</tr>
<tr>
<td>Inuit/Inuvialuit</td>
<td>70</td>
<td>23 0.33</td>
<td>32</td>
<td>0.46</td>
<td>15</td>
</tr>
<tr>
<td>First Nations</td>
<td>233</td>
<td>216 0.93</td>
<td>14</td>
<td>0.06</td>
<td>3</td>
</tr>
<tr>
<td>Métis</td>
<td>31</td>
<td>31 1.00</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>Non-Aboriginal</td>
<td>227</td>
<td>221 0.97</td>
<td>6</td>
<td>0.03</td>
<td>0</td>
</tr>
<tr>
<td>undefined</td>
<td>3</td>
<td>3 1.00</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>Yukon</td>
<td>325</td>
<td>312 0.96</td>
<td>13</td>
<td>0.04</td>
<td>0</td>
</tr>
</tbody>
</table>

CI: Confidence Interval
<sup>a</sup>Wild type
<sup>b</sup>Genotype frequencies deviated from HWE (p<0.05)

In NWT, the territorial allele frequency was substantially lower than in Nunavut, at 0.08 (95%CI: 0.06-0.10). The Inuvialuit had the highest frequency (0.44; 95%CI: 0.36-0.52), followed by First Nations (0.04; 95%CI: 0.02-0.06). There were 6 heterozygotes in the non-Aboriginal group. The P479L variant was not detected in those with maternal self-identification as Métis (n=31). The P479L allele distribution in the Inuvialuit was within HWE
but not in the NWT First Nations, where no P479L homozygotes would be expected at an allele frequency of 0.04.

In the Yukon, there were no P479L allele homozygotes but 13 heterozygotes. Since Aboriginal status was not available for Yukon DBS samples, it was not possible to determine if all heterozygous infants were of Aboriginal ancestry. In 2006, 21.7% (n=71) of Yukon births were to Status Indian mothers. If all 13 heterozygotes were Aboriginal, this would result in an allele frequency of 0.09 in the Aboriginal population (carrier rate of 1/6).

2.4 DISCUSSION

Hepatic CPT1A imports long-chain fatty acids into mitochondria for use in FAO (Figure 1.2) [63,70,73,74]. CPT1A is active during fasting to maintain energy and blood glucose levels. In the fed state, CPT1A is inhibited by malonyl-CoA, a product of glycolysis and substrate of fatty acid synthesis. Classic CPT1A deficiency is a rare autosomal recessive disorder and presents in infancy with hypoketotic hypoglycemia, which can lead to seizures, hepatoencephalopathy, and, in rare cases, sudden death [63,70,74].

A CPT1A variant, p.P479L (c.1436 C>T), is present in Canadian and Greenland Inuit, BC First Nations, and Alaska Natives [6,8,66]. In vitro, the P479L variant protein is constitutively active due to reduced inhibition by malonyl-CoA and it has decreased thermostability and functional activity (<50%) [6,65]. Although the pathogenic link between the variant and infant mortality and morbidity has not yet been established, it may confer risk when combined with secondary exogenous stressors, i.e. fever and illness. A number of Inuit and
BC First Nations children, all homozygous for the allele, have presented with clinical features such as hypoglycaemia, seizures, and sudden unexpected death; symptoms that are consistent with a condition of impaired FAO [6,66]. Autopsy findings of infants homozygous for the allele have included fatty infiltrates into the liver and, in one case, into the right ventricle (unpublished data). A study of the Kivalliq region of Nunavut found that 70% of infants who died unexpectedly during the study period were homozygous for the P479L variant, but this did not exceed the population homozygosity (69.7%; 294/422) [6].

This study suggests a high frequency of the P479L variant in Nunavut and Inuit/Inuvialuit infants born in 2006, which is consistent with results from previous studies of Inuit populations in the Kivalliq region of Nunavut and in Greenland [6,8]. The allele distribution was not consistent with HWE (p<0.001) in Nunavut as a whole, but this may not be true within the Inuit population of this region. Both Kitikmeot and Kivalliq were within HWE, as are the Greenland Inuit and coastal Alaska Natives [8,9]. However, in the Qikiqtani region, genotype frequencies did deviate from HWE. Approximately 90% of infants born in this region are to Inuit mothers [2]. Although it was not possible to identify ethnicity for the Nunavut samples; if it is assumed that all P479L homozygotes (n=162) and heterozygotes (n=89) in the Qikiqtani region were of Inuit ancestry, the genotype frequencies in the reduce sample size (n=272) do not deviate from those expected under HWE (p>0.05).

The P479L allele frequency in the Inuvialuit of NWT (0.44) was markedly lower than in the Nunavut Inuit and coastal Alaskan Natives (51%) [9]. The allele prevalence in the NWT First Nations and in the general population of Yukon was low (0.04, 0.02 respectively), with only
1% homozygosity in NWT First Nations and no homozygotes present in the Yukon during our study. The low homozygosity in these groups was unexpected as Gessner et al. [9] report P479L homozygosity of 33% (n=378) in central, southern and eastern Alaska Native populations and it is estimated that 9.8% of BC First Nations are homozygous for the allele (Sinclair and Vallance, personal communication). Ethnicity was not available for Yukon samples; however, if all P479L carriers detected in the Yukon were of First Nations ancestry, the allele frequency would 0.09 in this population.

Genotype frequencies in the NWT First Nations deviated from those expected under HWE. P479L genotype frequencies also deviated from HWE in the central, southern and eastern Alaska Native populations [9] and in BC First Nations (Sinclair and Vallance, personal communication). The Hardy Weinberg disequilibrium in First Nations and in Alaskan non-Inuit populations may represent admixture of these populations or it may represent an advantage of P479L and wildtype homozygosity over heterozygosity.

The high prevalence of the variant in the Inuit populations may be due to founder effect, genetic drift, linkage to another polymorphism that is advantageous, or it may represent an historical benefit for those with the P479L variant in these regions. The traditional diet of populations in Canada’s North was a high fat, moderate protein diet with little to no carbohydrate sources available [103]. The constitutively active, malonyl-CoA resistant, P479L CPT1A protein may have been advantageous by maintaining FAO and ketogenesis at all times; this would be especially advantageous during periods of diet change when high fat food sources were limited [6]. Study of plasma HDL-cholesterol and associated apoA-I in
Greenland Inuit found a possible protective effect associated with the variant against cardiovascular disease in adults, although this information alone does not likely explain a selective advantage for the variant [8]. The presence of the variant in the distantly related populations of Inuit and Inuvialuit of Nunavut and NWT, the Inuit of Greenland, and the Yupik Alaskan Natives indicates that this variant may have a place in the migration history of these populations. The relationship of the variant in BC First Nations as a dietary advantage remains unclear, as does the ancestral relationship to Inuit populations.

Although the high prevalence of the P479L variant reduces the likelihood that homozygosity for the variant was deleterious historically, it is possible that current dietary practices, including the consumption of carbohydrate rich foods and decreased length of breast feeding, could play a role in increasing risk for infants who might be affected with accompanying intercurrent illness [20,104]. Further study is currently underway to determine the prevalence of the P479L variant in infant mortality cases in all three territories. Results from the current study will be combined with that study to determine if the P479L variant plays a role in the excess infant mortality cases found in the Canadian Northern territories.
CHAPTER 3. DOES THE CPT1A p.P479L VARIANT PLAY A ROLE IN EXCESS INFANT MORTALITY CASES OF NUNAVUT, NWT, AND YUKON?

3.1 INTRODUCTION

Classical CPT1A deficiency is a rare autosomal recessive disorder, with only ~40 cases reported in the literature worldwide. CPT1A deficiency can cause hypoketotic hypoglycaemia, and metabolic decompensation. If untreated, this can progress to hepatic encephalopathy, seizures, coma, and, in rare cases, Sudden Unexpected Death in Infancy (SUDI) [63, 64, 70, 74].

Discovered by Brown et al. in 2001, the P479L variant of CPT1A has been found to be very common in Inuit/Inuvialuit populations of Nunavut, NWT, Alaska, and Greenland (64%, 21%, 51%, and 54% homozygosity) [6, 8, 9, 105]. The variant also has an estimated homozygosity of approximately 9.8% in BC First Nations (Sinclair and Vallance, personal communication). Although the variant is prevalent in these populations, more than 40 infants homozygous for the variant have presented with features of CPT1A deficiency, including seizures and sudden unexpected death [6, 10, 90]. To date, all reported affected individuals have been of Inuit, Alaska Native, or First Nations ancestry.

Classical CPT1A deficiency is detected during newborn screening by measuring free carnitine to long chain acyl-carnitines (C0/16+C18) using tandem mass spectrometry. Current cut-offs values of 130 for this ratio would need to be substantially reduced to
reliably identify infants homozygous for the P479L variant, which would reduce the specificity of the test [7,94]. Secondary genotyping for the P479L variant of CPT1A for those above a lowered cut-off would be costly but would reduce false positive results.

This study determined the P479L genotype for infant mortality cases in the three territories from 1999 to 2008 for deaths due to infectious disease and SUDI, which includes SIDS, and compared the frequency of the allele in these cases to the prevalence of the allele in newborns born in each territory in 2006. This data will aid in determining whether infants homozygous for the P479L CPT1A variant are at increased risk for infant mortality.

3.2 METHODS

3.2.1 ETHICS

Ethics and regulatory approval was obtained from UBC Research Ethics Board, Aurora Research Institute (NWT), Stanton Territorial Health Authority (NWT), Nunavut Research Institute, and the University of Manitoba. Territorial Aboriginal organisations consultation included: Nunavut Tunngavik Inc. (NTI), the Inuvialuit Regional Corporation (NWT), the Dene Nation (NWT), and the Yukon First Nations Health Commission.

3.2.2 SAMPLE COLLECTION

Newborn dried blood spots (DBS) cards were collected for infants born in the Yukon, NWT, and Nunavut and were genotyped for the p.P479L variant of CPT1A in collaboration with the Newborn Screening program at the BC Children’s Hospital, the Alberta Newborn Screening Program, and the Newborn Screening program at the Cadham Provincial
Laboratory in Manitoba. Infant mortality cases (birth to one year of age) during the period of January 1, 1999 to December 31, 2008 were reviewed by the coroner for each territory. To be included in the study, cases had causes of death listed as: infectious disease, SUDI, SIDS, unexplained death, or cause of death unknown. The dried blood spot cards (DBS) cards were matched with case information using date of birth, name, place of birth, maternal health identification number, and/or mother’s place of residence. All samples were anonymized and provided with a unique identifier. Individual Aboriginal identity according to genotype could be determined for samples from NWT, where the maternal health number is informative for First Nations, Inuvialuit, Métis, and non-Aboriginal ancestry. This method should identify all Aboriginal residents receiving benefits allocated to Aboriginal groups in NWT; however, there may be some Aboriginal individuals not identified as such by their health care number and, in some rare cases, individuals identified as Aboriginal by their health care numbers who are not ethnically Aboriginal.

Between the years of 1999 and 2008, 79 cases were identified in the three territories, 59 in Nunavut, 16 in NWT, and 4 in the Yukon. Of the 59 Nunavut cases, 20 samples were available for testing or had already been tested at time of birth (Kivalliq region) or death; 4/27 from the Qikiqtani Region, 5/15 from Kitikmeot region and 11/17 from the Kivalliq region. Of the 16 cases in NWT, 7 samples were available for testing. Of the 48 missing samples from Nunavut and NWT, 31 samples had been destroyed and 17 were either unavailable or could not be linked to cases. All 4 cases from the Yukon were available.
Newborn blood spots cards for the cases not previously tested were pulled and tested for the P479L variant (n=7). Data was anonymised and aggregated.

### 3.2.3 GENOTYPE ANALYSIS

Genotyping of samples from the Kivalliq region of Nunavut was conducted using the PCR-RFLP technique, as previously described [6]. All other samples were genotyped using TaqMan allelic discrimination RT-PCR assay as previously described in Chapter 2.

### 3.2.4 STATISTICAL ANALYSIS

Genotype frequencies were calculated and the \( \chi^2 \) test was used to analyse deviation from predicted frequencies from the Hardy-Weinberg equation with \( p<0.05 \) significance level. Hardy-Weinberg equilibrium (HWE) analysis was carried out in Aboriginal specific populations when possible. Odds ratios were calculated for case P479L homozygosity compared with known population homozygosity of infants born (see Chapter 2) in 2006 with 95% confidence intervals using STATA 10 (StataCorp. 2007. Stata Statistical Software: Release 10. College Station, TX: StataCorp LP). Due to small sample sizes, Fishers exact test was used to calculate \( p \) values and assess risk.

### 3.3 RESULTS

Homozygosity rates for each territory and for the NWT Inuvialuit are illustrated in Figure 3.1. Of the 59 cases documented in Nunavut that met the criteria, 20 were available for testing. The homozygosity rate within the Nunavut cases was 90%, with 18 cases homozygous for the variant, 2 were heterozygous, and no cases that were homozygous wild
type. The population allele frequency for the P479L variant in Nunavut using all infants born in 2006 as an estimate is 0.77 (95% CI: 0.75-0.79; see chapter 2). The odds ratio for homozygosity in cases was 5.15 (95% CI: 1.22-46.1; Table 3.1) based on 64% homozygosity in that population.

Figure 3.1  Distribution of CPT1A P479L homozygosity in the Canadian territories in infant mortality cases (1999-2008) and in the population. Population homozygosity estimated using data for all infants born in 2006 (see Chapter 1). Error bars indicate 95% confidence intervals. Nunavut and NWT population data were not in Hardy Weinberg equilibrium (HWE).
<table>
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<th></th>
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<th>95% CI</th>
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<tr>
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</tr>
<tr>
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<td></td>
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CI: Confidence Interval
*Genotype frequencies deviated from HWE (p<0.05)
Within the 18 homozygous cases, the leading causes of death were SIDS/SUDI (39%, 7) and respiratory infection (39%, 7). Most of the deaths occurred during the post-neonatal period (94%, 17). There was limited reporting of gestational age and no clear trends were evident (13 of 18).

There were 16 infant mortality cases documented in NWT from 1999-2008 that met the criteria. Samples were available for 7 cases (3 Inuvialuit, 3 First Nations, and 1 non-Aboriginal). Within those 7 cases, the P479L variant was present only in the Inuvialuit samples, 2 (67%) were homozygous for the variant and 1 heterozygous. All other samples were homozygous wild type. Using data from infants born in 2006, the estimated CPT1A P479L allele frequency for NWT Inuvialuit is 0.44 (95% CI: 0.36-0.52). Odds ratio for Inuvialuit cases was not significant.

Between the years of 1999-2007, there were 4 cases of sudden infant death in Yukon. All of the cases were available for testing and all were homozygous wild type at site 479. This is not surprising as the P479L variant is rare in this territory with an estimated allele frequency of 9% in the First Nations population within the territory. There were no cases of homozygosity in the population study.

3.4 DISCUSSION

Homozygosity for P479L variant of CPT1A is associated with symptoms consistent with CPT1A deficiency, including sudden unexpected death. All infants and children who have presented with these symptoms have been of Inuit, Alaska Native, or BC First Nations
ancestry [6,7,10]. However, population studies of the Alaska Native and the Canadian and Greenland Inuit have determined that the allele is very frequent in these populations [6-8,10,105]. Within Canada, the highest P479L allele frequencies are found in Nunavut (0.68-0.86) and NWT Inuvialuit (0.44) [105]. The high frequency of the variant in these populations has raised uncertainty regarding the variant’s clinical significance.

This study demonstrated that homozygosity for the variant is associated with an increased risk for sudden unexpected death in Nunavut infants. However, the numbers for the study were small, only 34% (31/79) of the cases identified in the three territories were available to be genotyped.

In a concurrent study of the variant in British Columbia (BC) (Sinclair and Vallance, presented with permission pre-publication), the P479L variant homozygosity was assessed in symptomatic and sudden unexpected death cases (SIDS, SUDI, and sudden death with infection) in infants and children (<2 years of age) with First Nations ancestry. Symptomatic cases presented with any number of clinical features associated with CPT1A deficiency, including hypoglycaemia, seizures, and liver dysfunction. Population prevalence of the P479L variant was estimated by genotyping newborn blood spots for First Nations infants born in 2004 (n=2332; identified using the maternal designation of “Status Indian” in BC Vital Statistics database).

The overall P479L homozygosity for BC First Nations was 9.8%. P479L variant homozygosity was highest in coastal First Nations communities on southern and mid Vancouver Island
(25.3% and 22.8%, respectively). P479L homozygosity was lowest in the BC South Interior and the lower mainland (4.3% and 4.6%, respectively). Within the high homozygosity regions, there were 32 sudden death and 84 symptomatic cases. Homozygosity for the variant was associated with an increased risk sudden death in the mid Vancouver Island region (OR: 3.36; 95% CI: 1.07-10.51), as well as in symptomatic cases in the high homozygosity regions (OR: 30.46; 95% CI: 10.94-84.68). However, symptoms of the clinical cases referred for testing were variable and non-specific including hypoglycemia, seizures, and liver dysfunction. It is difficult to interpret the results for the symptomatic cases without a more clearly defined phenotype.

The high prevalence of the P479L variant in Inuit, Alaska Native, and coastal BC First Nations populations suggests that this variant may have been historically beneficial for populations living in coastal areas subsisting on high fat, moderate protein diets rich in fish and marine mammals [17,103]. However, current transitions of diets to high carbohydrate sources and the reduction of breast-feeding may confer risk for infants homozygous for the variant, especially if there is accompanying intercurrent illness [20,21,104].

Risks for SIDS/SUDI and infant mortality in Inuit and First Nations populations of Canada are 2.2 and 7 times the national averages [2,4]. It is unlikely that it would be possible to demonstrate a large impact of the variant on such multi-factorial outcomes. However, study results from BC and Nunavut suggest an increase risk for sudden death with the P479L variant. This study did not assess other risk factors, but cannot exclude the interaction of social, medical, nutritional, environmental or other genetic factors. Risk factors for
SUDI/SIDS and death due to infectious disease include prematurity, not-breast feeding, maternal smoking or smoking in environment, crowded housing, and sleep position other than supine [33,34,45,46,56,106-108]. All of these are factors of concern for infants in Nunavut [1,30,109,110]. Nunavut has the highest rate of premature birth in Canada and many of these premature births have associated risk factors [18,30]. In a survey of maternal smoking in Canada, 64% of Nunavut mothers reported smoking during pregnancy, 5 times the national average of 16% [109]. In other studies of Qikiqtani only, the rate is even higher [18,111]. Placing infants to sleep on their backs is the key recommendation to reduce risk for SIDS and SUDI [45,112,113]. However, only 46% of Nunavut women report placing their infant to sleep on their back [109].

Classical CPT1A deficiency is normally detectable through newborn screening by analysing levels of free carnitine over acylcarnitine profiles (C0/(C16 + C18) > 130) using tandem mass spectrometry and indicates the activity of CPT1 indirectly by the accumulation of free carnitine in relation to its incorporation into fatty acylcarnitine [87]. However, the current cut-off value of >130 for this ratio does not identify most P479L homozygous infants. Substantially dropping the cut-off would identify most homozygotes, but would also lower the sensitivity of the test [9]. Although Alaska has been using the standard value of 130 to identify Alaska Native infants with abnormal acylcarnitine profiles, not all infants homozygous for the P479L variant are identified using the standard cut off values and many infants homozygous for the variant are asymptomatic [6,7,9]. An alternative method for detecting P479L homozygous infants is DNA testing, which could be conducted as a 2nd tier
after initial screening for C0/(C16+C18). However, this variant is highly prevalent in these populations. Further assessment of the clinical impact of the variant and effectiveness of treatment are needed. There is currently no P479L screening available to parents of infants born in any of Canada’s three territories. Screening for P479L in early infancy during the disease-free interval between birth and onset of symptoms would allow an early diagnosis and appropriate dietary management of infants, avoiding CPT1A deficiency clinical manifestations [101].

Although sample sizes for this study are small, homozygosity for the variant was associated with increased risk for sudden death in Nunavut infants. The information from this study will aid in determining appropriate management strategies for the variant. Any programs must include dialogue with health authorities, local medical professionals, and communities. Expanding newborn screening to include the P479L variant may not be appropriate without better understanding of the natural history of the deficiency and benefits of treatment. Currently, there is no evidence that preventative treatment will alter health outcomes for those homozygous for the variant. The high population frequency for the allele and low reported symptomatic cases in Nunavut indicates that the allele may have low penetrance, suggesting that larger population studies are needed to determine the penetrance of the allele [114]. Expanding newborn screening would have a significant impact on resources, primary care physicians, families, and communities. Clear and meaningful communication, appropriate medical follow-up, and strong social support are critical to the health and welfare of both the infant and the family when communicating a
screen-positive result [115,116]. Primary care physicians and public health nurses would be responsible for communicating screen-positive results in manner that avoids creating anxiety in parents and families, as well as for medical follow-up. In order to understand and communicate the implications of a positive screen result, local medical professionals would require detailed and concise information regarding the disorder and the P479L variant [117,118]. Many Inuit and First Nations individuals seek guidance on medical issues from informed community members and family, so information regarding CPT1A P479L should also come from within communities and incorporate culturally appropriate icons and local languages [115].

As with all studies where small numbers are used, the results from this study must be taken with caution. The new Nunavut Qiturngatta Surveillance System follows infants from the prenatal period to 5 years of age and includes medical conditions throughout childhood. If newborn screening for the variant is initiated in Nunavut, either as a pilot/research program or on a territory-wide basis, testing results could be included in this database. This would allow prospective study of infants through the first five years of their life and better characterisation of the clinical impact of the variant.
CHAPTER 4. RETROSPECTIVE REVIEW OF INFANT MORTALITY IN NUNAVUT (1999-2008)

4.1 INTRODUCTION

The northern Canadian territory of Nunavut has the highest infant mortality rate in Canada at 14.3/1,000 live births (1999-2007); a rate almost 3 times the Canadian national average of 5.3/1,000 live births and twice that of the bordering territory, the Northwest Territories [1]. The rate of infant mortality in Nunavut has remained consistently high despite substantial reductions in other jurisdictions of Canada [1-3]. Nunavut is divided into 3 regions, Qikiqtani, which contains the territorial general hospital (QGH), Kitikmeot, and Kivalliq. The Nunavut health care system depends on a series of community-based health centres and partnerships with southern tertiary care hospitals in Manitoba, the Northwest Territories, Ontario, and Quebec [13]. Infants requiring intensive care are evacuated to the QGH or out of territory to tertiary care centres in these other jurisdictions [14].

Nunavut has the largest Inuit population in Canada with 85% of Nunavut residents being Inuit [12]. Approximately 90% of the 700 births in Nunavut each year are to Inuit women [12,19,18]. Nunavut has the highest rate of preterm birth in Canada and preterm infants are at greater risk of mortality [25,30,119,106]. Factors that increase risk for preterm birth include maternal smoking, infection during pregnancy, and low maternal weight gain during pregnancy, all of which are factors that are associated with preterm birth in Nunavut [120-122].
Nunavut leads Canada with the highest post-neonatal mortality rate of 7.9/1,000 live births, a rate 5 times the national average (1999-2007) [1]. Sudden Infant Death Syndrome (SIDS), Sudden Unexpected Death in Infancy (SUDI), and infectious disease are the leading causes of post-neonatal mortality in Inuit regions [2]. Medical and environmental risk factors for SIDS and SUDI include sleeping in any position other than supine (i.e. sleeping on stomach or side), prematurity, young maternal age, age of infant less than 6 months, maternal (pre and post-natal) smoking, exposure to environmental smoke, male sex, not being breast-fed, bed-sharing, overheating, the presence of loose bedding, and soft sleep surface [34,41,44-51,106-108]. Genetic and/or biological factors, including cardiac conduction abnormalities (i.e. Long QT syndrome; LQTS) and fatty acid oxidation disorders (FAOD), may also increase risk for SIDS and SUDI [37,58-61].

This retrospective study presents an overview of all causes of infant mortality available in Nunavut from July 1, 1999-June 30, 2008. This report aims to provide insights into the overall contributors and the likely multi-factorial nature of the increased rate of infant mortality in Nunavut.

4.2 METHODS

All infant deaths occurring in Nunavut are reported to the Chief Coroner and subsequently to the office of the Chief Medical Officer of Health (CMOH). Detailed reports for 65 infant deaths as reported to the office of the CMOH from July 1, 1999-June 30, 2008 were reviewed and cross-referenced with Nunavut Bureau of Statistics where an additional 13 cases were documented (total=78). All prenatally occurring deaths (stillbirths) were
excluded for this review. Information collected, when available, included: cause of death, age at death, gestational age at delivery, infant chronic health conditions, condition of the infant prior to death, concurrent illnesses, breast feeding practices, sleep circumstances, known care giver use of alcohol and cigarettes, autopsy information, and CPT1A P479L genotyping results (Appendix Tables A.1 and A.2). Region of residence was determined using mother’s place of residence at the time of the infant’s death. Bed-sharing was defined as those infants sharing a sleep surface with another person as documented when the death occurred [123].

Case data were aggregated and reviewed for causes of death and trends in risk factors. Comparison data for Canadian mortality rates per 1,000 live births were obtained using the Statistics Canada CANSIM database. Nunavut Bureau of Statistics prepared total number of live births to mothers residing in Nunavut and by region within Nunavut for the years 1999-2008 (May 19, 2009). Gestational age specific infant mortality rates were calculated using cases born between 2000-2007 full calendar years (n=43) and compared live births by gestational age reported by Statistics Canada for Nunavut [30]. Cause specific rates were calculated using live births reported by Statistics Canada for Nunavut during the same period. These rates were compared to national rates when possible [30].

The rate of homozygosity for the P479L variant of CPT1A in cases was compared with known population rates of infants born in 2006. Odds ratios with 95% confidence intervals, and p values with Fishers exact test were calculated to assess risk.
Research ethics review approval was received by UBC Research Ethics Board and NRI. The review occurred under the supervision of the Deputy Medical Officer of Health (GO).

4.3 RESULTS

From July 1, 1999 to June 30, 2008, a total of 78 infant mortality cases were documented within Nunavut, 65 through the CMOH and another 13 in the Nunavut Bureau of Statistics. None of the 13 additional cases documented in the Nunavut Bureau of Statistics had cause of death information; however, 8 of those occurred during the first week of life. In total, 82% (64 cases) had sufficient information to determine cause of death, 97% reported exact age at death, 59% included gestational age at delivery, 45% had bed-sharing information, and 40% recorded sleep position placed and/or found (Table 4.1) The average infant mortality rate for Nunavut was 10.6/1,000 live births. Kitikmeot had a consistently higher infant mortality rate than the rest of Nunavut; Qikiqtani had the lowest (Figure 4.1).
Table 4.1  Information reported for infant mortality cases as documented in Nunavut (n=78; July 1, 1999-June 30, 2008)

<table>
<thead>
<tr>
<th>Category</th>
<th>Cases reporting* (% of total cases)</th>
<th>Risk association</th>
<th>n (%)** reporting cases with RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cause of death</td>
<td>64 (82.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at death</td>
<td>76 (97.4)</td>
<td>&lt;6mths</td>
<td>65 (86.0)</td>
</tr>
<tr>
<td>Gestational age</td>
<td>46 (59.0)</td>
<td>&lt;37 weeks gestation</td>
<td>18 (39.1)</td>
</tr>
<tr>
<td>Birth weight</td>
<td>44 (56.4)</td>
<td>&lt;2500g</td>
<td>7 (15.9)</td>
</tr>
<tr>
<td>CPT1A P479L status</td>
<td>22 (28.2)</td>
<td>Homozygous</td>
<td>20 (90.9)</td>
</tr>
<tr>
<td>Sleep position (found or placed)***</td>
<td>31 (39.7)</td>
<td>Prone or side</td>
<td>21 (67.7)</td>
</tr>
<tr>
<td>Bed-sharing</td>
<td>35 (44.9)</td>
<td>Present</td>
<td>25 (71.4)</td>
</tr>
<tr>
<td>Sleep surface</td>
<td>35 (44.9)</td>
<td>Sofa or other soft surface</td>
<td>6 (17.1)</td>
</tr>
<tr>
<td>Loose bedding</td>
<td>11 (14.1)</td>
<td>Present</td>
<td>11 (100.0)</td>
</tr>
</tbody>
</table>

*N indicates the number of cases during the study period (78) reporting on the category indicated. **Percentage of cases that reported information for the category and had the risk factor. ***4 cases did not indicate position found, but stated position paced, supine (3) or side (1)

Figure 4.1  Infant mortality rates as documented in Nunavut by region (n=78; July 1, 1999-June 30, 2008)
### 4.3.1 ALL CAUSES OF DEATH

Table 4.2 lists cause of death information for the 64 cases for which this information was available by Nunavut region. The leading causes of death were SIDS/SUDI (47%) and respiratory infection (19%) in Nunavut as a whole, as well as for each of the three regions (Figure 4.2). SIDS/SUDI accounted for 42% to 53% of deaths in the three regions. The male/female ratio for all infant mortality cases was equal, with small excesses of male deaths due to respiratory infection and female deaths due to infections other than respiratory, which were not statistically significant. Deaths designated as SIDS or SUDI were combined in this study, since differentiation of the two was often difficult even with careful review of autopsy reports.

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Qikiqtani</th>
<th>Kitikmeot</th>
<th>Kivalliq</th>
<th>Nunavut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant mortality rate</td>
<td>9.62</td>
<td>17.50</td>
<td>11.64</td>
<td>11.67</td>
</tr>
<tr>
<td>Neonatal mortality rate</td>
<td>1.41</td>
<td>3.68</td>
<td>2.43</td>
<td>2.09</td>
</tr>
<tr>
<td>Post-neonatal mortality rate</td>
<td>7.92</td>
<td>12.89</td>
<td>9.22</td>
<td>9.28</td>
</tr>
<tr>
<td>SIDS/SUDI</td>
<td>5.09</td>
<td>8.29</td>
<td>4.85</td>
<td>5.54</td>
</tr>
<tr>
<td>Respiratory infection</td>
<td>1.13</td>
<td>3.68</td>
<td>3.40</td>
<td>2.24</td>
</tr>
<tr>
<td>Other infection</td>
<td>1.41</td>
<td>1.84</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>Congenital defect / anomalies</td>
<td>0.57</td>
<td>1.84</td>
<td>0.49</td>
<td>0.75</td>
</tr>
<tr>
<td>Unknown</td>
<td>1.41</td>
<td>1.84</td>
<td>2.91</td>
<td>2.09</td>
</tr>
</tbody>
</table>

*Rates calculated using total births for each region and Nunavut as a whole, as reported by Statistics Canada for the study period (July 1, 1999 to June 30, 2008)
Figure 4.2 Nunavut infant mortality cases by cause of death categories (n=78; July 1, 1999-June 30, 2008). Medical causes were respiratory infection, other infections, and congenital anomalies. Unknown (18%) indicates those cases that did not have a cause of death recorded in the data available in Nunavut; 10% of the unknown cases were perinatal deaths that occurred out of territory.

The cause-specific infant mortality rates for SIDS/SUDI and respiratory infections were 5.54 and 2.24/1,000 live births respectively, based on the number of births reported by Statistics Canada. Deaths due to respiratory infections were higher in the Kitikmeot and Kivalliq regions (3.68 and 3.40/1,000 live births, respectively) than in the Qikiqtani region (1.13/1,000 live births). The proportion of post-neonatal deaths for SIDS/SUDI and death due to infectious disease were 57% and 29%, respectively.

4.3.2 NEONATAL AND POST-NEONATAL DEATHS

During the study period, 80% of the deaths recorded within Nunavut occurred during the post-neonatal period, providing a post-neonatal mortality rate of 9.28/1,000 live births;
more than 6 times the national rate of 1.4/1,000 live births for 1999-2006 [1]. The leading causes of death during the post-neonatal period were SIDS/SUDI (55%) and infection (respiratory or other; 31%). The neonatal mortality rate from the study data was 2.1/1,000 live births (n=14), which is substantially lower than the rates reported by Statistics Canada for Nunavut and for all of Canada (6.5 and 3.8/1,000 live births, respectively) for the same period [1].

4.3.3 PREMATURITY

Gestational age at birth was available for 46 cases with 39% of those (17 cases) reported as premature. The infant mortality rate (2000-2007; Table 4.3) for premature infants was 24.5/1,000 live premature births, 4 times the rate for term infants (5.4/1,000 live term births). The leading causes of death for both premature and term infants were SIDS/SUDI and respiratory infection.

Table 4.3  Gestational age specific rates for infant mortality cases documented in Nunavut (n=43; 2000-2007)

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Premature* (per 1,000 live premature births**)</th>
<th>Term (per 1,000 live term births**)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality rate</td>
<td>24.5</td>
<td>5.4</td>
</tr>
<tr>
<td>SIDS</td>
<td>12.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Respiratory infection</td>
<td>5.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Other infection</td>
<td>12.7</td>
<td>6.4</td>
</tr>
<tr>
<td>Congenital defect / anomalies</td>
<td>12.7</td>
<td>4.7</td>
</tr>
</tbody>
</table>

*Premature is defined as less than 37 weeks of gestation at birth. **Cases included in this analysis were restricted to those born between 2000-2007 calendar years, allowing comparison with reported live births by gestational age for Nunavut [30].
4.3.4 SIDS/SUDI

There were 37 SIDS and SUDI cases during the study period; 28 occurred between 1 month and 5 months of age and 33 were less than 6 months of age (Table 4.4). There was no evidence of excess of males among the SIDS/SUDI cases (male = 18).

Sleep position other than supine (found or placed) was reported in 17 cases and bed-sharing occurred in 29 cases. Sleep surface was a sofa or soft surface in at least 6 cases. Two or more sleep-related risk factors were present in 24 cases. Only 2 SIDS/SUDI cases reported no sleep circumstance risk factors.

Table 4.4 Information reported for SIDS/SUDI cases as documented in Nunavut (n=78; July 1, 1999-June 30, 2008)

<table>
<thead>
<tr>
<th>Categories</th>
<th>Cases reporting</th>
<th>Risk association</th>
<th>n (%)** reporting cases with RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age</td>
<td>25 (67.6)</td>
<td>&lt;37 weeks gestation</td>
<td>10 (40.0)</td>
</tr>
<tr>
<td>Birth weight</td>
<td>24 (64.9)</td>
<td>&lt;2500g</td>
<td>4 (16.7)</td>
</tr>
<tr>
<td>Age at death</td>
<td>37 (100)</td>
<td>&lt;6mth</td>
<td>33 (89.2)</td>
</tr>
<tr>
<td>CPTI P479L status</td>
<td>9 (24.3)</td>
<td>Homozygous</td>
<td>7 (77.8)</td>
</tr>
<tr>
<td>Sleep position (found or placed)***</td>
<td>24 (64.9)</td>
<td>Prone or side</td>
<td>17 (70.8)</td>
</tr>
<tr>
<td>Bed-sharing</td>
<td>29 (78.4)</td>
<td>Present</td>
<td>22 (75.9)</td>
</tr>
<tr>
<td>Sleep surface</td>
<td>26 (70.3)</td>
<td>Sofa or other soft surface</td>
<td>6 (23.0)</td>
</tr>
<tr>
<td>Loose bedding</td>
<td>8 (21.6)</td>
<td>Present</td>
<td>8 (100)</td>
</tr>
</tbody>
</table>

*N indicates the number of cases during the study period (78) reporting on the category indicated. **Percentage of cases that reported information for the category and had the risk factor. ***3 cases did not indicate position found, but stated position paced, supine (2) or side (1)
4.3.5 **THE P479L VARIANT OF CPT1A**

The P479L CPT1A genotype results were available for 22 cases, all had at least one copy of the variant, and 20 were homozygous (had 2 copies) for the variant. Most of the deaths occurred during the post-neonatal period (90%). The cases included deaths due to SIDS/SUDI (n=9), infectious disease (n=11), or congenital anomalies (n=2). The results for two cases of severe congenital anomalies were excluded from risk analysis, leaving 18 homozygous cases. The genotyping results were compared to the variant population homozygosity for Nunavut (0.64; Chapter 2), which resulted in a significant odds ratio of 5.15 (95% CI 1.22-46.1; p=0.016).

4.3.6 **OTHER FACTORS**

Maternal age was available for 40 cases; 11 mothers under 20 years of age, 21 between the ages of 20-29, and 8 over the age of 30. Information on maternal or household member smoking (n=8) or substance use (n=10) was limited for the 78 infant mortality cases. Information on breast-feeding was available for 21 cases. The deaths were evenly divided between breast-feeding alone, breast and bottle, and bottle alone.

4.4 **DISCUSSION**

Indigenous populations worldwide experience infant mortality rates that are substantially higher than national averages [1-3,26,32,124-126]. This is true in the Inuit regions of Canada and in the territory of Nunavut where infant mortality rates are, on average, 2 to 3 times the national rate, respectively [1,2]. Our review revealed that SIDS/SUDI and respiratory
infections were the leading causes of infant mortality in Nunavut with cause-specific mortality rates consistent with those reported by Luo et al. [2] (5.54 and 2.24/1,000 live births, respectively). During the study period, the proportion of post-neonatal deaths due to SIDS/SUDI and infectious disease in Nunavut were 2 and 3 times the reported averages for all of Canada (27.5 and 10.3%, respectively; 2004) [28]. The cause-specific mortality rates/1,000 for SIDS/SUDI and infection (including respiratory infection) were significantly higher than the national rates due to Nunavut’s disproportionately high post-neonatal mortality rate, which is 6 times the national average [1,28].

The neonatal mortality rate in this study is lower than rates reported by Statistics Canada and by Luo et al. (6.5 and 5.1/1,000, respectively) [1,2]. The reduced number of neonatal cases from data available in Nunavut may be due to under reporting of perinatal deaths occurring out of territory to Nunavut Bureau of Statistics. The post-neonatal mortality rate determined by this study was consistent with that reported by Statistics Canada for the region [1].

Premature infants have a 3-6 times higher risk for mortality than term infants, including higher risks for deaths due to infections and SIDS [107,127]. Although gestational age was not available for all cases, premature infants comprised at least 23% of the total infant mortality cases in this study and had a 4.5 times greater risk for mortality than term infants. Infants exposed to cigarette smoke in utero and in their environment are at increased risk for prematurity, lower respiratory infection, infant mortality, and SIDS and SUDI [56,128,129]. Nunavut women living in the Qikiqtaani region who reported smoking more
than 10 cigarettes per day had twice the risk of having a preterm infant [129]. Maternal or household smoke exposure information was rarely available for infant mortality cases in this review; however, between 60-80% of Nunavut women self-report that they smoked during pregnancy, which is 5 times greater than the national average of 16% [28,109,129].

Our study found that death due to respiratory infection was the second leading cause of infant mortality in Nunavut. Infants in Nunavut have the highest rate of hospitalisation for lower respiratory tract infections worldwide, with an average of 306/1,000 infants [14,57]. Environmental tobacco smoke is present in ~90% of Nunavut homes, which are small, house an average of 6 people, and have low air change rates [110]. Environmental tobacco smoke, overcrowding, and poor ventilation are critical risk factors for respiratory infection [56].

Information on maternal age was limited, but was consistent with population demographics for maternal age at birth compared to a 2003-2006 cross sectional study of woman giving birth in Nunavut [129].

There was a significant increased risk for sudden death associated with homozygosity for the P479L variant of CPT1A (OR 5.15; 95% CI: 1.22-46.1; p=0.016), however this result must be taken with caution, as the case sample size was small (n=20). These results are consistent with results from studies of the variant in mid-Vancouver Island, BC First Nations (OR: 3.87; 95% CI: 1.4-10.9, p<0.006; Sinclair and Vallance, personal communication). The clinical implications of the common P479L variant of CPT1A are not well defined. The results from this study suggest that infants homozygous for the variant may be at increased risk of sudden death, especially during periods of fever and intercurrent illness, however the
sample size for this study were very small. Prospective studies on the variant are planned in Nunavut.

Initial recommendations to reduce risk of SIDS were released in Canada in 1993 [130]. Despite this, SIDS and SUDI rates in Inuit regions of Canada have remained 7 times higher than the rest of Canada [2]. In this review, 89% of the deaths occurred before the age of 6 months and, although not all cases reported gestational age, the risk of SIDS/SUDI was 4 times higher for premature infants than term infants. Unsafe sleep circumstances, including factors like sleep position, sleep surface, loose bedding, and bed-sharing were reported in the majority of SIDS and SUDI cases, and 65% of the cases had 2 or more sleep related risk factors present. Placing infants to sleep on their backs (supine position) is the key recommendation to reduce the risk of SIDS [45,112,113]. In a 2006 survey (Canadian Maternity experiences), only 46% of women from Nunavut reported placing their infants to sleep in the supine position (on their backs) [109]. Information on sleep position was available for over half of the SIDS and SUDI cases and our results support that public health reminders about sleep position are needed.

Bed-sharing is a possible risk factor for SIDS, especially when it occurs on a soft sleep surface (i.e. sofa), if the infant is premature or had a low birth weight, or the bed-sharing is with a caregiver that smokes [113,131]. The issue of bed-sharing itself as a risk factor for infant mortality is controversial and could be a proxy for other risk factors which are associated with bed-sharing (i.e. loose bedding). Bed-sharing may be beneficial to infants by
promoting breastfeeding [112], as breast feeding may reduce risk for SIDS and SUDI [132,133]. The implications of bed-sharing require further population discussion and study.

4.5 CONCLUSION

A greater proportion of infants in Nunavut die of SIDS and infectious disease than infants in the rest of Canada. Factors like sleep position, bed-sharing, and exposure to cigarette smoke may play a role in these results, as well as homozygosity for the P479L variant of CPT1A. Studies are now planned to explore issues around sleep practices and to provide culturally appropriate information to the mothers and health care providers of Nunavut. Improved prenatal and post-natal data collection will enhance the understanding of the increased rates of infant mortality. The Qiturngatta Surveillance System, which is now underway throughout Nunavut, collects information from the prenatal period through to early childhood (to five years of age). The system includes key factors, such as pregnancy medical risk factors, prenatal care and nutrition, and substance use and would allow prospective study and analysis of risk factors not commonly available for this review.
CHAPTER 5. GENERAL DISCUSSION & FUTURE DIRECTIONS

5.1 THE P479L VARIANT IN CANADA’S NORTH

Since its discovery in 2001, the P479L variant of CPT1A has been of increasing concern regarding the possible associated risk for infant morbidity and mortality. A number of symptomatic and sudden death cases, all homozygous for the variant, have been reported in the literature and, subsequently, some jurisdictions have expanded newborn screening to include CPT1A deficiency in populations estimated to have high homozygosity for the P479L variant [6,10,11,65,90]. To date, there has not yet been evidence-based research to determine the prevalence and impact of the variant in those populations. This study is the first to systematically determine the prevalence of the variant in all three northern territories and to determine if there is an increased risk associated with the variant in sudden death cases from those territories. This study also examines other risk factors associated with infant mortality in Nunavut.

This study determined that the P479L variant is highly prevalent in Nunavut, results that are consistent with Rajakumar et al.’s [8] and Gessner et al.’s [9] reported prevalences for the Greenland Inuit and the Inuit and Yupik regions of Alaska. The prevalences of the variant in the Inuvialuit population of NWT and the BC coastal First Nations populations were similar and substantially lower than in the Inuit. There was very low frequency of the variant in the First Nations populations of the NWT and Yukon and interior populations of BC. Most of the First Nations populations in Yukon and NWT are in-land populations, so this may be
supportive of the hypothesis that the variant was historically beneficial to coastal populations.

The P479L allele frequency was in Hardy-Weinberg equilibrium in the Kitikmeot and Kivalliq regions of Nunavut and the Inuvialuit of NWT, which is consistent with results for coastal Alaska Native populations and Greenland Inuit [8,9]. However, the allele was not in HWE in the Qikiqtani region of Nunavut. It is possible that this represents admixture of this population, as an estimated 90% of the population are born to Inuit mothers in this region. Reduction of the sample to 90% and assuming that all homozygotes and heterozygotes were within that 90% gave values for P479L homozygosity (60%) and heterozygosity (33%) that did not deviate from those expected by HWE (p>0.05). However, the NWT First Nations population did deviate from HWE, as did results for Alaska Native populations in non-Inuit and Yupik regions [9]. In both populations there was a decrease in expected heterozygosity in favour of P479L homozygosity, suggesting either founder effect or a possible benefit to P479L homozygosity.

There was a significantly increased risk for sudden death in infants homozygous for the variant in Nunavut. The evidence for increased risk from this study alone is weak, but the results are consistent with those of BC First Nations and Alaska Native studies, which demonstrated similar, increased mortality risks for infants homozygous for the variant in mid Vancouver Island, BC (OR: 3.87; 95% CI: 1.4-10.9; Sinclair and Vallance, personal communication) and Alaska (OR: 7.6; 95%CI: 1.5-38.9) [94].
The information from this study is a first step and will aid public health efforts in determining how best to approach the management of the variant in their jurisdictions. It will also function as a baseline for studies on other health impacts of the variant in these populations, including the possible beneficial impacts in late life.

5.2 MODIFIABLE RISK FACTORS OF INFANT MORTALITY IN NUNAVUT

Nunavut has the highest infant and post-neonatal mortality rates in Canada. Earlier this year, Luo et al. [2] reported on birth outcomes in Inuit populations of Canada, including high rates of infant mortality, SIDS, and infant deaths due to infectious diseases. This study further describes the infant mortality cases in Nunavut and associated risk factors. The majority of post-neonatal infant deaths in Nunavut were attributed to preventable causes such as SIDS/SUDI (55%) and infectious disease (31%), at proportions that were 2 and 3 times greater than the national averages [28].

The CPT1A P479L was associated increased risk for sudden death and may be playing a role in the excess cases of post-neonatal mortality cases in Nunavut; however, small case numbers and the inability to obtain all case samples limited the strength of the study. Prospective studies are planned in Nunavut to further explore the effect of this variant in early childhood.

This study has highlighted possible factors that can be explored in efforts to reduce infant mortality in Nunavut such as public health efforts to encourage safe sleep positioning and other sleep practices, and exposure to cigarette smoke. The results of this study add to
existing data and will aid in development of future health programs and research regarding birth outcomes in Nunavut as well as other jurisdictions with large Inuit populations. Studies are now planned to explore issues around sleep practices and smoking and to provide culturally appropriate information to the mothers and health care providers of Nunavut. Improved prenatal and post-natal data collection will enhance the understanding of the increased rates of infant mortality. The Qiturngatta Surveillance System, which is now underway throughout Nunavut, collects information from the prenatal period through to early childhood (to five years of age) and includes key factors often not available for infant mortality review, such as pregnancy medical risk factors, prenatal care and nutrition, and substance use. Future study of infant mortality in Nunavut should look beyond the descriptive data and identify those modifiable factors that will improve and support individual and community health [22,134].

5.3 IS THERE A P479L VARIANT ADVANTAGE?

This study demonstrates a high frequency of the P479L variant in the Inuit population of Nunavut, which is consistent with prevalence reported for other Inuit populations [8,9]. A gene variant/polymorphism may occur with such high frequency in a population due to the founder effect, genetic drift, a conferred advantage, or linkage to another polymorphism that confers an advantage. The variant is especially frequent in the Canadian and Greenlandic Inuit, the northern and western regions of Alaska [6,8,94]. It is also very frequent in the NWT Inuvialuit and coastal BC First Nations populations. This suggests that
there may have been an advantage to individuals with the P479L variant living in northern coastal environments subsisting on diets rich in marine fats [10].

The P479L CPT1A variant has lower enzymatic activity and reduced sensitivity to malonyl-CoA (over controls, *in vitro*), suggesting that the variant protein is persistently active, even when glucose is present [65,6]. The reduced sensitivity to malonyl-CoA may be due to the position of the P479L substitution near or within the malonyl-CoA binding site [77]. The traditional Inuit diet is a ketogenic diet with high fat, moderate protein, and minimal carbohydrate [20]. However, historically, there was often a temporary increased reliance lean protein (i.e. rabbit) during the spring, which may have shut off ketogenesis and was associated with symptoms of lethargy and headaches [135]. P479L homozygous and, to a lesser extent, heterozygous individuals may have avoided the negative effects of diet transitions by preserving ketogenesis [6,135].

The P479L variant may be also beneficial in adulthood by reducing risk for cardiovascular disease by benefiting adult lipid-profiles. Rajakumar et al.’s [8] study the P479L variant’s impact on lipid profiles and cardiovascular disease in adult Greenland Inuit found a possible protective effect for P479L homozygous adults against cardiovascular disease. Homozygous individuals had higher levels of HDL-cholesterol and associated apoA-I than heterozygous and homozygous wild type individuals. Interestingly, heterozygous were intermediate between P479L homozygous and wild type homozygous. However, the potential dietary and lipid profile benefit of the variant is one that is noted in adult health and, subsequently,
but does not explain why there might be a selective/reproductive advantage for those homozygous for the variant.

Alternatively, the P479L variant may have been benign or may not have been subject to negative selective pressure in populations surviving on a low carbohydrate diet. Free LCFAs have been found to induce hepatic CPT1A expression in both rats and mice [85,136]. Female neonatal mice offspring of dams fed a high-unsaturated fat, high protein, low carbohydrate diet during gestation and lactation had hepatic CPT1A protein levels that were ~52% higher than those female offspring of dams fed a carbohydrate diet [93]. Alternatively, inhibition of CPT1A activity in the hypothalamus reduces feeding behaviour in obese rats [137]. The traditional high fat diet of the Inuit may have compensated for the reduced activity of the P479L CPT1A variant by increasing overall CPT1A expression.

5.4 CONCLUSION AND FUTURE RESEARCH DIRECTIONS

5.4.1 PUBLIC HEALTH PROGRAMS

This paper demonstrates that SIDS, SUDI, and infectious disease comprise the majority of infant deaths in Nunavut. It also demonstrates that infants homozygous for the P479L variant have a moderate but significant increased risk for death due SIDS/SUDI and infectious disease. While the results from this study must be viewed with caution due to small numbers, it is still appropriate to consider public health programs targeted to affected communities and the medical professionals serving those communities. This may include public health education programs advising parents and extended families to ensure infants
are carefully monitored when ill, and inclusion of the P479L variant testing in newborn screening. Any public health programs will need to consider the importance of family and consultative structure in Aboriginal communities [138]. Programs should be developed in partnership with community members and leaders regarding how information regarding the P479L variant and CPT1A deficiency should be conveyed to parents and families.

Traditional lifestyle, breast-feeding, and diet may play a protective role for those homozygous for the P479L variant. A cohort study assessing factors around infancy and through childhood may allow better characterisation of the disorder and those factors that may ameliorate the presence of the variant. This could include assessment of whether consumption of a traditional high-unsaturated fat diet during gestation and lactation improves outcomes for P479L homozygous infants. Information from the Nunavut Qitturngatta Surveillance System may be helpful in the identification of such factors.

5.4.2 IMPLICATIONS OF NEWBORN SCREENING FOR THE P479L VARIANT

Newborn screening for the variant, combined with a surveillance system, would allow territorial health authorities to prospectively study the impact of P479L variant, including rates of sudden unexpected death and effectiveness of treatment. Screening would also allow parents and health care providers to monitor P479L homozygous infants closely during times of fever and illness. Lowering standard cut-offs (C0/(C16+C18 > 20) followed by secondary genotyping would capture most homozygous and some heterozygous infants [9]. However, the high prevalence of the variant in Inuit, Inuvialuit and BC First Nations populations suggests that further characterisation of the clinical impact of the variant and
clarification of effective treatment are needed to allow primary care health care providers to properly advise parents of homozygous infants. The intent of the newborn screening program is to avoid harm to infants and families, therefore, information regarding CPT1A deficiency and the risks associated with homozygosity for the P479L allele must be disseminated in a manner that does not unduly alarm parents or health care professionals. Education programs will need to be respectful of the different cultural perspectives of the affected Inuit and First Nations communities and avoid ‘medicalising’ healthy children.

5.4.3 CHARACTERISATION OF THE P479L VARIANT

This research demonstrates the high prevalence of the variant in Inuit populations of northern Canada and demonstrates that the natural history aetiology and clinical impact of the P479L variant needs better characterisation. This could be addressed in a variety of ways, including clarification of the symptomatic phenotype, prospective studies of infants homozygous for the variant, and mouse modelling to better characterise the biochemical significance of the variant as well as to clarify environmental factors that may affect the penetrance of the variant.

Study of symptomatic cases has been difficult due to the highly variable symptoms of cases. A clearly defined phenotype is needed to determine risk associated with the allele and the benefits of treatment, including recommending fast avoidance is appropriate. This issue can only be addressed by prospective study of cases sent for CPT1A P479L testing.
A mouse model for the P479L variant of CPT1A would allow characterisation of the variant under a variety of physiological conditions as well as the impact of maternal diet and health of neonates. It would also aid in determining if the variant is thermodabile and its activity and malonyl-CoA binding affinity \textit{in vivo}. A CPT1A knock-out mouse has been developed and haplo-insufficiency of the heterozygous mice demonstrated information that characterised CPT1A deficiency [139]. A P479L CPT1A mouse model could also help determine the presence of increased sensitivity to fasting and fever in P479L CPT1A homozygous mice and be compared to the CPT1A knock-out heterozygous mice to assess how the two genotypes differ in CPT1A activity, response to treatment, and overall health outcomes. As CPT1A is also expressed in the hypothalamus and is involved in feeding behaviour, the effect of P479L CPT1A variant on satiety could also be investigated. This model could also help determine any benefits of an Inuit traditional style diet for those homozygous for the variant.

\textbf{5.4.4 HISTORICAL SIGNIFICANCE OF THE P479L VARIANT}

The presence of a variant in the distantly related Inuit, Yupik, Inuvialuit, Alaska Native, and coastal First Nations populations of BC raises questions as to an advantage associated with the allele, and whether the mutation occurred in a common ancestor of all populations, or whether the mutation occurred independently in some or all of these populations. Determining the prevalence of the variant in the Yupik of the Russian Chukotka peninsula would aid in answering this question.
5.4.5 INFANT MORTALITY IN NUNAVUT

This paper has identified a variety of risk factors and data concerns relating to infant mortality cases in Nunavut. Better surveillance will improve data collection of risk factors surrounding infant mortality cases in the territory and will allow analysis for adverse health outcomes. Better communications tools regarding sleep position and smoke exposure are also needed. These tools should utilise the extensive communication networks within and between Nunavut communities and should be developed in partnership with community leaders, parents, families, and public health nurses to ensure that the message is consistent and culturally meaningful.

Dialogue with community members and leaders would help researchers and health care professionals to characterise factors that may hinder access to information and resources as well as to better understand both traditional and modern perceptions of infant mortality and associated risk factors. Engagement of all stakeholders, including elders and community members, would help to identify positive and modifiable factors that could aid in reducing infant mortality in communities.

5.5 LIMITATIONS

This study was the first study to assess the prevalence of the P479L variant of CPT1A in infants of all three territories, and compared it with the frequency of homozygosity of those who died unexpectedly. There are, however, several limitations to this study, which must be kept in mind when reviewing these results. The low samples size for infant deaths
greatly reduced the ability to determine risk associated with the variant. Although the case numbers analysed in this study were small, they were higher than those reported in Gessner et al. [94]. The case numbers were further limited by the unavailability of dried blood spot samples for many of the cases identified for inclusion in the study. Newborn blood spot samples were only available for 39% (31/79) of sudden death cases for all three territories and 34% (20/59) of Nunavut cases. This reduced ability to assess risk associated with the variant, especially for a multi-factorial outcome like SUDI or death to infectious disease. However, the study was still able to demonstrate a moderate increased risk for infants homozygous for the variant in Nunavut. This study was a candidate gene study, which may be influenced by population stratification. Although we tried to control for this in the study, it was not possible to completely control for this in Nunavut or Yukon samples as no ethnicity information was available for population samples, as discussed in Chapter 2, and this is a concern for these data. Another limitation was the inability to conduct cluster analysis of territorial data to determine whether there are geographical clusters trends in P479L variant frequency in the three territories.

This review of risk factors for infant mortality cases in Nunavut was a retrospective case review study, with limited available risk determinant information. Many mortality subgroups presented in this study were too small for statistical analysis. Caution must be used when interpreting mortality data in such small numbers. Review of Nunavut Bureau of Statistics indicated that out of territory perinatal deaths were likely under-reported to
Nunavut. In particular, detailed information for these deaths was unavailable to the Chief Medical Officer of Health limiting analysis of contributing factors.
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APPENDIX A.

Table A.1 Variables included from coroner’s report for infant mortality cases documented in Nunavut (n=78; July 1, 1999-June 30, 2008)

<table>
<thead>
<tr>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of Birth</td>
</tr>
<tr>
<td>Date of Death</td>
</tr>
<tr>
<td>Cause of Death</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Gestational Age (wks)</td>
</tr>
<tr>
<td>Place of Residence</td>
</tr>
<tr>
<td>CPT-1 P479L Tested</td>
</tr>
<tr>
<td>Sleep Position - found</td>
</tr>
<tr>
<td>Sleep Position - placed</td>
</tr>
<tr>
<td>Bed-sharing</td>
</tr>
<tr>
<td>Loose bedding present</td>
</tr>
<tr>
<td>Sleep surface</td>
</tr>
<tr>
<td>Smoking present in environment</td>
</tr>
<tr>
<td>Alcohol present in environment</td>
</tr>
<tr>
<td>Breast feeding</td>
</tr>
</tbody>
</table>

...
Table A.2 Causes of death as stated by autopsy report for infant mortality cases documented in Nunavut (n=78; July 1, 1999-June 30, 2008)

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Known medical cause</strong></td>
<td></td>
</tr>
<tr>
<td>Respiratory infection including pneumonia, bronchiolitis</td>
<td>15</td>
</tr>
<tr>
<td>Other infection, including H influenza type A Sepsis, other sepsis, H influenza</td>
<td></td>
</tr>
<tr>
<td>type B meningitis, other meningitis/encephalitis, pericarditis, and viral</td>
<td>7</td>
</tr>
<tr>
<td>myocarditis.</td>
<td></td>
</tr>
<tr>
<td>Congenital defect / anomalies, including heart defects, neuromuscular, brain</td>
<td>5</td>
</tr>
<tr>
<td>malformation, and respiratory system malformation.</td>
<td></td>
</tr>
<tr>
<td><strong>SIDS/SUDI</strong></td>
<td>37</td>
</tr>
<tr>
<td>SIDS</td>
<td>24</td>
</tr>
<tr>
<td>SUDI</td>
<td>13</td>
</tr>
<tr>
<td><strong>Unknown</strong></td>
<td>14</td>
</tr>
<tr>
<td>Unknown - perinatal death - died out of territory</td>
<td>8</td>
</tr>
<tr>
<td>Unknown - died out of territory</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>78</td>
</tr>
</tbody>
</table>