# A Palaeopathological and Immunogenetic Assessment of Archaeological Canadian Inuit Populations

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# Abstract

For centuries there has been a disparity in the health of Canadian Aboriginal populations and the colonizers who came to inhabit their territories. In contemporary times, this disparity is translated into a number of growing health concerns that appear at higher rates in circumpolar populations than in many other Canadian communities. Tuberculosis rates in Arctic and circumpolar communities, particularly those with a high Inuit demographic, remain more than 20 times higher than in any other population demographic. Understanding the factors that contribute to the continued prevalence and high incidence of TB in the Arcticcompared to the remainder of Canadian populations demonstrate requires a longitudinal analysis of a number of factors related to overall health.

This dissertation explores this disparity through the examination of the immunogenetics and palaeopathology of an archaeological Inuit population. This archaeological cohort was assessed using palaeopathological techniques of to establish the disease burden experienced by the Inuit in the pre-contact and early contact period. The palaeopathologicual inventory also established individuals with possible TB pathologies as candidates for further molecular analysis. Molecular analyses focused on the establishment of Inuit ancestry and the examination of the presence of four polymorphic sites in the promoter regions of IL-6, IL-10, TNFα and IFNγ. Polymorphisms for the Th2 cytokines IL-6 and IL-10 are associated with the down regulation of Th1 cytokines activated to combat TB infection, while the Th1 cytokines TNFα and IFNγ are essential for the effective immune response against TB infection. These analyses resulted in the establishment of genotypes and phenotypes detected utilising a novel molecular method and protocols developed specifically for this research.

Osteological observations indicated an increase in risk of disease in early contact populations, particularly those associated with infectious disease or the co-infection of multiple conditions compared to the pre-contact cohort. In contrast pre-mortem tooth loss decreased with contact, and degenerative pathologies maintained a relatively balanced presence. TB pathologies were observed in both pre-contact and contact groups, with an increased level of pathologies observed in contact individuals.

Molecular results suggest immunogenetic profiles similar to First Nations groups, with only a single cytokine SNP exhibiting a unique phenotype in comparison. Immunogenetic profiles suggest Inuit have maintained a Th2 immune response for many generations, and this remains unchanged with contact.

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# Table of Contents

# List of Tables

# List of Figures

# Chapter 1 Introduction

The Canadian Arctic has seen a flow of migratory peoples for tens of thousands of years (Goebel 2002; Goebel et al. 2003). Archaeological evidence supports sporadic human occupation prior to the Last Glacial Maximum (between 4,300 – 12,000 ya) (Pitulko et al. 2004). Early migration models based on mtDNA data suggest one to two major expansions from Siberia-Asia, however new research supports a third and final expansion from Alaska 5400 years ago (Zlojutro et al. 2006) resulting in the expansion of groups that would later become ascendants of the contemporary Inuit. Some have suggested that this final expansion began the development of early Inuit cultures, (McGhee 1978, 2005; Park 1993; Jordan 1984; Hayes et al. 2003; Fagan 2005; Hayes et al. 2007) developing into contemporary Inuit populations from the combination of cultural and genetic characteristics from the Dorset and Thule cultures (Helgason et al. 2006). Both of these early Inuit precursor populations were important to the development and diversity of Inuit populations across the Canadian and Greenlandic Arctic.

Inuit is a term that can be used to loosely amalgamate a circumpolar population that stretches from the easternmost tip of Russia to the eastern coast of Greenland. A contemporary Inuit population of approximately 167 000 individuals reside in four circumpolar nations: Greenland (as part of Denmark) and Canada comprise the largest populations (50,000 individuals respectively), followed by Alaska (44,000) and the continental USA (13,000), Denmark (8,000) and Russia (1,700) (Bjerregaard and Young 1998). There are two main linguistic families the Inuit/Inupiaq found in parts of Alaska, Canada and Greenland and the Yup'ik found in Alaska and the Chukotka peninsula in Russia

(Bjerregaard et al. 2004). Early "Eskimo" populations are thought to be descendants of the last waves of Old World migrants across the Beringia land bridge circa 5 000 years ago (Szathmary 1993; Helgason et al. 2006) moving across the Canadian Arctic and into Greenland by 4-4,500 ybp (years before present) . In Canadian populations, The Dorset culture developed between 3-3,500 ybp in the Hudson Bay region, while the Thule culture began in Northern Alaska circa 1000 ybp, reaching Greenland relatively quickly ca. 800 ybp (McGhee 2000; Morrison 2000; Helgason et al. 2000). Inuit genetic and cultural heritage is heavily influenced by the Thule cultural group (Merbs 1976), however rcent mtDNA research in Kitikmeot and Greenlandic populations reveals evidence of between Dorset and Thule populations (Helgason et al. 2006).

In response to fluctuations in environmental conditions, the Inuit population segmented into smaller and more diverse geographic and/or cultural groups. The Little Ice Age (AD 1600 – 1850) resulted in changes to climate, biomass, sea levels, and the advance of glaciers onto Arctic islands, as well as the retreat of the tree line to its current position. In response to these changes the Thule ancestral Inuit population segmented and became groups adapted to specific regional resources (McGhee 1994). The degree of distance between populations resulted in the creation of different dialects and the development of highly varying cultures (Merbs 1976). What resulted was several culturally distinct and yet similar Inuit culture groups generally described by their eponymous regional territories (McGhee 2005). The expansion and migration of the Dorset, Thule and subsequent Inuit groups, accounts for the genetic similarity found in many contemporary Inuit populations (Merbs 1976) as well as the similarity reported in the Inuktitut dialects.

The health of historic and pre-historic ArcticArctic peoples has been analyzed using osteological and historical informationArctic.. Studies about the Aleut (Zimmerman et al. 1971; Zimmerman et al. 1981; Lantis 1984; Keenleyside 1998, 2003a, 2003b), other various Alaskan populations (Dayydov 1977; Tikhemenv 1978; Veniaminov 1984; Fortuine 1989), Greenlandic Inuit (Jordan 1984) and Canadian Inuit (Merbs 1963, 1968, 1969, 1983, 1995; Cassidy 1977) demonstrate that Arctic peoples suffered from a wide range of historical health conditions: degenerative disorders, activity induced conditions, viral and fungal infections, and even cancer (Cassidy 1977). However, there remains a lack of skeletal and molecular evidence supporting the presence of TB in archaeological Canadian Inuit populations. Historical accounts describe tubercular conditions such as "consumption" (Dayydov 1977; Tikhemenv 1978; Veniaminov 1984) in historical populations and tuberculosis (TB) is prevalent in recent Aboriginal populations throughout Canada (Ferguson 1950; Davies 1967; Fortuine 1989, 2005; Grygier 1994; Fagan 2000; Health Canada 1999).

Compared to many global communities and nations the overall annual incidence rate and the pulmonary TB rate in Canada are amongst the lowest globally (2/100 000 vs. e.g. Swaziland 510/100 000, World Health Organization 2011). However, amongst the Canadian population the Inuit maintain the highest annual tuberculosis rates found within Canada as a whole, and often within individual provinces (Public Health Agency of Canada 2011). In a ten year period the Nunavut territory has maintained a tuberculosis rate multiple times higher than any other province or territory in Canada (174/100 000 in 2009), comparable to some of the most highly affected countries worldwide (Public Health Agency of Canada 2011). Provinces with high First Nations' populations, and a high Inuit

population in particular, such as Nunavut, the Northwest Territories, Manitoba and Newfoundland and Labrador maintain the highest overall incidence rates, and the highest prevalence rates[1] for the Inuit population (Public Health Agency of Canada 2011).

The lack of archaeological evidence for pre-contact Inuit populations combined with the disproportionately high rates and prevalence of TB in Canadian Inuit populations provided the basis for thisresearch. The present observed vulnerability of Canadian Inuit populations to tuberculosis infection lead to the three specific questions: 1) If Canadian Inuit populations did not experience high levels of TB in the historical past, would this contribute to their current vulnerability? 2) Inuit populations post-contact will have higher levels of observed TB 3) Do both pre and post contact populations maintain immune profiles reflective of their environments? 3)Were there changes in the observed health and vulnerability over time between pre and post European contact Inuit populations?. Resolving these questions required the use of osteological analytical techniques and the development of molecular techniques specific to ancient DNA.

Osteological analysis included the assessment of skeletal remains from 20 Inuit archaeological sites in the Canadian Arctic, in an attempt to identify pathological conditions present within the population over time, and specifically the presence of TB. A diversity of sites allowed for an assessment of pathological conditions present in different Inuit cultural groups (or their Arctic predecessors), as well as between pre-contact and post contact individuals.

The analysis of ancient DNA (aDNA) allows for the examination not only of pathogens but of the immune regulatory system in past populations. New techniques were

[1] Incidence describes the number of new events occurring in a population within a specific time
Prevalence is the proportion of the population with a specified condition at any one time (Waldron 1994)

used to analyze polymorphisms in promoter genes of cytokines that influence the immune response and regulation of tuberculosis infection. Establishing immunogenetic profiles from single nucleotide polymorphisms (SNPs) through molecular analysis enabled an understanding of Inuit health status and their immunogenetic capacity for combating diseases, infections, trauma, and degenerative disorders. Results from aDNA analysis were compared to contemporary First Nations immunogenetic profiles to determine the range of immunogenetic variability within Inuit and between First Nations and Inuit populations. Molecular analyses of aDNA were used to 1) identify mtDNA markers that are indicative of Inuit ancestry, 2) determine immunogenetic profiles in order to assess possible population based differences in immune regulatory genes, and 3) to confirm or refute the osteological observation of TB within the population sample.

Aboriginal health in North America is discussed in Chapter two, introduces what the archaeological record and historical documents can suggest concerning diseases in the New World prior to colonisation. This provides the foundation for understanding health differences between prehistoric, historic and contemporary Canadian indigenous populations. Chapter three introduces the human immune system, cytokines and population variation in immune characteristics which play an important role in understanding health differentials from a genetic and immune function level. Chapter three also elaborates on the function of ancient DNA (aDNA) and aDNA analyses within anthropology. This introduces the foundation for understanding the methodologies used to analyze the immunogenetics of the ancient population cohort for this study. Chapter four introduces the samples used and provides the demographics of the entire osteological sample (n. 152) examined during the palaeopathological assessment at the Canadian

Museum of Civilization and elaborates on traditional methods for examining a number of pathologies which manifest in the skeletal system, tuberculosis being the key focus. Chapter five provides further analysis of twenty individuals chosen as candidates for molecular analysis. The chapter explains the protocols employed during this research: primer design, sex determination, mtDNA analysis and haplogroup determination, SNaPshot analysis and microbial amplification and analysis. Chapter five also describes the techniques developed for the aDNA analysis of immunogenetic variants in archaeological populations; specifically the analysis of polymorphisms in promoter genes of cytokines linked to immune regulation in response to tuberculosis and other pathological conditions in past populations. Chapter six illustrates the results obtained and compiled during both the osteological inventory and the molecular analyses. Results were obtained from two types of sample material, bone and teeth, resulting in a varied success rate for each protocol. Results were obtained for mitochondrial, nuclear and microbial analyses with varying rates of success dependent upon the extraction material (rib vs. molar), the type of DNA (mitochondrial vs. nuclear), the type of analysis (sex determination, SNaPshot, mitochondrial sequencing, microbial amplification and sequencing), the cytokine SNP (IFNγ +874, TNFα -308, IL-10 -1082, IL-6 -174) and finally the size of the amplicon (ranging from 71 bp to 331 bp). Chapter seven discusses the results and concludes the thesis with an explanation of the importance of immunogenetic analysis to anthropological research. It demonstrates the unique opportunities provided, and as yet underdeveloped in archaeological populations, for interpretation of interactions between historical populations, hosts and pathogens and environments and individuals. This unique approach

to anthropological population analysis has the potential to provide insight into health differentials in contemporary populations through the analysis of historic counterparts.

# Chapter 2 Pathological Conditions and Health Status in the New World: Pre-contact to Contemporary Populations

The human condition of health evolves in response to a wide variety of interconnected influences. Understanding the evolution of a population's "health" requires understanding a number of basic tenets. Primarily, populations grow and decline naturally through time, due to the impact of numerous interactions of biological and socio-cultural factors. The following factors are related to population fluctuation: population growth impacts mortality and disease, as well as the movement of people; travel, trade and contact with people to diversify economy and subsistence leads to the influx of novel diseases, sometimes with devastating effects on the population (Crosby 1972; Dobyns 1983; Roberts and Manchester 2007). The local environment of a population, including the exact location and climate, has a profound effect on the host and consequently the pathogen (Brimblecombe 1982; Patz et al. 1996); migrating populations encounter new environments which may winnow the existing pathogen load[2] while introducing new dangers. Changes in diet and economy, particularly subsistence practice changes such as the advent of agriculture or urbanisation and industrialisation have wide reaching impacts: an increase in sedentism, a closer association with domesticated animals and denser population clusters. Concomitantly, the living environment, such as housing and sanitation, tend to decline during the early stages of transition, which can lead to an easier dispersal of certain diseases. Cultural factors such as occupation and the method in which pathological conditions are treated also impact a population's health and are in continuous flux through

[2] Pathogen load refers to the amount of microorganisms within a given environment or host. This can be the amount of different microorganisms present or a measure of microbial growth (Murphy et al. 2008; Schneider and Ayres 2008)

time (Roberts and Manchester 2007). In the Americas, the advent of agriculture and associated changes in mobility, subsistence, economy and diet, led to a consistent decline in the health condition of many Aboriginal populations indicated by a number of pathological conditions (Cohen and Armelagos 1984; Larsen 1995).

Understanding how ascendant populations existed in a pre-contact environment can lead to a greater understanding of the impact of colonialism on Aboriginal populations and subsequent changes to their health and environment. Populations and environments develop synergistic relationships that can exist within a fragile balance; the introduction of new factors such as colonists and novel infections can result in a period of stress and imbalance before a new equilibrium is established. Examples of morbidity and mortality imbalances created by colonists have been explored in the idea of "virgin soil epidemics". Virgin soil populations are groups thought to be nearly immunologically defenceless to novel pathogens introduced from an outside source (Crosby1976; Dobyns1966; Ashburn 1947). For New World Aboriginal populations this defencelessness was thought to come from a lack of previous exposure to Old World diseases such as smallpox, measles and yellow fever (Crosby 1976). Aboriginal Comparing health differentials such as changing pathogen loads and comparing morbidity and mortality rates across time and territory can demonstrate changing influences on a population, and result in a new interpretation of current health differentials.

The evidentiary resources available to anthropologists researching pre-contact New World populations are limited. Of those available, the archaeological record is the main source of information concerning pathological conditions in Aboriginal populations. Skeletal remains collected from this time period manifest pathological alterations

indicating disease as well as other abnormal health conditions. Skeletal remains are the only direct resource available to researchers for a population without a written record. Sources such as a written records offer indirect evidence of the impact of a particular disease, and in some cases may be too general to be attributed to any specific condition. Much of the record of health in pre-contact populations is collected from archaeological remains which manifest pathological alterations associated with disease.

Other evidentiary resources exist, both indirect and those commonly used as analogues. Researchers can deduce that diseases present in the natural environment (e,g, the soil or zoological specimens) would also have an impact on the human populations existing iwthin it. Indirect sources such as certain socio-cultural and magico-medical practices within cultures may also demarcate the populations' encounter with different diseases and abnormal conditions. Morbidity and mortality rates at the time of contact act as evidence for the potential interaction of naive populations with novel pathogens. The seemingly explicit vulnerability and higher mortality rates of Aboriginal populations to common diseases that many conquistadores, explorers and subsequent waves of settlers and colonisers of European origin generally survive, has also suggested a difference in pathogen load between the Old and New Worlds. Whether this is a difference in endemic and epidemic disease presence or a complete absence of the pathogen in either "World", is dependent on the geographical territory and the pathogen itself.

## Health in the New World prior to contact

Lacking archaeological remains from the Arctic, historical accounts of the reactions of native peoples to novel diseases carried or experienced by European colonisers were

one of the key indicators of pre-contact health. This is a highly subjective form of in-direct evidence, relying on the ability of laypeople to accurately and objectively describe a pathological condition. Early accounts, described below, of New World native populations expressed the belief that these groups did not experience disease or ill health of any kind prior to contact. This assumption created a division between the alleged health status of pre and post contact Aboriginals.

The supposition of a pristine disease free New World environment with a healthy, sturdy native population was based on observations of the high rates of mortality and morbidity among the natives when they encountered infectious diseases that were common among the early sailors and explorers. As mentioned above, these early contact Aboriginal populations were often described as virgin soil populations decimated by successive waves of infectious diseases carried by colonizers (Ashburn 1947, Crosby 1976, Dobyns 1983).

Accounts of not only the high morbidity and mortality experienced by First Nations people upon exposure to Old World diseases, but also conquistadores' and explorers' initial observations of the condition of the natives fed the belief for many years. Early accounts by New England settlers described,

> the Indians ...[as] lusty and healthful [in] bodies, not experimentally knowing the Catalogue of those health-wasting diseases which are incident to other Countries, as feavers, Pleurises, Callentures, Apoplexies, Dropsies, Gouts, Stones, Tooth-aches, Pox, Measlees, or the like, but spinne out the threed of their days to a faire length, numbering three-score, four-score, some a hundred years…(Wood, 1634:92-93).

Similarly, Oveido described Honduras as very fertile and its Aboriginal population as very healthy (Ashburn 1947), while the Jesuit priest Brebeuf described the Huron of Southern Ontario as being supremely healthy, healthier in fact than the European settlers (Thwaites 1897-1901). These accounts created and sustained the enduring myth of the "noble savage"(Ellingson 2001). It was not just the accounts of those arriving and encountering New World natives for the first time that influenced this concept of a pristine and healthy native population; the natives from the early post-Contact period often reflected back onto times they perceived as happier and healthier, before the arrival of European settlers. A native from the Yucatan wrote of his people,

> There was not sickness; they had no aching bones, they had no high fever; they had no smallpox; they had no burning chest; they had no abdominal pain; they had no consumption; they had then no headache…The foreigners made it otherwise (Crosby 2003).

A French Jesuit Recollect named Le Clercq recorded a similar sentiment expressed by the Micmac in New France (modern province of Quebec). While the bulk of the Micmac speaker's account concerned the contentedness of the Micmac compared to the French, the Micmac native also made reference to the decrease in "longevity" of the Micmac people after the arrival of the French (Martin 1978 quoting Le Clercq). A contemporary of Le Clercq, Denys, commented that the Micmac were not subject to diseases, and knew nothing of fevers before the arrival of the "whites" (Denys 1672).

The concept of a completely disease free New World environment or native population is inaccurate (Cook 1998; Denevan 1992). The examination of osteological material, coprolite evidence and native materia medica indicates that native populations

suffered from environmentally acquired infections, as well as, diseases that were part of humankind's shared ancestry. These sources have suggested that a number of infections, conditions and disorders were present prior to contact with European descent peoples including: yaws, hepatitis, encephalitis, poliomyelitis, tuberculosis (possibly not pulmonary), rheumatism, intestinal parasites, gastrointestinal disorders, non-specific respiratory infections (Martin 1978), bacillary and amoebic dysentery, viral influenza, arthritides, pneumonia, Espundia, streptococcus, staphylococcus, non-venereal syphilis, pinta, round worms, Chagas' disease, nutritional deficiencies, salmonella (Newman 1976); arbovirus groups A, B, and C, encephalitis, bartonellosis, coccidiodomycosis, American leishmaniasis, American trypanosomiasis, tunga infection (Dillehay 1991; Waguespack 2002) and many more all existed in at least one pre-contact native population. The populations that migrated across Beringia to initially populate the New World would also have brought some of diseases that Old World populations would have shared prior to migration. Possible examples are hookworms, and as already mentioned, many varieties of intestinal parasites (Cockburn 1971); parasitic yaws and head as well as pubic lice, are thought to have travelled with the humans across the land bridge as well, rather than being locally acquired (Waguespack 2002). However, there could be many other infections with no current extant record. These infections would need to survive the sparsely populated territories, with migrating human reservoirs, as well as the harsh Arctic conditions during initial settlement. The Arctic climate has been likened to a screening process for pathogens entering the New World (Ashburn 1947; Newman 1976); those that could not survive these harsher conditions naturally decreased and were eliminated. Factors that might have resulted in the eventual denouement of a number of Old World conditions include: the

harsh Arctic temperatures and prolonged winter seasons, the lack of sufficient human and zoological reservoirs, migrant mortality, and the effects of genetic bottlenecks and genetic drift.

The New World environment itself would have contained new pathogens in its soil (e.g. blastomycosis, Buikstra 1976) that populations might have been exposed to upon arrival to the New World. Arthropod reservoirs such as ticks provide vectors for diseases such as Lyme disease and Rocky Mountain spotted fever, both believed to be strictly New World infections linked to specific geographical locations (Steere 2001).

Domesticated (non-herd) animals were thought to be too few in number (both in species variety and density) to greatly impact pathogenic load in the New World (Cohen 1989). St. Hoyme (1969) has speculated that scattered groups of Aboriginals would have come into contact with populations of wild animals who could have acted as reservoirs for endemic diseases such as brucellosis and tuberculosis (TB). Arctic environments do not support an extensive variety of faunal populations but one of the few animals readily available in Arctic territories is caribou. The Peary caribou extend into the High Arctic Islands and the Woodland caribou can extend into the boreal and woodland areas of the Canadian provinces. However, it is highly improbable that significant levels of tuberculosis among human populations were caused by infection from caribou. Zoonotic transmission of tuberculosis is caused by the consumption of milk or raw flesh from a *Mycobacterium bovis* infected animal; humans typically contract this species of tuberculosis through infection of the gastrointestinal track (O'Reilly and Daborn 1995). The Inuit of many coastal Arctic communities were reliant on a marine animal diet, supplemented by caribou meat and not caribou milk, making transmission probabilities low. Given the caribou

migration patterns, extended contact with caribou populations was unlikely. While it is probable that TB was present in the environment in animal or soil reservoirs, it is unlikely that an epidemic level was experienced until after European contact.

## Inuit Health

The pre contact Inuit experienced health problems relevant and extant in all populations, illness brought upon by environmental factors and food shortages. However, each population experiences health and illness in its own unique way. The disease experience is reliant upon the populations' overall health, social, economic, genetic and environmental condition, which differs globally as well as locally. Therefore, each group is influenced by its own unique biological history. Arctic populations experienced radical shifts in social and economic factors with the influx of European peoples. Changes in nutritional patterns, health conditions and even the incursion of new genes had varying degrees of effect with many populations experiencing detrimental shifts in verall health status of multiple members (Hegele et al. 1997; Bjerregaard et al. 2004). ArcticAboriginals of the Caribbean, Central and South America, as well as North American Aboriginals not living in Arctic and circumpolar regions, experienced contact in a much more compact time frame, with more intensity, and with swifter corollaries.

In the Canadian Arctic the contact experience was relatively gradual until the latter half of the twentieth century when the process was greatly accelerated with the influx of a more continued colonising presence (Bjerregaard et al. 2004). The major impact on contemporary Inuit communities is the erosion of their life ways with an inadequate substitution of a quasi-European lifestyle ill-suited for Arctic conditions. Pressures from

governmental and religious institutions have resulted in the decline of many social practices and networks that supported Inuit communities. Mineral and petroleum exploration have affected ecosystems, resulting in the disappearance of some Arctic species, and the removal or alteration in other species from geographical locales. These social and economic factors greatly influence the reaction of populations to other health problems. Contemporary populations face not only a continued risk from high rates of infectious diseases (Koch et al. 2008), but other conditions such as cardiovascular disease and diabetes, often the result of assimilation into a more Euro-Canadian society (Jorgensen and Young 2008). Today one of the most prevalent and urgent concerns in the Arctic is the effects of pollution and environmental contaminants; lead, mercury and PCB saturation are well above acceptable levels in Inuit populations (AMAP 2009) and can lead to developmental problems and chronic conditions, and in the most severe and highly affected communities, even death.

The most persistent health problem in the Arctic remains infectious diseases. Most can be considered emerging diseases; many are more accurately classified as re-emerging. However, the circumpolar region is also vulnerable to the introduction of wholly new diseases currently restricted to warmer climates. With the rising temperatures of polar waters which were once too cold to sustain many microbial life forms, the introduction of new infectious threats emerges. These new microbes include viral infections, avian influenza in particular and bacterial agents which cause gastroenteritis (Koch et al. 2008). However, the most prevalent as well as persistent form of infectious disease threat in many Arctic communities continues to be tuberculosis. TB prevalence in Arctic communities continues to be higher compared to their southern counterparts, and less than 50 years ago

the incidence rates were higher in Greenlandic and Canadian Inuit, as well as American Eskimo than any other global population (Koch et al. 2008). Tuberculosis remains a significant health threat in the majority of Arctic communities and populations.

Close contact with animals such as sled dogs and prey animals predisposed Arctic peoples to a variety of zoonotic and parasitic infections. Cultural practices and environmental interactions exposed them to risk of traumatic injury, hypothermia, frostbite and drowning (Fortuine 1989; Keenleyside 1998), while other cultural practices, such as the eating of raw or undercooked foods also placed them at risk of parasitic infections, and ingestion of contaminated resources could lead to infections of the gastrointestinal tract (Keenleyside 1998). Periodic cycles of plenty/starvation created the potential for vitamin and iron deficiencies, and an increase in risk for the development of infectious diseases (Keenleyside 1998). Migratory and habitation practices, as well as poor sanitation provided conditions that were ideal for the spread of disease.

## Contact

The initial European encounter with the New World occurred in Canada with the arrival of Norse explorers from Norway, most likely via Iceland and Greenland in the last decade of the 10$^{th}$ and first decade of the 11$^{th}$ century (Linderoth Wallace 2000; Magnusson 2000; Stefánsson 2000). While the Norse Sagas describe the arrival of Norwegian Vikings and prolonged encounters between Norsemen and Icelandic and Greenlandic natives, there are no such sagas describing the interactions of Canadian Inuit and the Norsemen (Magnusson 2000). The brevity of the occupation and the lack of both historical

documentation outside of the Sagas or osteological record suggests no significant prolonged contact occurred between the Vinland settlers and First Nations or Inuit groups of the modern Maritime Provinces. In rare circumstances, osteological evidence of violence between Norse settlers and First Nations communities has been encountered (Quinn 1997). This skeletal evidence is supported by documentary evidence which relates the death of one of the expeditions' leaders (Quinn 1997) and suggests interaction at some level. Despite this, the early encounter with Norse explorers does not appear to have had an impact on early northern Aboriginals' ability to mount an immune response to infectious disease nor increased later generations' vulnerability to Old World disease or biological conditions. So, while this initial contact does present interesting possibilities in terms of an impact on cultural and historical knowledge (McGhee 1994), there is no skeletal evidence to support any major impact on the biology of either population. After the departure of the Norse settlers, prolonged and continuous contact in the north was not to occur for more than 600 years.

Initial disease transmission came from the periphery of the New World northern territories, from islands such as Hispaniola, and through Mexico and Florida; various trade routes and Aboriginal contact mechanisms spread the diseases from one settlement and cultural group to another with pathogens often preceding actual European settlers to native territories (Dobyns 1983). With the spread of Old World pathogens and deteriorating political conditions (war, receding territorial boundaries, competition for food), a decline of Aboriginal population commenced and lasted for nearly 400 years, largely due to the staggered occurrence of epidemics within already detrimentally affected populations (Crosby 1986; MacNeill 1977). Entire native populations were highly

susceptible to European pathogens in the first wave of exposure to disease causing pathogens (Dobyns 1983). Diseases had a further impact on the population because of mitigating factors such as alterations to socio-cultural practices. One of the main aspects supporting the virulence of host-pathogen interactions was the resultant lack of life-support and care systems. With the whole population of a village or territory equally susceptible and likely to develop the sickness, there was no one to carry water or hunt for food, or care for the ill. This lack of fundamental care during illness increased the risk of death from secondary causes such as dehydration and starvation (Dobyns 1983).

## Arctic Contact and the Impact on the Inuit Environment

While whaling and exploration ships had sailed through many Arctic territories since Norse contact, there is no evidence of significant interaction until the occupation of the Western Arctic by the Russians. Despite occasional encounters with whalers in Hudson Bay, overall contact in the Arctic was delayed compared to the remainder of the New World territories (i.e. as late as 1824 on Southampton Island) (Lyon 1825) . Arctic Aboriginal populations appear to have remained relatively isolated from many of the epidemics that appear to have decimated many southern First Nation populations. Epidemics that occurred in the continental United States could take decades or more to reach Arctic communities (Haggett 2000). An example of this slow progression is the introduction of measles to the Arctic. The initial outbreak is thought to have begun in Oregon in AD 1829 and travelled northwards arriving in Alaska sometime after AD 1848, spreading to the rest of Alaska and achieving epidemic levels in 1900 (Haggett 2000). While a previous

outbreak had occurred in the Aleutian Islands around 1875, and routes for transmission to Alaska was possible, the impact of the later epidemic in 1900 had a devastating effect on the Alaskan native populace.

The Aleuts were the first Arctic Aboriginals of the North American Arctic to have extended contact with Russians in Alaska (Fortuine 2005). Historical records indicate that the Aleutians were negatively impacted by the presence of Europeans and that numerous infectious disease outbreaks occurred post contact: in AD 1791-1792 on Unalaska Island an outbreak of "chest diseases" with high mortality (Merck 1980); on Atka island in AD 1802 "contagious fever" also with high mortality (Davydov 1977); in the eastern islands during the years between AD 1807 and AD 1808 an outbreak of dysentery with high mortality rates, and in AD 1838 and AD 1848, smallpox (Veniaminov 1984) and measles (Tikhmenev 1978; Bancroft 1959), respectively.

Tuberculosis has been found in many pre-Columbian populations and many South American and south-western American populations experienced TB outbreaks and epidemics (Roberts and Buikstra 2003) prior to contact. However there is no apparent linkage of these southern outbreaks with later historical and contemporary outbreaks in the Arctic and Canadian northern First Nation communities. One of the first suggestions of TB's presence in the Arctic was described by Veniaminov, a Russian Orthodox priest, in the latter half of the eighteenth century by the name of Veniaminov who describes a disease in Alaskan populations as *chakhotka*, roughly translated as phthisis in Russian; a modern translation might construe this as tuberculosis (Veniaminov 1984). Additionally a number of colloquial historical remedies were found within Inuit, Inupiak, Aleut, and Alutiiq peoples that deal with tuberculosis or similar infections (Fortuine 2005) suggesting some

familiarity with TB symptoms. Some scholars have argued that the existence of these remedies is suggestive of the presence of pre-contact TB. However, the first reported case of tuberculosis in Northern natives is generally attributed to two Aleuts from Akutan who were taken to Russia in AD 1770 and died of consumption en route to Siberia (Fortuine 2005). Other records from this period make frequent reference to other common ailments, such as venereal diseases, colds, consumption, itch and ulcers, but mention of TB is sporadic (Lisiansky 1814). The lack of evidence orf TB in North American Arctic pre-contact populations does not preclude the actual presence of infection; however it is suggestive of an absence of the epidemic form in pre-contact populations prior to its introduction by Russian sailors. Historical documents from the time (late 1700s), including the journals of Lakov Netsvetov, Father Veniaminov and Carl Heinrich Merck, make reference to the ill health of sailors, sometimes describing the condition in terms suggestive of tuberculosis (Bearne and Pierce 1976; Merck 1980; Nestvetov 1980). There is no evidence that the epidemic form of tuberculosis travelled to the northern portions of North America. While it is true that South America and the south-western portions of the United States suffered earlier from the epidemic form of tuberculosis (Roberts and Buikstra 2003), there is no apparent linkage of these epidemics to the current and later historical epidemics in Alaska and Northern Canada.

The supposition that infectious diseases reached different portions of Aboriginal populations in Canada at different times is supported by the historical and oral documentation of ill-health. The longest exposure of native groups to European colonists is found in the Eastern portion of Canada. While initial contact occurred in AD 1001 in Newfoundland (Linderoth Wallace 2000; Magnusson 2000; Stefánsson 2000), the actual

entry point for many of the infectious agents that affected native populations in Canada occurred in the southern portions of the country. Measles and smallpox both appear to have entered via the Huron or Iroquois nations (Dobyns 1983) and spread northwards and westwards from each of these outbreaks. There is no evidence to substantiate an episode of initial contact, at least the contraction of a pathogen, in the north with delivery from a southern source. Most of the initial entry points for pathogens into the New World are documented as occurring in South America, Latin America and the Caribbean (Dobyns 1983; Cook 1998; Crosby 2003). This suggests that epidemics spread northwards either as propagated or common vehicle epidemics (Haggett 2000) from a southern and/or eastern sea board originating incidence.

Pathogens travelled via human to human contact in a chain reaction or spread by a causative agent such as food or trade goods (Haggett 2000), and because of this, it is very possible for two disparate groups such as the Inuit and the early Norse explorers to have had biological exchanges without any human interaction. Despite this, the populations in the Canadian Arctic appear to have been spared the numerous early epidemics experienced by their southern counterparts. The logical theory is that a delayed direct contact with foreign sources in concert with restricted inter-group interactions and low population densities, resulted in numerous decades of a less hostile biological environment. While diseases such as smallpox, measles, influenza, cholera, diphtheria, typhus, bubonic and pneumonic plague, scarlet fever, and many unidentified diseases and epidemics contributed to the large-scale depopulation of North America in what many historians refer to as the conquest of the Americas by the Europeans through large scale depopulation,

there is little evidence to suggest transmission on significant scale to their Arctic counterparts (Dobyns 1983).

With these risk factors, a number of skeletal indicators of poor health can be expected to be apparent within many Arctic populations. Commonly discussed pathological modifications include vitamin and mineral deficiencies, trauma, degenerative disorders and infection. Common vitamin and mineral deficiency-related pathologies are cribra orbitalia and porotic hyperostosis. These conditions are attributed to iron deficiencies and anemia caused by low dietary intake or problems with absorption (Steinbock, 1976; El-Najjar et al., 1976; Goodman et al., 1984; Ortner and Putschar, 1985; Keenleyside 1998). Porotic hyperostosis is typically characterized by symmetrically disturbed cranial lesions involving the outer table only of the frontal and parietal bones and much less frequently the occipital. A thickening and expansion of the diploic layer and resorption of the outer table occur in fully developed lesions. In less developed cases multiple discrete pinhole sized perforations are visible in a concentrated area (Aufderheide and Rodriguez-Martin 1998).

Accidents and cultural practices lead to certain levels of trauma in all cultures (Aufderheide and Rodriguez-Martin 1998), but fractures are the most commonly recorded form reported in Arctic cultures (Alaskan) (Keenleyside 1998). A fracture is a break of any type (e.g. crack, complete transaction, discontinuity) in the skeletal material. It can occur with or without injury to overlying soft tissues, and a number of varieties could be described dependent upon the external or internal causal factors (Aufderheide and Rodriguez-Martin 1998). In archaeological materials the most common form of evidence is not the fracture itself, but the evidence of its healing. Evidence of fractures include poor

alignment, which is common in historic and prehistoric societies, bone shortening, possible signs of infection, necrosis due to interruption of the blood flow, and articular changes. During the course of healing, the development of extraneous bone in the form of a callous over the area of discontinuity is observable to the naked eye, over time and in young adults this may heal to a degree as to be unobservable (Aufderheide and Rodriguez-Martin 1998).

Alaskan populations demonstrate the presence of parasites in Arctic populations, some of which are capable of producing the iron deficiencies that cause anemia and porotic hyperstosis. Examples of these parasites include *Cryptocotyle lingua* (Zimmerman and Smith, 1975), *Trichinella spiralis* (Zimmerman and Aufderheide, 1984) and *Echinococcus granulosus* (Ortner and Putschar, 1985). Specific and non-specific infections are also common, with non-specific infections being more common in archaeological skeletal material (Goodman et al. 1984). Both Aleut and Eskimo populations have been recorded as having non-specific infections attributed to either streptococcal or staphylococcal bacteria (Keenleyside 1998). Pulmonary infections are also common and may be exemplified by the presence of rib periostitis on the visceral surface of the rib (Keenleyside 1998). Other forms of infection known to have been observed in pre-contact Arctic populations include emphysema (Zimmerman et al.., 1971), fibrosis (Zimmerman et al.., 1981; Zimmerman and Aufderheide, 1984; Zimmerman and Smith, 1975), and anthracosis from smoke inhalation (Zimmerman and Aufderheide, 1984; Zimmerman et al.., 1971, 1981; Zimmerman and Smith, 1975). Dental pathologies are also common in Arctic population, with enamel hypoplasia occurring as an indicator of systemic stress, caries, and pre-mortem tooth loss (Keenleyside 1989; Merbs 1963, 1968, 1983). Isolated examples of other conditions have

been reported such as cancer (Cassidy 1977), but malignancies in Arctic skeletal populations are rare.

## New World Tuberculosis

The evidence for tuberculosis in Arctic populations prior to contact is lacking. There is no conclusive evidence of tuberculosis in any Eskimo or Aleut samples (Keenleyside 1998), findings which are consistent with other palaeopathological studies of Arctic populations suggesting that TB in the Arctic was introduced by European populations rather than developing in situ from either pre-contact endemic infections or animal reservoirs (De Laguna, 1956; Hrdlicˇka, 1931; Oswalt, 1967; Lantis, 1984; Keenleyside 1998).

Despite minor successes in the treatment of TB, modern estimations suggest that approximately two billion people worldwide (almost one third the world's population) are infected with tubercle bacilli (WHO 2006; Kochi 1991) and more than 5000 deaths a day are currently attributed to tuberculosis (Roberts and Buikstra 2003). The World Health Organization (WHO 2000) estimated that new cases of tuberculosis would exceed 8.08 million per year in the coming years. In a global context, those affected by TB infection come from myriad environments and socio-cultural backgrounds. Only about 10% of those infected will progress to active disease (Donoghue 2008); those most likely to develop active TB are those with weakened immune systems, the old and very young, those inhabiting substandard housing, living with poverty and malnourishment, those co-infected with other diseases especially HIV, and those suffering from high levels of mental and physical stress (Donoghue 2008; Roberts and Buikstra 2003; Kochi 1991).

Tuberculosis is currently regarded as having had a long history in both the Old World and New World, with a definite pre-Columbian presence in North America, specifically in the Northeast (Starna 1992; Thornton 1987; El-Najjar 1981; Pfeiffer 1984). To date no osteological evidence of a definite case of pre-contact TB has been found in an Arctic archaeological context (Keenleyside 1998). Outside of major finds in South America and the United States, there are other isolated examples of osteological evidence suggesting the presence of TB in Canada prior to contact. The Bennett site in southern Ontario and the Woodlawn site in Manitoba have each yielded one individual with TB (Roberts and Buikstra 2003) that can be dated to a pre-contact population. The Fairty Ossuary and Uxbridge Ossuary (Pfeiffer 1984) have also yielded data suggestive of low level tuberculosis; each site yielded two or more pre-contact individuals that manifest tuberculous lesions (Roberts and Buikstra 2003). These sites represent the few examples that exist outside of the mid-continental and south-western United States clusters that provide the definitive evidence of pre-contact tuberculosis. These clusters include two large urban centres that had dense populations during the 1300's (Roberts and Buikstra 2003), which may account not only for the presence of individuals with skeletal TB, but perhaps an increased presence of the mycobacterium.

It has been suggested that TB was absent from the Arctic prior to the arrival of the Russians in the mid-eighteenth century (Fortuine 2005). Early medical historians and anthropologists support this hypothesis by pointing out the paucity of pre-Contact individuals with unequivocally diagnosed with from skeletal lesions (Hrdlicka 1909, Morse 1961, Cockburn 1963). They also emphasised the lack of anecdotal evidence of the disease from early explorers, colonists, and the Aboriginal populations in Alaska and Canada

themselves. Other evidence suggesting the lack of TB in the Arctic pre-contact period is the extreme susceptibility of Native American groups to the disease in a historical context, and in contemporary populations (Wilbur and Buikstra 2006).

Osteological evidence has proven to be problematic as a method for proving the presence of TB in the Arctic. Many cases originally thought to represent pre-contact TB in Arctic human skeletal remains were later attributed to fungi that exhibited similar healed granulomas (Zimmerman and Smith 1975), resulting in a reinforcement of the hypothesis that TB was non-existent in Alaska and surrounding areas prior to its "introduction" by the Russians.

Contemporary research is now testing this hypothesis. Many scholars now agree that although tuberculosis was present in the New World prior to contact, it was rare in the northern reaches of Canada and Alaska (De Laguna, 1956; Hrdlicka, 1931; Oswalt, 1967; Lantis, 1984; Keenleyside 1998). The lack of early population density and urban centres in northern Canadian territories perhaps explains the lack of skeletal evidence for TB in the north. Tuberculosis was therefore most likely endemic to the New World prior to contact, but was less of a historical threat to First Nations populations especially in circumpolar and Arctic Aboriginal communities (Thornton 1987). Fortuine (2005) suggests that endemic TB came over on one of the waves of migration across the Bering land bridge approximately 12 000 years ago. He speculates that the disease remained endemic and sporadic in its presentation for thousands of years and was most likely a low virulence form of the pathogen (Fortuine 2005). The Arctic population's long experience with this endemic form allowed them to spontaneously heal their lesions through fibrosis and calcification (Fortuine 2005). This ability would have lowered the mortality rate of the

infection, possibly the morbidity rate, allowing the pathogen to exist for thousands of years within a sparsely populated environment. In 1999 the discovery of an ancient male individual eroding out of a glacier near the Yukon-Alaska border of British Columbia resulted in positive amplification of TB pathogenic DNA from preserved lung tissue (Swanson 2008). This individual, *Kwäday Dän Ts'ìnchi*, has been dated to two different time periods, resulting in an unclear depiction of the date of his death. Initial radiocarbon dating of his clothing indicated an age of 500± 30 years before present (Beattie et al. 2000). More recently, radiocarbon dating of his tissue resulted in dates between AD 1670 and AD 1850 cal. Both dates represent periods possibly pre-dating European contact in the region (Richards et al. 2007) but without greater specificity or agreement the date of death cannot be established as either pre and post contact. Therefore data for pre-contact TB in the Arctic is problematic. It is possible that, rather than being a pre-contact individual as originally suggested, he was from the early contact period. Molecular testing showing positive amplification of TB DNA may be the result of infection from an introduced source to the region, rather than from a local endemic infection. Prior to the discovery of *Kwäday Dän Ts'ìnchi,* there was a lack of evidence, both skeletal and historical, to support the presence of TB in the Arctic. With further analysis of the remains, and a more definitive dating, this may be the first example of an infectious pre-contact individual in the Arctic. The issue of pre-contact TB in the Arctic remains debated, and further evidence, both osteological and molecular, is needed to elucidate the circumstances of its introduction and virulence in isolated Arctic populations.

# Chapter 3 Human Immunogenetic Diversity Present and Past

The only methods of observing the immune system in archaeological populations is through analysis of pathological conditions manifested in the skeleton in conjunction with molecular analysis. Establishing the presence of pathologies within a given population can provide insight into morbidity and mortality. However, the presence or absence of skeletal lesions does not measure the effectiveness of human immune response. Immune function and variability in anthropological studies is observed through the analysis of three forms of ancient DNA (aDNA): microbial, nuclear and mitochondrial. This chapter introduces how the human immune system functions in general, the function of cytokines and the overall response to tuberculosis (TB). The second half of the chapter introduces the ways that molecular anthropology can be utilised to study archaeological populations. In particular the chapter discusses how aDNA is utilised in this research to establish the basic tenets of this thesis: establish Inuit ancestry, detect cytokine genotypes, and confirm or refute the presence of TB. These analyses work in conjunction to determine the reason for current discrepancies between TB rates of infection in Inuit, First Nations and non-Aboriginal Canadian populations.

## The Human Immune System

The human body is varied in its actions and responses, by individual and by population. Much of the variation depends upon the history of the population, pathogens and environments encountered and experienced throughout the evolution of its inhabitants. The responses afforded by the human immune system against infection of any form are part of immune response. A major aspect of immune diversity is the Human

Leukocyte Antigen (HLA) complex of genes in the major histocompatibility complex (MHC). HLA genes are important in the development of activated t-cells and their associated response to foreign microbes. Diversity comes from the over 200 highly mutable possible alleles (Eren and Travers 2000) in the HLA complex which are categorized into two classes I (-A, -B, -C) and II (HLA-DRB1, -DQA1, -DQB1, -DPA1, -DPB1) (Solberg et al. 2008). HLA class I and class II proteins are highly polymorphic and are essential in distinguishing self and non-self during immune response (de Bakker et al. 2006).

Hundreds of HLA alleles have been documented in human populations globally, many with high relative frequencies.. This diversity makes the collective HLA loci the most polymorphic in the human genome (Eren and Travers 2000). The combination of alleles on a chromosome is referred to as a haplotype (Murphy et al. 2008); a number of HLA haplotypes have developed amongst various populations. HLA diversity can often be utilised as a tool for assessing the relationship between populations. Anthropologists have used the distribution of HLA haplotypes to investigate human genetic relationships, reconstruct human migration patterns (Bodmer et al. 1997; Clayton et al. 1997), and assess genetic admixture and/or isolation in New World populations (Blanco-Gelaz et al. 2001). The role of the HLA complex is to provide individuals with protection against pathogens that invade the body and there is a direct link between a population's disease history and evolution and its HLA composition (Hill 1998). The HLA complex is responsive to myriad pathogens and their changeable nature provides immunological protection through selective pressures, often related to human biological and cultural behaviours (Blanco-Gelaz et al. 2001). HLA haplotypes, of which there are many, are partially responsible for a

population's ability to resist and combat different pathogens (Hill 1998). The frequency of these alleles can be highly different between individuals and populations.

At a cellular level the human immune response is designed to differentiate between self and non-self (Coico and Sunshine 2009). However, there are four main tasks required to fulfill the proper functioning of the immune system as a whole. The first is the ability for the immune system to recognize foreign intrusion into the body, referred to as immunological recognition; this initial function is provided by the innate system which provides an immediate response through the white blood cells and later by the lymphocytes of the adaptive system. Secondly, the "intruders" must be quarantined or eradicated from the system; this demands the response of immune effector functions, which often work in concert with each other and may consist of a host of different cell types: the complement system of blood proteins, antibodies, and the destructive capabilities of lymphocytes and other white blood cells. The immune system must be controlled in order to prevent destruction of healthy cells, which can occur in individuals suffering from autoimmune diseases such as rheumatoid arthritis and lupus. This third property is referred to as immune regulation, or self-regulation; the lack of this ability to control leads to the aforementioned auto-immune diseases as well as allergies and other detrimental conditions. The final task of the immune system is one of the primary functions of the adaptive portion of the immune system, that of immunological memory. This allows an individual to elicit an immediate and progressively stronger response with each successive exposure to a pathogen, what is known as protective immunity. However, there are many pathogens to which the immune system never develops a long-lasting

immunity; this is the major concern of many health researchers who study complex diseases (Murphy et al. 2008).

### Innate Immune System

The innate immune system is the dominant immune system in plants, fungi, and insects and in primitive multi-cellular organisms. It is thought to represent an evolutionarily older defence mechanism than the remainder of immune responses in modern populations (Janeway et al. 2002). It is found in all orders of animals and plants as the immediate or general defence mechanism that responds instantaneously to changes in the host with a multitude of responses, but lacks specificity. Unlike the adaptive immune system, the innate system does not confer lifelong immunity or the ability to respond accordingly with each successive infection (Alberts et al. 2002), however, it is the mechanism that provides the initial discrimination between the self and the other.

The defences of the human immune system range from physical barriers such as our skin and mucosal membranes to highly sophisticated response systems. Physical and chemical barriers are the frontline defences against foreign body intrusion. Most foreign organisms cannot penetrate skin unless it is damaged; some microorganisms enter through sebaceous glands and hair follicles but are initially combated by the presence of sweat, sebaceous secretions, fatty acids and hydrolytic enzymes (Coico and Sunshine 2009). The more sophisticated systems are utilised once the chemical and physical barriers have been penetrated and include a number of intracellular and extracellular mechanisms. The myeloid lineage comprises most cells in the innate immune system, including macrophages, granulocytes, mast cells, neutrophils, eosinophils, basophils, and dendritic cells. Some of

these cells coordinate immune responses, induce inflammation, secrete signalling proteins for other cell activation and recruitment, use anti-microbial agents to destroy microorganisms, defend against parasites, and scavenge within the body and eradicate other dead cells. The lymphoid lineage matures in the bone marrow or thymus and then collects in lymphoid tissues throughout the body, and are found in large numbers in lymphocyte congregates known as lymphoid tissues and organs (Murphy et al. 2008). Mucous membranes and the skin are the first defence against invasion; cells that prevail and break through these barriers are met by cells of the myeloid and lymphoid lineages.

The immune system is regulated by soluble mediators known as cytokines (Coico and Sunshine 2009). "Cytokine" is the general term for any protein that is secreted by immune cells in both the innate and adaptive systems (Coico and Sunshine 2009) and affects the behaviour of other cells with complimentary receptors. A subgroup of cytokines known as chemokines are secreted proteins that attract cells with complimentary receptors from the body to the infected or foreign cells. It is through the release of cytokines and chemokines that the primary response of the innate immune system, inflammation, is triggered. Most infectious agents activate the innate immune system initially and induce inflammatory response, which is usually generated in the early stages of immune defence. It is stimulated by cytokines released by affected cells and results in localised heat, swelling, and redness, all which serves to establish a physical barrier against the spread of infection, and promote healing in affected tissue once the injury has been resolved (Stvrtinova et al. 1998). Inflammation also results in additional beneficial responses, particularly the attraction of neutrophils which alert the immune system to injury or infection and summon other immune cells such as leukocytes and lymphocytes.

Macrophages (a specific type of leukocyte), when stimulated by bacteria, produce cytokines; these include tumour necrosis factor (TNFα), and interleukin 1 (IL-1), interleukin 6 (IL-6), Interferon gamma (IFNγ), and IFN-beta (IFNβ) (Lotze and Tracey 2005). IL-6, IL-1, and INFα initiate a wide spectrum of biologic activities that help coordinate the host's responses to infection by microbial and viral pathogens. Most commonly they induce fever, which is why they are sometimes referred to as endogenous pyrogens (Roth and DeSouza 2001; Kluger 1991; Jansky et al. 1995; Roth et al. 1993; Lemay et al. 1990; Coico and Sunshine 2009).

### Adaptive Immune System

The adaptive immune response is characterized by its ability to recognize a vast assortment of antigens (Medzhitov and Janeway 1998). It has a number of mechanisms to combat infection; the main method is by specialized white blood cells known as lymphocytes (B cells and T cells) which can recognize and target pathogenic micro-organisms or infected cells (Murphy et al. 2008). However the ability and function of the adaptive immune system is predicated by the presence of the innate system. Adaptive immune responses occur when the innate system is unable to clear infection.

If the infection persists for an extended period of time then the adaptive immune response takes over. Once a pathogen has been introduced to specialized B-cells and T-cells these cells are "educated" to remember the pathogen as a threat in the event of future infections. This is called immunological memory, a phenomenon which bestows lifelong protective immunity to the individual. An example of this type of protective immunity is

the resistance to re-infection by chicken pox after the initial infection. This response and adaptation is particular to the individual and to each distinctive pathogen.

## Th1 vs. Th2

The type of T helper cell response to pathogens is a major factor in the production of key cytokines. A Th1 or Th2 response can affect the host's individual ability to eradicate an infectious agent (Romagnani 1996; Hurtado et al. 2003; Larcombe et al. 2005; Wilbur and Buikstra 2006). Th1 and Th2 responses are also linked to the system's response to external and internal stimuli. Normative secretion of a cytokine is increased in response to intrusion by a particular foreign element. Suppression, down regulation or an increase in production is continually affected by the secretion and production of a number of other immune cytokines promoted by the two branches of the T helper response (Romagnani 1996, 1999; Flynn and Chan 2001; North and Jung 2004) and individual genotypic profiles will affect these mechanisms of Th1 and Th2 responses.

T helper lymphocytes or T cells can be divided into two unique subsets of immune cells based on their function and associated profile of cytokines (Romagnani 1996, 1999; Constant and Bottomly 1997; Flynn and Chan 2001; North and Jung 2004). The two subsets are referred to as either Th1 or Th2 immune responses; both develop from naive T cells and are activated dependent upon what type of microbial antigens have been introduced to the host (North and Jung 2004). A Th1 immune response and associated cytokines is activated and produced in response to intracellular bacteria and some viruses (Romagnani 1999), such as tuberculosis or influenza, while Th2 immune responses and

associated cytokines are stimulated by complex parasites such as gastrointestinal nematodes (Romagnani 1999).

Th2 helper cells promote the production of cytokine responsible for strong antibody production, eosinophil activation and inhibition of several macrophage functions, thus providing phagocyte-independent protective response. Cytokines such as IL-4, IL-5, IL-6, IL-10 and IL-13 are associated with the Th2 or humoral form of immune response (Romagnani 1999). Th1 type helper cells produce a specific contingent of cytokines typified by the production of interleukin (IL) 2, interferon gamma (IFNγ) (Surcel et al. 1994) and tumour necrosis factor beta (TNFβ) (Romagnani 1999). These cytokines are generally considered pro-inflammatory cytokines and are involved in cell-mediated immunity. However, a number of functional reactions are instigated by a Th1 immune response including: the production of opsonizing[3] and complement-fixing antibodies, macrophage activation, anti-body-dependent cell cytoxicity and delayed type hypersensitivity (DTH) (Mosmann and Coffman 1989; Romagnani 1994, 1996, 1999). Key to an immune response to tuberculosis infection is the ability to stimulate phagocytosis[4] and destruction of microbial pathogens. The polarised nature of Th1 and Th2 cells creates a greater significance on the phenotype of the host's immunogenetic profile. The host's differential production of a wide variety of cytokines is often dependent upon the SNPs that affect the gene and function responsible for that cytokine concomitantly to alleles for all

[3] Opsonizing or opsonization is the alteration of the surface of a pathogen so that it can be ingested by phagocytes. Antibody and complement opsonize extracellular bacteria for destruction by neutrophils and macrophages (Murphy et al. 2008)

[4] Phagocytosis is the internalization of particulate matter by cells. In the case of tuberculosis the particulate matter is the infectious bacilli and the cells can be macrophages. The ingested material is contained in a vesicule called a phagosome. In most cases the bacilli remain encased inside the phagosome and resist eradication (Murphy et al. 2008)

other cytokines. SNP's in the promoter region of a cytokine create a cytokine genotype. The association of SNPs in a number of cytokine promoter regions associated with disease prognosis can create the overall ability for a host to actively and effectively combat foreign intrusion.

## Cytokines

Both innate and adaptive immune responses depend on the production of leukocytes, which are an unrestricted immune cell that combats abnormalities in the body (Murphy et al. 2008). However there is a vast range of cells that are produced by the immune organs, as well as by cells which identify a pathogen and signal for the presence of a specific combative cell, some of the cells that combat and signal are known as chemokines and cytokines. Cytokines are small proteins, usually around 25 kiladaltons that are released by various cells in the body, usually in response to stimulus from other cells within the immune system (Murphy et al. 2008). There are two major lineages or families of cytokines, the haematopoietic and the TNF family. The haematopoietic family includes growth hormones and interleukins with roles in both the innate and adaptive immune systems. The TNF family also functions in both immune response systems. The importance of identifying cytokine polymorphisms in association with certain diseases, like tuberculosis, can lead to a better understanding of disease pathology; identification of potential markers of susceptibility, severity and clinical outcome; identification of potential markers for responders vs. non-responders in clinical and therapeutic trials; identification of novel strains, either extinct or extant, and strategies to prevent diseases or improve existing preventative measures, such as vaccines (Bidwell et al. 1999). Understanding the

way a population combats disease at the immunological level can reveal important factors about the evolution of the human immune system anddifferences between individual and population expression of cytokines.

Cytokine polymorphisms are common immune modifiers (Wagner et al. 1999); however they are rarely linked to disease causation, except in a limited number of isolated cases. Instead cytokine polymorphisms act as disease modifiers by affecting disease severity, the ability to effectively combat the disease and mount an immune response, and in some cases, increase or reduce susceptibility to infection (Curfs et al. 1997; Wagner et al. 1999). Cytokine SNPs most commonly affect disease outcome in inflammatory, allergic, autoimmune or immunodeficiency diseases. Cytokine SNPs have also been linked to transplant outcome, individuals with certain polymorphisms may be more likely to reject or accept an organ based on their cytokine genotype profile (Turner et al. 1997; Asderakis et al. 2001). Chemokine and cytokine SNPs all demonstrate variability between populations (Borman et al. 2004; Rovin et al. 1999; Hoffman et al. 2002; Kaur et al. 2007).

When the innate system fails to eradicate TB infection, the adaptive system is triggered by a number of immune cells. The Th1 immune response is favoured over cytokines and immune cells produced by Th2 immune response.

## Immunity and Tuberculosis

The protective and immunological response to tuberculosis is a complex and layered system, involving many different aspects of the human immune system (Hoal 2002), which once activated is generally successful in containing the active pathogen, but not eradicating the infection itself (Flynn and Chan 2001). Tuberculosis is caused by

multiple strains of mycobacteria: *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium africanum, Mycobacterium microti, and Mycobacterium canetti*. There are also strains of mycobacterium that cause other diseases, such as *Mycobacterium leprae* and *Mycobacterium avium* neither of which result in the pathogenesis of tuberculosis. Tuberculosis is caused by the pathogen *Mycobacterium tuberculosis*, an aerobic intracellular pathogen that can survive and multiply inside macrophages and other human cells. It has evolved to avoid destruction by innate and adaptive mechanisms of immunity in certain immune competent humans. Initial infection occurs via the respiratory system (Dannenburg 1994) and a small percentage develops active infection (North and Jung 2004). Approximately 5% of immunocompetent adults will develop active disease within 2 years, and a further 5% will develop active disease ("reactivation disease") at some time later within their lifetime. Ninety percent of immunocompetent adults who develop latent TB infection will never develop disease during their life. Resistance to tuberculosis depends critically on the ability to produce an adequate Th1 immune response (North and Jung 2004) through the production of antigen-specific T-cell mediated activation of macrophages[5] (Surcel et al. 1994). Macrophages, acting as antimycobacterial effector cells, mediate killing or inhibition of bacterial pathogens. Individuals with an immune response that is unable to resist infection or bind the tubercle bacilli develop primary TB. The mycobacteria multiply and grow resulting in further immune activation. A second form of infection results from the successful containment of bacilli resulting in a latent stage, whereby the infection persists within granuloma in the organs of infected hosts (Flynn and Chan 2001). This form is known as reactivation TB (Schluger and Rom 1998).

[5] Macrophages are the major effectors of cell-mediated killing of intracellular pathogenic mycobacteria (Surcel et al. 1994)

In reactivation TB, activation of the disease might occur later in life, when the immune system has a weakened ability to contain and isolate the bacilli or if the immune system is compromised by another disease such as HIV/AIDs (Hoal 2002).

The innate immune response at the time of infection is crucial for the initial isolation, identification and containment of the bacilli. This response determines whether the bacilli are eradicated, or take residence within their macrophage niche environment resulting in active infection (Hoal 2002). If the innate system is unable to eradicate the infection upon exposure, the adaptive system is triggered by the growth and multiplication of the tubercle bacilli. This in turn results in a Th1 immune response through the production of antigen specific T cells, primarily the cytokines IFNγ (Lurie 1942; Suter 1952; Mackaness 1969; Flynn and Chan 2001) and TNFα (Flynn et al. 1995), which work synergistically to produce macrophage activation (Schluger and Rom 1998). Macrophage activation results in the inhibition of bacilli growth through phagocytosis. This process results in the internalization of the bacterium and growth inhibition or bacilli eradication (Schluger and Rom 1998). There is evidence that MTB can be killed by several immune system mechanisms and a variety of complicated interactions, mediated by a number of Th1 cytokines. The Th1 induced immune response (CD4+ T-lymphocytes) and natural killer (NK) cells secrete IFNγ, activating macrophages which in turn produce cytokines such as IL-12 which reinforces this pathway. Murine models of tuberculosis infection suggest that the granulomas induced by the production of TNFα restrict the growth of the bacilli and slow progression of the infection (Flynn et al. 1995). Murine models also demonstrate that Th1 immunity is responsible for control of Mtb growth (North and Jung

2004) and that inhibition of IL-12 results in the incapability to express anti-Mtb immunity through the generation of Th1 immune responses (North and Jung 2004).

There are many different Mycobacteria strains. Strain differences have been noted in terms of transmissibility, virulence, and invoked immune response in terms of macrophage growth (Hoal-van Helden et al. 2001a) and cytokine release (Hoal-van Helden et al. 2001b).

## Population-based immunological variation

All humans are capable of Th1 and Th2 immune responses. This allows individuals to respond to a wide variety of zoonotic, bacterial, parasitic and fungal infections. Variation in these responses is linked to adaptation to environment and is highly linked to the population's evolutionary history. A major factor in the ability of a population to mount an effective immune response is its historical interaction with a pathogen. The adaptive immune response available generally depends on the pathogen load, the virulency of each pathogen and the duration of exposure. . For example, "crowd diseases", those that developed with the urbanization and increased population density of cities (Diamond 2002), have existed for a longer period of time in the Old World, where domestication and city states first arose (Lipsitch and Sousa 2002; MacNeill 1998). Here the population went through selective pressure in favour of a stronger Th1 immune response, and an exchange of genetic information through admixture. These populations have most likely encountered a wide range of diseases and conditions over extended periods. Crowd diseases such as TB, measles and influenza require efficient Th1 immune responses.

Individuals who were highly susceptible and lacked an effective Th1 immune response would not have survived epidemics caused by bacterial and viral pathogens..

Aboriginal populations in the New World did not experience the same selective pressures for bacterial and viral pathogens (crowd diseases) compared to Old World populations (Hurtado et al. 2003; Wilbur and Buikstra 2006). New World Aboriginal populations evolved effective strategies for combating parasitic and fungal infection which predominated their environment. We may hypothesize that Aboriginal populations evolved efficient Th2 responses well suited to fighting parasitic and fungal infections (Hurtado et al. 2003), but possibly less efficient in response to viral and bacterial infections. Researchers have concluded that populations who share similar ethnicity (biological and cultural history) exhibit shared variable responses and rates of infection (Larcombe et al. 2005; Ducati et al. 2006). This condition would not be independent of socio-economic and socio-cultural factors, but exacerbated by the detrimental aspects of them. Similarly, the environment, including host-pathogen interaction and the pathogen load of past populations, cannot solely explain a current population's immunological response (Rempel et al. 2011). However, understanding the various forms of immune diversity present within a population can shed light onto the population's susceptibility or immunity to various pathological conditions (Rempel et al. 2011). Research comparing the genetic structure of the host population (Ewald 1994, for example) suggests that hosts within an ethnic population may maintain a wide variety of genetic characteristics affecting susceptibility and resistance to disease. A large population with appreciable genetic variety maintains the ability to better combat infection by a pathogen. The main reason for this is that a pathogen may encounter a number of individuals with an immune profile able

to effectively combat infection before encountering a viable host (Wilbur and Buikstra 2006).

Many pathogens therefore never become highly virulent in these populations but are forced into fairly benign forms so that they do not decimate the population and self-eradicate (Wilbur and Buikstra 2006). In a population lacking genetic variability in immune response, pathogens may not evolve to a less virulent form, but instead spread quickly, and may result in high morbidity and mortality within the population. Evidence indicates that Native American populations are more genetically homogenous at many loci including those that affect the immune system and response for TB (Wilbur 2005). The immunogenetic variability of the Aboriginal populations, both Arctic and southern, was likely reduced by genetic bottleneck events, war, poverty, starvation, and previous epidemic infection, possibly leading to a more vulnerable population (Wilbur and Buikstra 2006).

Wilbur and Buikstra (2006) argue that a number of factors can contribute to the effect of an infection within a population; social and cultural disruption such as the increase in population densities and widespread relocation of native groups would have altered susceptibility of native populations (Wilbur and Buikstra 2006). They suggest that rather than assuming co-evolution of a host and pathogen would result in acquired immunity, that some populations may never gain an adequate immune response to certain pathogens. There is adequate evidence to support this supposition in Native South American groups; in certain populations individuals who have active TB or have been treated for TB are unable to mount and effective a Th1 response, and thus react as if they were immunologically "naïve" to the pathogen (Wilbur 2005; Wilbur and Buikstra 2006).

In North American Aboriginal groups such as the Inuit, Eskimo, Tlingit, Tsimshiam and Na-Dené unique HLA haplotypes have been identified (Pablo et al. 2000). Amerindian populations also exhibit significantly reduced allelic and haplotypic HLA diversity when compared to populations from Africa, Europe and Asia. Amerindian populations are often clustered together and show less "genetic distance" between each other compared to all other populations (Tsuneto et al. 2003). When compared to Old World populations Amerindian groups often cluster together and analyses sometimes reveal interesting linkages between populations (Pablo et al. 2000; Tsuneto et al. 2003).

Hoffmann and colleagues (2002) found a striking difference in allelic frequencies between American black and whites for IL-2 and IL-6. They also observed that Asian descent populations compared to Caucasians (referred to in their paper as "whites") have a considerably higher proportion of genotypes that result in high IL-6, and low IL-10 and IFNγ production. A different study saw a higher frequency of the down-regulating IL-10 (-819) T/T genotype and IL-10 (-1082) A/A genotype in Black American women compared to those described as "white" American women. The genotypes were 3.5 times and 2.8 times more common in black women (Ness et al. 2004). The study observed that African-American women were more likely than Caucasian women to have allelic variants known to increase the production of the pro-inflammatory cytokines and less likely to have allelic variants known to increase production of the immune-system-suppressant cytokine IL-10 (Ness et al. 2004). Hispanic descent groups differed from Caucasians in the inheritance of the high IL-6 producing allele and a reduction in the inheritance of the high IL-10 polymorphic alleles (Hoffmann et al. 2002).

In reference to tuberculosis, while the polymorphism in the promoter region of TNFα (-308) is associated with TB susceptibility in India (Selvaraj et al. 2001) there appears to be no significant association between the same polymorphism and TB in Cambodia (Delgado et al. 2002). IL-10 SNPs were not associated with disease susceptibility in Gambian populations (Bellamy et al. 1998), however, a significant correlation was observed between the A/G genotype and TB disease in Cambodian populations (Degaldo et al. 2002). In China there is a high association between the A/A genotype of IFNγ (+874) and TB infection (TSo et al. 2005). This genotype results in a low producer phenotype, which may impair activation of macrophages and result in an increased risk for the development of TB in individuals exposed to the bacilli. In this particular population the risk is calculated at 3.79 times higher than if the individuals had an intermediate or high production phenotype. The A/A genotype is related to TB severity and/or reactivation of the infection (Tso et al. 2005). Similarly in Sicilian populations the T/T genotype is associated with protective factors in TB development. Sicilian populations show a decreased frequency of the A/A genotype and an increased frequency of the T/T genotype in comparison to the Chinese population, which shows a significant difference in frequencies (Tso et al. 2005).

In a recent study it was found that a Caucasian cohort maintained a high frequency of cytokines associated with a strong Th1 immune response. In contrast a First Nations cohort as well as Filipino descent populations favoured a Th2 immune response (Larcombe et al. 2005; Larcombe et al. 2008) based on the high frequency of Th2 cytokine promoter SNPs. The Th1 is characterised by the release of IFNγ and TNFα which has been proven to provide protection against TB infection (Kamijo et al. 1995) while the Th2 immune

response is characterised by the production of cytokines associated with parasitic and fungal infections, such as IL-4, IL-6 and IL-13 (Marquet et al. 1999). Caucasians maintain a higher frequency of TNFα (-308) and IFNγ (+874) allele SNPs, whose phenotypic expression enhanced cytokine production ( Wilson et al. 1997; Kroeger et al. 1997; Allen 1999; Larcombe et al. 2008). Aboriginal and Filipino descent populations examined within this study (Larcombe et al. 2008) generally maintained a higher frequency of IL-6 (-174) SNPs associated with high production. First Nations' examined also appear to maintain lower production phenotypes for IL-10 (-1082) compared to Caucasian populations. The Dené have allele frequencies associated with low production of TNFα, IFNγ, and IL-10, and high production of IL-6; the Cree also demonstrate allele frequencies associated with low producers of TNFα, but they may also exhibit alleles associated with low to intermediate production of IFNγ and IL-10 (Larcombe et al. 2008). The osteological record has shown that parasitic and fungal infections, as well as malnutrition, were common in the pre-Contact New World (Marquet et al. 1999). It has been shown that helmiths and macro-parasites have an impact on the Th2 immune response in New World Aboriginal populations (Hurtado et al. 2003; Wilbur and Buikstra 2006). Some Aboriginal populations produce large amounts of antibodies while healthy; this is thought to be a result of the stimulus of macro-parasites on the Th2 pathway (Hurtado et al. 2003; Wilbur and Buikstra 2006). Both the Dené and the Cree exhibited cytokine SNPs associated with high production of IL-6 and low production of IFNγ(Larcombe et al. 2005; Larcombe et al. 2008), which suggests that First Nations immune system adapted a specific environment

No previous genetic research exists on the immune system of the Inuit in historic or pre-Contact periods. What information that is available about the occurrence of infectious

diseases in the Inuit is based on historic records such as hospital records, birth and death records, and information recorded in personal journals. Genetic analyses on contemporary Inuit populations focus on human leukocyte antigen (HLA) diversity, generally in comparison to other populations (Chu et al. 2001; Tsuneto et 2003). There still remains a gap in the knowledge concerning the genetic effect of the immune system on disease progression in the Inuit as well as an understanding of the synergistic relationship between the Inuit and the Arctic environment. Even given the fact that the highest annual rates of tuberculosis prevalence and incidence occur in Nunavut and northern Manitoba Inuit communities, research has been focused thus far on Canadian non-Aboriginal populations, primarily Caucasian, and Canadian First Nations reserve and non-reserve individuals (Larcombe et al. 2008). By examining the SNPs in promoter regions of specific cytokines for archaeological Inuit populations, and by attempting to identify the genotype and phenotypic profile of these cytokines, inferences can be made about possible selective pressures of the Arctic environment on the Inuit's immune system. Fluctuations in selective pressures and immune response can be achieved through the analysis of archaeological immune profiles in comparison to contemporary Inuit profiles as well as other contemporary populations Linkages can then be made between prevailing TB rates and the immune profiles of previous Inuit populations by examining the differences between not only longitudinal Inuit populations but other First Nations' populations and non-Aboriginal Canadians.

Membership in a specific ethnic group likely plays a role in the variable response and rate of infection in different populations (Larcombe et al. 2005; Ducati et al. 2006) regardless of disease. Cytokine expression, in particular IL-10, IL-6, IFNγ and TNFα,

significantly impact the clinical outcome of TB, as well as the vulnerability of a population to initial and re-infection by tuberculosis bacilli. Ethnic groups have differing cytokine promoter phenotypes, which may contribute to the differential rates of TB in Canada. The duration of ancestral selection for resistance to TB (Tso et al. 2005) may also contribute, but more likely it is the interaction between environment, genetics and socio-economic conditions.

## Cytokine Polymorphisms and Disease

A number of associations have been made between cytokine polymorphisms and a number of pathological conditions (for a summary see Bidwell et al. 1999). Research has identified linkages between specific cytokine polymorphisms and cardiovascular diseases, cancers, neurodegenerative disorders, periodontal disease, immune-mediated diseases such as allergic asthma and other autoimmune diseases, and transplant rejection or acceptance (Hobbs et al. 1958; Turner et al. 1995; Turner et al. 1997; Lim et al. 1998; Noguchi et al. 1998; for a summary database see Bidwell et al. 1999; for a review see Yucesoy et al 2007).

Allelic variants of cytokine genes are associated with higher or lower production of cytokines both *in vivo* and *in vitro* (Perrey et al. 1998). The polymorphisms studied in this research are all biallelic, meaning single genes with two alleles present; the genotype is expressed as homogenous for either A, T, C, or G, or heterogeneous. The genotype is linked then, to a high, low or intermediate expression of cytokine production (Table 3.1) based on the pair of nucleotides represented at the locus. Important polymorphisms reside in the promoter regions of IL-10, TNFα, IL-6 and the first intron of IFNγ (Perrey et al. 1998).

Important to this dissertation are IL-6 (-174) (rs1800795) (C/**G**), IL-10 (-1082) (rs1800896) (**A**/G), IFNγ (+874) (T/A) (rs3087456), and TNFα (A/G)(AF247607.1)(-308).

**Table 3.1 Cytokine Genotypes and Associated Phenotypes**

| Cytokine Polymorphism | Genotype | Phenotype |
|---|---|---|
| **TNFα (-308/)** | **A/A** | **High** |
| | **A/G** | **Intermediate** |
| | **G/G** | **Low** |
| **IFNγ (+874)** | **T/T** | **High** |
| | **T/A** | **Intermediate** |
| | **A/A** | **Low** |
| **IL-6 (-174)** | **G/G** | **High** |
| | **G/C** | **Intermediate** |
| | **C/C** | **Low** |
| **Il-10 (-1082)** | **G/G** | **High** |
| | **G/A** | **Intermediate** |
| | **A/A** | **Low** |

### Analysis of Cytokine Gene Polymorphisms

Genetic diversity related to the human immune response is a key factor in individual and population survival throughout human history. Differences in disease susceptibility and resistance have been identified and linked to differences in cytokine mRNA and protein

expression levels (Larcombe et al. 2005). It has been reported that Canadian Aboriginal individuals have a higher frequency of cytokine single-nucleotide polymorphisms favouring a low production of Tumour Necrosis Factor alpha (TNFα), IFN gamma (IGNγ) and IL-10 and high production of IL-6 as compared to a Canadian Caucasian population (Larcombe et al. 2005). Researchers suggest that the evolution of this unique cytokine genotype profile may be linked to Canadian First Nations, and possibly other Aboriginal populations adaptations to selective pressures related to an environment in which helminthic, parasitic and fungal infections predominated, as well as a shorter period of exposure and acquired immunity to the specific causative agent. To truly understand the importance of genetic analysis of both pathogens and polymorphisms related to the human immune response, it is necessary to understand how the immune system functions. Variation in immune function can be found at the individual level of genetic diversity and biological adaptation, as well as at the population level. Differences caused by the inheritance of unique gene combinations can result in specific susceptibility or resistance to bacterial, viral and parasitic infections. These gene frequencies can be studied at the population level and frequently coincide with biological ethnicity and in some cases "environmental" ethnicity, referring to closely related biological groups who are affected by differing geographical and environmental conditions. In fact, a direct correlation has been suggested between the ability to effectively fight off certain parasitic infections (tropical in origin) and a bias towards the expression and retention of a Th2 mediated immune response (Candelaria et al. 2007). This bias has also been linked to geographic and environmental location, both historic and current, for a population (Candelaria et al. 2007).

IFNγ is a key Th1 pro-inflammatory cytokine (Miyazoe et al. 2002; Gonsalkorale et al. 2003) produced primarily by T cells and natural killer cells (Tso et al. 2005) and has pleiotropic effects in immunoregulation and inflammation dependent upon context. Macrophage activation by IFNγ is essential for adaptive immunity (Lopez-Maderuelo et al. 2003). This is also a major function of the cytokine in combating and protecting the body against specific pathogens such as *Mycobacterium tuberculosis* (MTB or TB) (Tso et al. 2005). The basic pathway of immunity to mycobacteria involves the production of IFNg, IL-12 and IL-18 by the infected macrophage. This occurrence is probably confined to the early phase of infection process and is required to establish a Th1 response. Th1 cells secrete IFNγ and TNFα, which activates macrophages to kill mycobacteria via mechanisms involving nitric oxide production (Flynn 1999; Murray 1999). Polymorphism in the promoter region of IFNγ (+874) may affect the quality of immune response to tuberculosis. Research has demonstrated that populations which have high rates of TB prevalence and incidence express higher frequencies of the A/A genotype compared to the T/A and T/T genotypes. IFNγ low producers (A/A) are therefore at a higher risk of developing TB (Tso et al. 2005).

IL-6 is a multifunctional (Akira et al. 1990) inflammatory cytokine (Fishman et al. 1998) whose major function is antibody induction (Akira et al. 1990) and it reinforces the differentiation of CD4 cells into Th2 cells. The lack of regulation of IL-6 can lead to autoimmune diseases and disorders. IL-6 is found in large amounts, and is produced in the synovial tissue in patients with rheumatoid arthritis (Houssiau et al. 1988). Similar to TNFα it has both systemic and localised effects on both the innate and adaptive immune responses. TNFα is a Th1 pro-inflammatory cytokine (Miyazoe et al. 2002; Gonsalkorale et

al. 2003) and a protein produced by macrophages and other immune cells (Tracey 1994). At the local level it activates vascular endothelium and increases vascular permeability, which leads to increased entry of IgG, complement, and cells to tissues and increased fluid drainage to lymph nodes. At the systemic level, TNFα produces fever, shock and mobilizes metabolites. It is one of the few cytokines directly associated with disease causation (Yucesoy and Luster 2007). TNFα receptor polymorphisms have been directly linked to periodic fevers (Aganna et al. 2001; Aksentijevich et al. 2001; Leonard 2001). It is produced in response to bacterial presence such as recognition of tuberculosis bacilli. The polymorphism in the promoter region of the gene that encodes for TNFα at nucleotide position -308 is associated with a number of diseases (Van Dullemen et al., 1995; Present et al., 1999; Yucesoy et al. 2001). In some cases the association results in an improved outcome, in others the association of TNFα (-308) is pathogenic (ex. Rheumatoid arthritis, Sander and Rau, 1997; Feldman and Brennan 2001).

Interleukin-10 is an anti-inflammatory Th2 cytokine (Tso et al. 2005; Gonsalkorale et al. 2003) and considered a potent inhibitor of most Th1 effectors. It down regulates the IFNγ production of T cells, TNF, and nitric oxide (Tso et al. 2005). Research has discovered that in some populations there is no association of IL-10 with either an increased or decreased TB susceptibility (Tso et al. 2005), however in mice models over expression potentially increases chances of reactivation of latent TB (Tso et al. 2005). IL-10 can be highly toxic and has been implicated as a lethal mediator of acute and chronic infection (Tracey 1994). There is a weak association with IL-10 and IFNγ with relapse and extrapulmonary/miliary TB (Tso et al. 2005).

## Canadian Aboriginal and Inuit Specific Immune Diversity

Immunological diversity in Canadian First Nations and Inuit populations has been observed for several immunological characteristics. Amongst them cytokine SNP diversity, HLA diversity and Killer immunoglobulin-like receptors (KIRs), which are largely responsible for regulating the function of natural killer cells and some T-cell subsets, thus affecting both innate and adaptive immune responses (Rempel et al. 2011). The KIR cluster contains 15 genes and 2 pseudogenes, 6 which are segregated within the centromeric and telomeric portions of the cluster. Significant differences have been observed in Canadian Aboriginal populations in comparison to control (Caucasian) populations for a number of genes within this KIR cluster. The Canadian Aboriginal population's KIR cluster demonstrates a greater immune activating phenotype associated with genes of the B haplotype situated within the telomeric region (Rempel et al. 2011).

HLAs function as receptors for foreign antigens and are involved in immunologic responses to a number of diseases and conditions (Wiseman et al. 2001). HLA diversity in Inuit, particularly the presence of HLA-DRB1*14, HLA-DRB1*04 and DRB1*0407 have been linked with actinic prurigo (AP), an idiopathic photodermatitis. The HLA-DRB1*14 allele in particular may be associated with the initiation of immune responses that cause AP. In conjunction with certain clinical criteria, this suggests that AP in Inuit is unique and may have a distinct immunopathogenic basis (Wiseman et al. 2001). It has been demonstrated that HLA diversity in Inuit populations can also be linked with rheumatic conditions (Oen et al. 1986). In Greenlandic Inuit, the rarity of certain autoimmune diseases (e.g. rheumatoid arthritis, insulin-dependent diabetes mellitus, Graves' disease and psoriasis) has been tentatively explained by the absence of the HLA allele B8, while the high relative

frequency of the HLA-B27 allele has been linked with the high prevalence of reactive arthritis (Harvald 1989).

## Ancient DNA and Anthropology

The first successful application of molecular analysis to human remains occurred in 1985 (Paabo 1985) and began a ``revolution`` in not only how we study past populations but how we understand them. Research that explores some of the same questions that are part of traditional anthropological research is still undertaken, but the methods used to answer them, and the depth of the information now available has increased exponentially. The application of ancient DNA (aDNA) research methods to anthropology and archaeology are unlimited, and the development of new technologies and methodologies is continuous. Initially aDNA studies focused more on information garnered from mitochondrial DNA (mtDNA) rather than nuclear DNA (DNA), this is directly related to the degraded and highly fragmented nature of aDNA. Unlike mtDNA, which is multi-copy in form, nuclear DNA is single copy in form and damage to the nuclear sequence is more likely to be problematic in comparison to mtDNA (Hummel 2003).

Traditional methods utilised by physical and biological anthropologists provide the foundations to molecular studies; the analysis of pathogens requires that a researcher be able to ascertain a provisional diagnosis through lesion identification, and also provide a differential diagnosis to argue that the destructive sampling procedures of aDNA analysis are plausible, required and valid. Likewise, cultural and familial affiliation should be assessed via archaeological survey of a burial; individual identification should be undertaken using traditional methods for aging, sexing, and health indicators. Constructing

a palaeopathological inventory of skeletal remains is a reliable method for determining the overall health of an individual. Molecular analysis can confirm the presence or absence of a pathogen; however pathogen identification cannot indicate the severity of the infection, the extent of dissemination, or its interaction with nutritional and degenerative diseases. Molecular analysis should be undertaken only when a definitive conclusion cannot be drawn from the osteological record; DNA analysis is by its nature a destructive process. Despite the limitations and hazards of undertaking aDNA studies, the outcome of ancient DNA (aDNA) analyses is a greater understanding of anthropological issues concerning the familial history of hominins, the peopling of the New World, the presence of pathogens in certain environments and basic demographic information. The ability to resolve these issues makes aDNA analyses invaluable despite the destructive quality of its methodologies and the somewhat prohibitive cost of its utilization.

The main source of DNA used in many laboratories for ancient investigations is bone or teeth (Keyser-Tracqui and Ludes 2005); soft tissue is also used, but differential preservation of interred remains sometimes precludes the availability of this particular tissue type. Multiple skeletal elements of a single individual are often found, resulting in the possibly of significant amounts of DNA-rich samples. Teeth which are represented in multiple copies are the ideal choice for sampling. The enamel encapsulated dental pulp of teeth provides a uniquely preserved source of DNA (Pfeiffer et al. 1999). Regardless of environment, if the enamel is intact, lacking cracks and caries that have exposed the dental pulp to contamination and degradation, a rich source of well preserved, highly concentrated DNA is available for both the host and pathogen. Other skeletal elements that are candidates for molecular sampling include ribs, which also appear in the skeleton as

multiple copies. A single bone, rib, femur or humerus, can yield varying concentrations of DNA depending on the aspect sampled. This is generally due to varying rates of preservation and the environment in which the bone was preserved (Schultes et al. 1997; Hummel 2003). In pathogenic analysis it is sometimes required that direct sampling of a lesion occurs, to ensure pathogenic DNA is collected along with the endogamous human DNA. Regardless of the skeletal element chosen, the destructive process of retrieving aDNA permanently removes the sample from the osteological record.

The preservation and quality of skeletal remains, as well as the environment from which the remains were excavated and then later curated, impact the quality and concentration of the DNA. Preservation depends upon the coincidence of a number of environmental factors. In terms of archaeological preservation one of the most important aspects is the occurrence and amount of microorganisms in the soil and surrounding environment. Two additional factors that impact the quality of skeletal remains and by relation the quality of preserved DNA are that low pH levels restrict the formation of certain minerals that can completely destroy a skeleton (Herrmann and Newesley 1982) and low levels of alkaline and urea, usually caused by high levels of excrement in the soil, prevent inhibition during molecular analysis and degradation of the skeletal material as well as encourage molecular stability and quality (Hummel 2003). A major factor for post excavation preservation during curation is the temperature at which skeletal remains are stored. Remains kept at room temperature result in the degradation of DNA quality in a relatively short period of time (Hummel 2003). Ergo, remains excavated from an environment with low pH levels, low alkaline and humic acid levels, cool or cold

temperatures (Gilbert et al. 2004; Lindahl 1993), with low levels of microorganisms result in functional molecular samples.

Methodologies can also play an important role in the ability and viability of DNA amplification. One of the most crucial aspects of ancient DNA analysis is the extraction process. Mistakes at this level can lead to contamination, low concentration and even destruction of DNA. There are many protocols available; some are originals while many others have been adjusted over time with experimentation. There are four basic categories of extraction protocols: phenol, silica, boiling and chloroform. The phenol and silica based methods are those that are more widely used in contemporary research (Hummel 2003) and varying degrees of success for each methodology have been published (Lassen et al. 1994; Loffler et al. 1997; Yang et al. 1998; Hummel 2003). Each of these extraction methods may utilise a different sample preparation procedure (i.e. removal of cortical bone, powdering, washing solutions). Mitochondrial DNA has been successfully extracted and amplified using all of the abovementioned methods, while nuclear DNA is more successfully amplified via the silica and phenol methods (Hummel 2003). Kit methods are primarily designed for modern and forensic DNA analyses and are not currently available for aDNA analyses. Ancient DNA studies in anthropology have been used to address a wide range of topics. The study of the migration of early hominins and the structure of their populations through mitochondrial analysis has led to a better understanding of how groups migrated from Africa to populate the rest of the world (Kahn & Gibbons 1997; Ward & Stinger 1997; Hoss 2000). The ability to discriminate between individuals, both forensically and anthropologically, has led to changes in the methods in which the legal system carries out justice, which was based on methodologies created for the study of

ancient DNA (Kaestle and Horsburgh 2002). Methodologies and technologies developed through analysis of aDNA are also applicable to topics peripherally associated with anthropology or that through the use of molecular methods have only recently become important to anthropological studies. Some of these areas include the examination of museum specimens for identification and conservation of faunal and floral remains, known as conservation biology and genetics (Hummel 2003; Spencer et al. 2010), the screening of genetically modified organisms in microbiology, and the study of infectious diseases and their evolutionary record, in particular their interaction with the human species and the co-evolution in different temporal and geographic populations (O'Rourke et al. 2000; Hummel 2003).

Methods developed to analyse the immunogenetic profiles of past populations though the amplification of aDNA may eventually reveal links between historical, cultural, and biological events with contemporary health issues. The protocols in this study are an example of how these methodologies can be implemented in archaeological Inuit populations as foundations to further knowledge. A limited number of molecular studies examining immunogenetic profiles in past populations currently exist. The majority of comparative information is extrapolated from analyses of past Oji-Cree Canadian populations from Manitoba (Larcombe 2005) and from analyses of contemporary Canadian Dené, Caucasian and Cree populations (Larcombe et al. 2005; Larcombe et al. 2008). These studies provide comparison groups for the study of genotype frequency fluctuations that may have occurred between different ecological niches, cultural groups, and temporal populations. By examining the genotype frequencies and immunogenetic profiles of past Inuit populations, it becomes possible to understand the effect of past selective pressures

on contemporary health issues, such as the Inuit's disproportionately high susceptibility to tuberculosis.

## Mitochondrial DNA

Mitochondrial DNA (mtDNA) is a circular multicopy DNA found within the mitochondria of an organism's cell; there are multiple mitochondria containing up to 1000 copies of the same genetic information in each cell. In humans each mitochondria contains 16 570 base pairs, coding for a total of 37 genes (Hummel 2003). Unlike chromosomal nuclear DNA, mtDNA is only inherited through the female portion of the previous generation, and therefore represents a haplotype, as all members of a maternal lineage show the same mtDNA (Hummel 2003). Each ovum carries a complete copy of the whole mitochondrial genome; in contrast the sperm does not contribute the mitochondria it carries to the zygote. This process results in the transfer of only the maternal mitochondrial genetic information through the generations, and each member of the maternal line is a member of the same lineage and forms a haplogroup. Mitochondrial DNA does not recombine (Stoneking and Soodyall 1996; Mesa et al. 2000) and contains an abundance of polymorphisms in the non-coding regions (Bianchi et al. 1998) which makes it ideal to study short group associations. The fact that mtDNA does not experience recombination means that identical copies are located throughout members of the same haplogroup, and differentiation occurs only through mutation, which occurs at a much higher rate than seen in nuclear DNA.

Hypervariable regions I and II (HVR I and HVR II), located on the D-loop control region of the mitochondrial genome, manifest the most sequence polymorphisms in

mtDNA. In total this region spans 900 bp, of which 600 bp are the HVR I and HVR II regions (Hummel 2003). These regions reveal approximately 3% variability between individuals, and the associated polymorphisms often cluster around so-called "hotspots" (Hummel 2003: 23). This enables researchers to identify individuals at the family lineage level (i.e. Butler and Levin 1998; Schneider et al. 1999), but these "hotspots" are also valuable in the analysis of population genetics or phylogenetic studies (Boles et al. 1995; Stone and Stoneking 1998). Therefore the mitochondria of each generation will be identical to the maternal population of their direct ascendants and descendants. These mitochondria accumulate mutations, or substitutions, at a much higher rate than the nuclear genome (Linnane et al. 1989). In each successive generation, as mutations are acquired, genetic distance will accumulate (Linnane et al. 1989), this permits researchers to trace lineages backwards and estimate chronological distance. A comparison of the hypervariable regions of two random individuals would reveal differences in 3 out of every 100 nucleotide positions (Stoneking 2000), indicating a non-relation, or a very distant relation. Closely related individuals, such as mother and child, should manifest no differences in the same comparison although mtDNA can also be used to identify individuals (Brown 1980).

For this research mtDNA was employed to ensure that the individual being analysed was a member of the target population (Inuit). Elimination of individuals with mitochondrial profiles outside of expected haplogroups was essential to accurately assess and authenticate the results extracted from nuclear DNA analyses. A limited number of haplogroups are known to exist within ancestral groups of North, South and Central America allowing for simple exclusion based on haplogroup membership. Due to the fact that the Arctic has experienced only a short period of prolonged contact with populations

outside of the Inuit ethnic group it is highly unlikely that a significant amount of gene flow had occurred within the population analysed. Individuals from the pre-contact period could be definitively assigned to the five known haplogroups of North America, and any individuals exhibiting a haplogroup outside this limited number are easily assigned the determination of contaminated and excluded from analysis.

## Haplogroups

Polymorphisms in the HVR regions of mtDNA can be used to trace descent populations and migration patterns (Hagelberg and Clegg 1993). Haplogroups, groups of individuals that share similar mitochondrial genetic markers but that are not necessarily closely related (De Benedictis et al. 1999), have been traced for the global population. To date five haplogroups, and a variety of haplotypes, have been assigned to New World Aboriginal populations based on mitochondrial restriction/deletion polymorphisms: A, B, C, D, and X (Torroni et al. 1992), however haplogroups A-D represent virtually all of North and South America's mtDNA diversity (Tamm et al. 2007). A2, B2, C1, D1 are the four major pan-American haplotypes and are thought to be the founding New World haplogroups (Kolman and Tuross 2000), however some researchers have suggested that a 5$^{th}$ haplogroup of X, and especially X2a is also a founding lineage, not an example of post migration mutation (Bourgeois et al. 2009). In fact besides the four pan-American lineages, three minor lineages are also found in higher frequencies throughout the New World; X2a, D2 and D3 (Tamm et al. 2007) and an additional two X haplotypes are common in select areas of North America, X6 and X7 (Torroni et al. 1994). While these haplotypes are

significantly rarer than the major four, they are found in pockets of high frequency throughout the New World. The discovery of these haplogroups and their linkage to Eurasian haplogroups have allowed anthropologists to more accurately recreate descendent populations and migration patterns to the New World.

Ancient DNA analysis of these groupings has altered the manner in which anthropologists view not only overall population distribution in the New World, but the specific relationships within geographical regions. While skeletal indicators of ethnicity can be used to assign the broad category of Amerindian, DNA sequencing can be utilised to further elaborate ethnic identity. For example Algonquian-speaking groups such as the Chippewa/Ojibwa, and Micmac are characterised by very high frequencies of haplogroup X (Smith et al. 1999), and Kiowa-Tanoan, Siouan, Wakashan, Penutian, and Northern Hokan speakers also exhibited high frequencies across North America. Whereas in Manitoba, the X haplogroup is associated with Algonkian speaking groups only (Malhi et al. 2002). These findings have altered the manner and method of examining human migration in the past, and displayed how the continuity of a population may have developed or resulted in the development of a single haplogroup into several cultural groups. Ancient DNA methods and technology have enabled anthropologists to compare ancient and modern populations to establish different levels of genetic relationships. The comparison of these haplogroups has aided in highlighting other socio-cultural, linguistic and immunogenetic differences between populations within Canada and the United States. For example the abovementioned differences in Algonquian speakers.

It has been suggested that the initial number of migrants that crossed from the Old World into the New World via Beringia was relatively low based on the paucity of mtDNA

diversity within the New World (Tamm et al. 2007). There has been research into the distribution of haplogroups throughout the American continents, in some cases the results have indicated temporal and geographical continuity (O'Rourke et al. 2000; Malhi 2001), while in others it has been discovered that some modern populations in a geographic territory have been shown to be "unrelated" to their geographic predecessors (Kaestle and Smith 2001). This is likely a factor in ancient and contemporary Inuit populations that share cultural traits but may not necessarily share biological or molecular traits.

While some haplogroups and haplotypes have a high distribution throughout almost the whole of the New World, others are restricted to specific populations or territories. There are a number of factors that may have contributed to this happenstance, but continued or restricted migration is generally thought to be the major factor. Lorenz and Smith (1996) typed 497 distinct Aboriginal groups from the Inuit of Canada and Greenland to the Zapotec and Maya of Central America. They found that haplogroup A had the greatest frequency at 40% and D the least common with a frequency of at least 7% in most populations. Intraregion genetic homogeneity is substantially less than intragroup genetic homogeneity; however the Arctic and subArctic regions are genetically distinct from the rest of North America and almost as distinct as individual groups. Frequencies expressed in South America vary greatly from those expressed in North America, suggesting that a restricted gene flow transpired with bottlenecking occurring in Central America (Lorenz and Smith 1996). The Arctic, populated at a much later date than the remainder of North America, and substantially later than South America, may represent yet another area in which populations evolved haplogroup frequencies independent of each other. This has much to do with the manner in which the Arctic was peopled, and the movement and

migrations of peoples from the Old World to the New World and from the Western Arctic to the Eastern Arctic.

Exactly how and when the Arctic was peopled is still a highly contentious topic of research. Mitochondrial DNA analyses have been used to substantiate not only a single wave migration but also a multi-wave migration (Merriwether et al. 1995; Starikovskaya et al. 1998). As a result, a broad range for the timing of New World migration has evolved; early estimates put the initial entrance from Eurasia at as early as 40 000 years before present, while later estimates place it at as late as only 10 000 years before present (for a review, see Eshleman et al. 2003). Isolated haplotype migration estimates have been calculated for Arctic populations in Greenland and the Eastern Canadian Kitikmeot populations (Helgason et al. 2006).

Origin times for A2a and A2b haplotypes, two of the dominant haplotypes for Inuit populations, have been calculated (Helgason et al. 2006) using a variety of colonization models resulting in a number of derived migration times. Under a combined Siberian Kitikmeot model the origin of A2a in Greenland has been calculated as 426± 159 years (based on the pedigree mutation rate) or 1160± 434 years (based on the evolutionary rate model). Haplotype A2b has been calculated at 376± 160 years and 1024± 435 years, respectively. While these estimates are based on geographical regions existing outside of the majority of the sample populations' boundaries, information can be extrapolated from these dates. Similar migration times can be presumed to coincide with the remainder of the Eastern Arctic populations outside of the Kitikmeot regions, with slightly earlier origins based on the assumption that migration occurred from a west to east direction. Populations that arrived in Greenland would have passed through the rest of the Arctic at

an earlier date. In some cases the absence of a particular haplotype in Asia has been used as evidence of the timing; for example, the lack of Hg C1b,c, and d in Asia has been used to place the initial timing for entrance to the New World at 13 900 ± 2 700 years before present (Tamm et al. 2007). Regardless of how the initial migration occurred, mtDNA analyses reveal an obvious discrepancy in haplogroup and haplotype frequencies between the North and South Americas, as well as Arctic regions and the rest of Canada.

Different areas of North America in general and Canada in particular, have exhibited particular haplogroup frequencies. Haplogroup A is the most common haplogroup in all geographic areas with the exception of the Southwest and Great Basin regions of America, where its frequencies dip to 26 and 7 % respectively (Lorenz and Smith 1996). Hg A is thought to have had two variants that migrated with initial populations from Beringia to the New World, A1 and A2 (Merriwhether and Ferrell 1996). Hg A and its associated haplotypes find their highest frequency in Canada, the eastern United States and central Mexico (Malhi et al. 2002) and is one of the two most frequent haplogroups in Inuit populations. Amerind populations generally have varying frequencies of all four haplogroups, but the NaDené and Eskimo populations have mtDNA sequences mainly from haplogroup A (Merriwhether et al. 1995; Wallace 1995; Bonatto and Salzano 1997), similar to Canadian Inuit populations. In contemporary populations haplogroups A, along with C and X, have increased (Shook and Smith 2008). Historical changes in frequencies, especially when they are coupled with the increase in a rare or low frequency haplogroup (such as X6 or X7) and a drop in previous common haplogroup frequencies, is generally attributed to the effects of European contact (Shook and Smith 2008). Some researchers

claim that a drastic reduction in population for certain areas contributed to the discontinuity in ancient and modern frequencies (Shook and Smith 2008).

Haplogroup B is characterised by a nine base pair deletion, as well as a number of single nucleotide polymorphisms. The deletion is present amongst all Amerind populations but its frequency varies amongst other populations (Lorenz and Smith 1996). Similar to haplogroup A, B is thought to have two founding lineages in the New World, B1 and B2. However, it has been noted that B has a smaller within lineage divergence time than the other three main haplogroups, suggesting that individuals with the mtDNA sequence for B entered the New World in a separate migration and at a later time than those with the mtDNA sequence for A, C and D (Torroni et al. 1992, 1993). It finds its highest frequency in the Southwest region of the United States, and sees a sharp decline in occurrence to the West, South, East and North of this region; in fact it is almost wholly absent in the Arctic and rare in circumpolar populations, but has an overall North American frequency of 32% (Lorenz and Smith 1996). B is absent in most Eskimo and Inuit groups and rarely if ever present in the NaDené groups (Lorenz 1997), it is also absent from Siberian populations and like the Eskimo populations is virtually absent from all other Aboriginal groups within the Alaskan territory (Merriwhether and Ferrell 1996).

Similar to haplogroups A and B in the New World, haplogroup C is also thought to have two founding lineages, C1 and C2. C exhibits a fairly consistent frequency throughout North America, but exhibits a notable decline in the Arctic and subArctic regions (Malhi et al. 2002). This haplogroup is rare in Eskimo and Inuit populations (Lorenz 1997), but exhibits an increase in frequency in contemporary populations compared to ancient populations, and in certain groups such as the Algonquian peoples of southwestern Ontario

and the northeastern United States (Shook and Smith 2008). Haplogroup C manifests a fairly consistent expression throughout North America. Similar to D, it is never the most frequent haplogroup of any geographical location. However, it does see an increase in frequency in the United States compared to Canada, and a lower frequency in Northwest Coast populations. It is absent in Arctic populations of Canada and rare in Eskimo and Inuit populations of Alaska (Lorenz and Smith 1996).

Haplogroup D is the rarest of all North American haplogroups with an expression of only 7% (Lorenz and Smith 1996); it sees a lower frequency in the southern portions of North America, but a slightly higher frequency in Alaska (Malhi et al. 2002). Its expression is most prevalent in the northern Paiute/Shoshone at more than 40% and is generally expressed at less than 6% in most other regions of the United States. Two small areas of moderately high expression are seen in the northwest Coast and California/Great Basin Aboriginals where frequencies can reach 18 and 22% respectively (Lorenz and Smith 1996). It is also the second haplogroup most commonly present within both modern and ancient Inuit populations.

Haplogroup X is relatively rare within the New World; however its two main lineages X6 and X7 are thought to represent alternative founding lineages from X2a for specific populations. These populations are generally characterised by the complete absence of the definitive four founding populations A, B, C, D. The Yanomami peoples of South America exemplify a population that is devoid of the four main haplogroups and instead expresses the X6/X7 lineages (Merriwhether and Ferrell 1996). The X haplogroup has seen an increase in frequencies since ancient populations; it is rare or absent from the majority of geographical populations prior to contact (Shook and Smith 2008) and is absent

from Caucasian and African populations (Torroni et al. 1994). X is seeing higher frequency, along with A and C, in modern Algonquian populations (Shook and Smith 2008) and is found in varying frequencies in some Northwest, Siberian and Asian populations (Torroni et al. 1994). While many of the Inuit populations in North America exhibit similar haplogroup frequencies for A and D, Inuit populations of Greenland have higher frequencies of the X haplogroup, with similar higher frequencies seen around the Great Lakes Region of North America (Shook and Smith 2008).

While these five haplogroups and their associated lineages and haplotypes constitute the vast majority of haplogroups expressed in the New World there is evidence that suggests that other haplogroups may have existed at one time. Common sense suggests that it is possible that some catastrophic event could have eradicated haplogroups that have no extant expression. Current data suggests that only five haplogroups were present during the foundation of contemporary populations in the New World, however, it is possible that remains may eventually substantiate a different theory of migration and population demography during the initial stages.

## Nuclear DNA

Nuclear DNA, so called because it is found only in the nucleus of each cell, is expressed in only a single copy for each nucleus. However, the vast majority of genetic information for an individual is contained within the nucleus rather than the mitochondria. Nuclear DNA is densely packed into each nucleus within diploid chromosomes. Each individual has a set of 46 chromosome inherited in 23 pairs. One set of 23 is inherited from

the paternal sperm and another 23 inherited from the maternal ovum. Unlike the mitochondrial exchange of genetic material, nuclear DNA is exchanged equally by the sperm and the ovum. This method of inheritance is complex and results in recombination of genetic information. Each individual ovum and sperm may pass on a different combination of genes to each offspring resulting in a vast amount of recombinatory possibilities. There is, therefore, a considerable amount of individual variability possible, especially given that 95% of the nuclear genome is non-coding and contains polymorphisms and mutations (Hummel 2003).

Nuclear DNA is used to analyse genes and traits that are more individual in nature, rather than at the population level. However, it is possible to study the frequency of trait inheritance within and between wider populations. The majority of biological characteristics are encoded within the 23 sets of chromosomes in the nuclear DNA genome; one set encode for genetic sex (the X and Y chromosomes) and the remainder of the 22 sets encode for every other biological function, including the immune system. The analysis of DNA can be used to establish individual genetic discrimination, paternity and maternity, and profiles concerning immune response, health and inheritance of genetic disorders such as Alzheimer's disease, and characteristics of physical appearance. In anthropological studies sex determination is one of the basic and most common nuclear DNA analyses.

### Sex Determination

The amelogenin gene is a single copy gene located on Yp11.2 of the Y-chromosome and Xp22.31-p.22.1 of the X chromosome, which encode for a protein of tooth enamel (Hummel 2003). A 6-9 base pair deletion was discovered in the intron of the X

chromosome and allows for a distinction between biological sexes (Sullivan et al. 1998; Manucci et al. 1994). Amplification with the correct primers results in a single amplification product for females and a double band for males, or on an electropherogram or chromatograph two peaks for males at 106 bp and 1012 bp and a single peak at 106 bp for females (Hummel 2003). However, because the amelogenin gene is present on both the X and Y chromosome a false positive may occur if for some reason the 112bp fragment for the Y chromosome does not amplify. Therefore while the amelogenin amplification suggests female it is possible that these individuals are false positives and actually male. There are some issues with this gene and various methodologies involved in its amplification and analysis; in rare cases, similar to the X chromosomal region, the Y-chromosomal region may also present the necessary deletion; with aDNA a larger problem is allelic dropout where only one of the two alleles is amplified by chance (Hummel 2003) usually resulting in a false positive for the determination of female sex. However, these problems can be resolved through the application of repeated amplification; results in ancient DNA sex determination should not only be confirmed against osteological methods, but through repetition of the amplification and analysis methods. Repeatable results withstand greater scrutiny and often resolve the issue of allelic drop out.

The discovery of the amelogenin deletion and its application to sex determination have allowed for a greater understanding of the demographics of past populations. Understanding the distribution of sex within a population can relay information about birth rates, infant mortality rates, and cultural practices. For example, changing rates of male or female infanticide can reveal changes in cultural attitudes toward gender and the importance placed upon the acquisition of a son or daughter to a kin group and the wider

cultural community. Determination of biological sex may also reveal the function of archaeological building remains based on the presence of an all female or an all male burial site (Faerman et al. 1997, 1998). The basic application of sex determination is the confirmation of osteological sex, but it can have a far reaching impact on the cultural profile of a population.

### Analysis of Microbial DNA

The analysis of nuclear DNA for genotypes and phenotypes associated with the human immune system and health, and by association the amplification of pathogenic DNA, has been one of the most common forms of aDNA analysis since its introduction. Infectious diseases have accompanied humankind from its evolutionary origins (Cipollaro et al. 2005) and the identification of pathogens in ancient remains can provide information on host-pathogen interactions, human migration patterns and the health profile of past populations, while providing new possibilities for methods to answer questions raised by medical historians and palaeopathologists concerning actual prehistoric and historic frequency and occurrence (Cipollaro et al. 2005). From its inception the majority of studies in ancient DNA have dealt with population genetics, diet, domestication, and evolution of mammals and birds (Donoghue and Spigelman 2006). The analysis of ancient microbial DNA developed soon after, and has resulted in unique studies and the extraction and amplification of microbial and microbial DNA information from a diverse range of situations, circumstances and environments. Examples include the extraction of bacterial

DNA in amber (Bada et al. 1999; Greenblatt et al. 2004) and microbes retrieved from the depths of the permafrost (Christner et al. 2003).

Of particular interest to anthropologists is the ability to accurately assess the health of past populations, and particularly in Canada in First Nations and Inuit populations. Comparisons between present First Nations or Inuit populations and their ancestors can shed light on current health concerns and reveal historical trends, changes and impacts. Ancient DNA analyses have drastically altered the manner in which health status is evaluated in the osteological record and how researchers can reconstruct not only human migration patterns through pathogen load and infectious diseases (Swanson 2008), but also in reconstructing the evolution of a particular disease within different populations or confirming its ancestral or modern form. A distinctive branch of ancient DNA research is the study of pathogenic microbial DNA and its relationship with infected human hosts (Kolman and Tuross 2000; O'Rourke et al. 2000). There is increasing interest in this field but, in order to critically assess this body of research, one should be aware of the differences caused by the characteristics of different microbial pathogens, and their distribution and dissemination within the host (Donoghue and Spigelman 2006), both in archaeological and modern samples.

Since they should undoubtedly be ancestral to pathogens found in modern genetic diversity, the rapid evolutionary rate of many pathogens offers a unique means to establish the authenticity of ancient pathogen sequences (Willerslev and Cooper 2006). Such classification of pathogen evolutionary rates would also be of major importance to studies of the origin and spread of diseases, which may aid researchers in the eventual prevention of resurgent, emerging and new microbial diseases (Musser 1996). Changes in human

behaviour, simple processes of microbial evolution and increasing resistance to antimicrobial agents will continue to supply humankind with new infectious disease challenges, and, therefore, motivation for molecular population genetic studies (Musser 1996) and the continued study of human genetic adaptability.

*Mycobacterium tuberculosis* and *Mycobacterium leprae* are the focus of the vast majority of papers analysing ancient microbes (Donoghue et al. 2004; Drancourt & Raoult 2005; Donoghue 2009), however, many other pathogens and pathological conditions are also studied. Some have been and are studied indirectly via assessment of immune functioning through genetic analyses of polymorphisms and allele frequencies, while others are analysed directly through whole genome sequencing and phylogenic studies. Assessment is often hindered by the nature of the microbe or pathogen itself; microorganisms differ in the vulnerability of their DNA to primary decay and eventual degradation processes over time (Donoghue et al. 2004). Mycobacteria, including the causative agents of tuberculosis and leprosy, have resistant hydrophobic cell walls that have been revealed to physically persevere for approximately 250 years (Donoghue et al. 2004). In addition, they are members of a genetic family with DNA rich in guanine and cytosine, which presents superior stability (Pääbo et al. 2004) due to the number of hydrogen bonds between base pairs. This stability makes them attractive subjects for analyses in archaeological specimens. Pathogenic DNA will always be a negligible portion within the osteological specimen and often localised and isolated, so it is necessary to be able to identify particular pathogenic lesions. Initially, attention should be directed to bony lesions idiosyncratic to a condition. However, even in individuals who suffered and died from a chronic condition, these sites are rare; in tuberculosis, the majority of patients die

from the acute form before lesions had time to form in the skeletal system, a process which requires the development of a chronic and disseminated disease condition. Lack of understanding of this point led to classical palaeopathologists questioning reports that *M. tuberculosis* DNA could be detected in bones without lesions (reviewed by Donoghue et al. 2004; Zink et al. 2005). It has been shown that *M. tuberculosis* DNA can be detected, in over 50% of the cases, where ribs from a population manifested less than 5 percent of pathological modifications (Fletcher et al. 2003). The pathogenic study of *Mycobacterium tuberculosis*, a pathogen that has no environmental reservoir, and has likely evolved along with its human host, allows for a more holistic understanding of co-evolution and long term coexistence between a pathogen and its host population (Hershkovitz et al. 2008). Recent studies suggest that *M. tuberculosis* is not a descendant of *M. bovis* as originally thought, thus the domestication of cattle was not directly responsible for the transmission of the infection to humans (Brosch et al. 2002). It continues to be debated whether TB pathogens co-migrated with the initial peopling, or were already present in the New World prior to peopling in either environmental or zoonotic vectors. However, cattle domestication did play an indirect role in the sustainability of TB within human host populations by creating the ability for previously nomadic populations to remain more sedentary with greater population densities, facilitating an ease of transmission between individuals. Regardless of how TB came to be a human pathogen, the co-existence of TB and human populations spans thousands of years. The similarity of the historic and ancient strains of *M. tuberculosis*' genetic signature with those found in today's populations also lends credence to the theory of a long-term co-existence of host and pathogen (Hershkovitz et al. 2008). This co-existence is suggestive of a correlation between human immune response and

cytokine expression differences within and between different populations and has implications on the understanding of TB pathogen-host co-existence and varying rates of prevalence and vulnerability globally.

# Chapter 4 Skeletal Analysis

## Skeletal Samples

The Inuit individuals (n= 152) who comprise the osteological sample for this dissertation are currently curated by the Canadian Museum of Civilization, Gatineau, Quebec in climate controlled storage facilities. Curation conditions are longterm and variable. Many of the burials have been previously handled and analyzed and may have been stored with burial goods, non-human remains and other human remains throughout curation. The 152 individuals are a small proportion of a larger collection of over 1000 skeletal remains from across Nunavut, Canada. These individuals represent archaeological remains excavated or discovered as surface finds from burials sites from the High Arctic Islands of Devon, the Western Hudson Bay area, and a large section from Southampton Island in Hudson Bay.

The remains were excavated over the 20th century and include osteological sex and age, burial site, a description any burials goods, and the assignment of a temporal context. This temporal categorization is based on the excavator's discretion and the museum's analysis. The categories included, Late Prehistoric, Historic, and Modern burials. Modern burials were excluded from the research sample for this study and the categories Late Prehistoric and Historic are classed as pre-contact and contact respectively.

### Inclusion/exclusion criteria

The original skeletal database provided by the Canadian Museum of Civilization contained 1004 individuals. Of this, 684 were excluded from the working sample based on provenience, age, or lack of skeletal elements. The remaining individuals (n= 320) were either adults or sub-adults from burial sites within the modern political territory of

Nunavut, with the majority of skeletal elements present. Individuals were excluded from this point on only after visual inspection to assess preservation quality and the presence of pathological modification. The final sample consisted of 152 individuals; the remainder (n= 168) were excluded based on poor preservation.

Preservation of both cranial and post-cranial remains is important for a detailed palaeopathological analysis and for the potential recovery of DNA. The skeletons were required to be well preserved with little taphonomic/ post mortem damage, little to no research damage (i.e. previous sampling for thin sectioning, DNA testing, radiocarbon dating and so on), and the presence of the majority of the post-cranial elements. The essential elements included, cranium, mandible with intact molars, vertebral column, 50 – 100 percent of the ribs, and the majority of the long bones. Burials lacking the majority or all of these elements were excluded from the study. Ideal candidates for inclusion in the sample possessed a complete cranium and mandible, although presence of either set of dentition was also considered viable. In terms of molecular sampling, individuals with encased and intact molars were selected as sample individuals, while those lacking definitively individual dentition (i.e. those not loose) were disregarded. Skeletal elements with a small degree of post-mortem damage were included; individuals with highly fragmented, burned or lichen covered elements were excluded. Note was taken of elements that possessed damage, especially when pathological modification was later observed.

Preservation and completeness of skeletal remains were vital to establishing working palaeopathological and molecular samples. Individuals excavated and recovered from permafrost represent unique opportunities for aDNA analysis (Smith et al. 2001;

Gilbert et al. 2004; Hebsgaard et al. 2005). Individuals that remained buried in a continuously frozen state, such as those in permafrost (Hansen et al. 2006), are considered individuals from "optimal conditions" for molecular analysis because they lack the damage caused by cycles of thawing and freezing (Smith et al. 2001; Hansen et al. 2006).

The assessment of provenience ensured burials were from within Nunavut[6] and were interment style burials and not surface finds. Surface finds were excluded because they lack archaeological context and cannot be assigned an approximate date of internment based on stratigraphy or burial goods. Basic dating was especially important for individuals estimated to have been interred during the contact period to establish the population affiliation of burials.

The high rate of mortality, the plasticity of developing skeletal elements and the vulnerability of pre-adults to succumbing to infections quickly are all reasons for exclusion from the population cohort. Individuals were grouped into two temporal categories, contact and pre-contact, based on the previous assignment of historical context by the excavator. Individuals who were assigned the label "Late Pre-historic" were grouped together as pre-contact individuals. This is a category to describe individuals who lived before prolonged continuous contact with European travellers. Late prehistoric individuals refer to those before the 17th century and back several hundred years. Individuals assigned the label of historic or modern by the museum were grouped together as contact individuals, referring to the period of Inuit history occurring after a continuous European presence in the aArctic. A general acknowledgement must be stated that the pre-

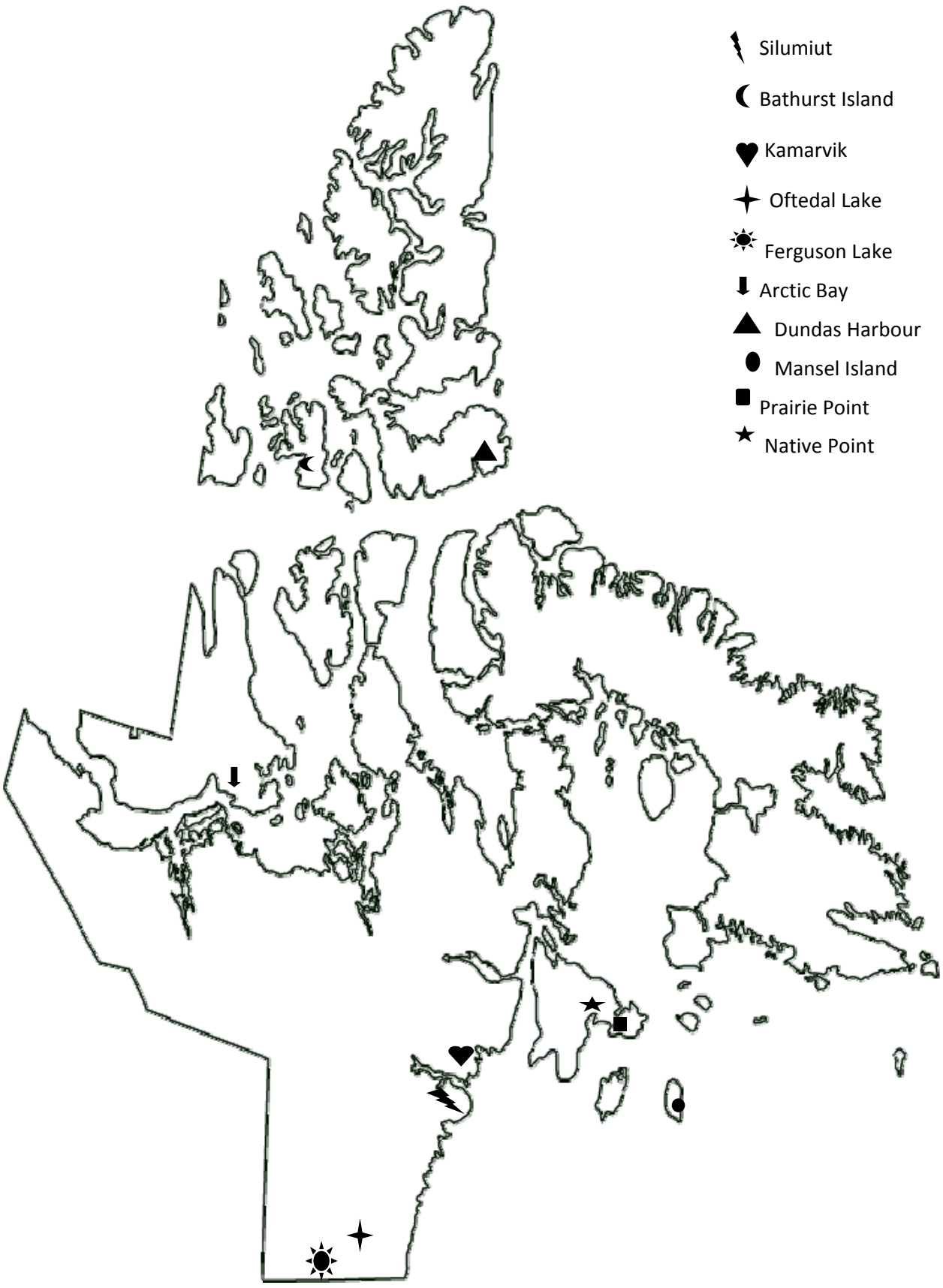
[6] A single individual originally designated as a burial within the Nunavut territory, was later designated as a northern Manitoba burial and excluded from the analysis. Burials from within Nunavut are considered of Inuit ethnicity.

historic period lasted for a much longer period in the aArctic relative to the remainder of Canada. In general the pre-historic period in the contemporary territory of Nunavut can be generalised from the commencement of the 19th century when a fairly regular written account or history began for many communities. This occurrence delineates the pre-historic (no written record) from the historic (presence of a written record). For a distribution and frequency of individuals within these periods, please refer to Table 4.1 below.

The sample includes individuals from 20 burial sites across the eastern Canadian AArctic, in the Nunavut Territory (Figure 4.1). While a portion of these sites yielded a single appropriate individual to the sample, two sites encompass the bulk of individuals examined. The Sadlermiut culture site of Native Point on Southampton Island consists of over 50 percent of the individuals analysed, all from the contact period, but none dated to the years after 1902-1903, when both the culture and the biological population ceased to occupy the island. From this point onward the cultural group was no longer represented in the continuum of Arctic peoples, and the biological population is scattered and intermixed within other populations at minute levels. The second sample population is the Kamarvik, from the western shores of Hudson Bay, located between the inlets of Chesterfield and Rankin (Figure 4.1).

Only a single burial (XIV-C: 315) has been included that cannot be definitively assigned to a temporal era or definitive burial site. Of the remaining 151 burials, 94 are classified as contact and 57 as pre-contact. Previous osteological assessment assigned osteological sex for approximately 91% of the archaeological sample (n= 138): 44 contact females, 28 pre-contact females, 44 contact males, 29 pre-contact males and 14 individuals

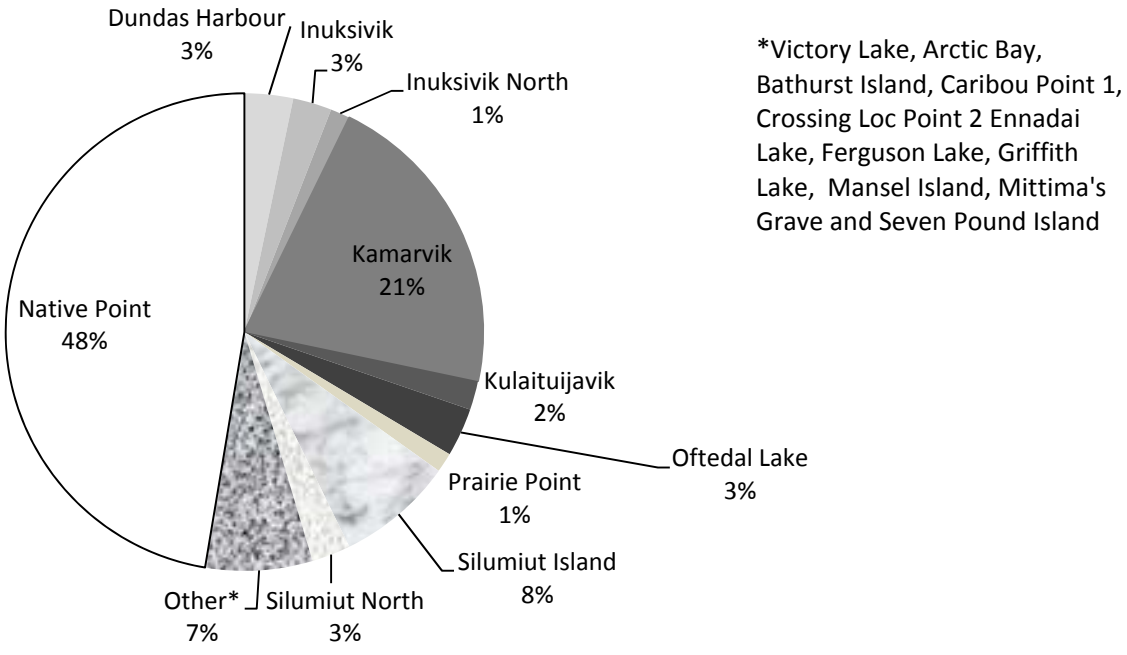
## Figure 4.1 Map of Nunavut with Selected Sites



of indeterminate sex from either the pre-contact and contact period. The total skeletal sample incorporates 152 individuals, of which only ten are sub-adult. Sub-adults (13- 18 years old) were included when the skeletal remains were well preserved, of a more advanced maturation, and presented something unique or important to the overall sample.

The ten adolescent individuals are found in the Crossing Point Loc 2, Kamarvik, Oftedal Lake, and Native Point sites, and represent a minor percentage of the overall sample. The remainder of the sample are distributed between twenty sites. Ten burial sites contribute a single individual; seven sites contribute between 2-5 individuals; and the remainder of the osteological sample was obtained from three major sites, Native Point, Kamarvik and Silumiut Island (Table 4.1 and Figures 4.1 and 4.2).

**Figure 4.2 Frequency of Individuals from Burial Sites**



| Table 4.1 Demographic Distribution and Frequencies | | | |
|---|---|---|---|
| **Sites** | **Pre-Contact** | **Contact – Post Contact** | **Total** |
| **Arctic Bay** | 1 Male | | **1** |
| **Caribou Point 1** | | 1 Female | **1** |
| **Crossing Point Loc 2** | | 1 Indeterminate Adolescents | **1** |
| **Dundas Harbour** | 2 Females<br>3 Males | | **5** |
| **Ennadai Lake** | | 1 Male | **1** |
| **Ferguson Lake** | 1 Female | | **1** |
| **Griffith Lake** | | 1 Male | **1** |
| **Inuksivik** | 2 Females<br>1 Male | 1 Male | **4** |
| **Inuksivik North** | | 2 Male | **2** |
| **Kamarvik** | 10 Females<br>19 Males<br>1 Indeterminate Adolescent | 1 Male<br>1 Female | **32** |
| **Kulaituijavik** | 1 Female<br>1 Male | 1 Male | **3** |
| **Mansel Island** | 1 Female | | **1** |
| **Mittimatalik/ Mittima's Grave** | 1 Female | | **1** |
| **Oftedal Lake** | | 3 Males<br>1 Male Adolescent<br>1 Female Adolescent | **5** |
| **Sadlermiut/ Native Point** | | 29 Females<br>27 Males<br>4 Male Adolescents<br>2 Female Adolescents<br>10 Indeterminate | **72** |
| **Sadlermiut/ Prairie Point** | | 1 Indeterminate<br>1 Female | **2** |
| **Seven Pound Island** | | 1 Male | **1** |
| **Silumiut Island** | 6 Females<br>4 Males | 1 Male<br>1 Indeterminate Adolescent | **12** |
| **Silumiut North** | 4 Females | | **4** |
| **Victory Lake** | | 1 Female | **1** |
| **1 undetermined** | | | |
| **Total** | **57** | **93** | **152** |

## Osteological Methods

### General Assessment

Initial evaluation of the osteological collection required the elimination of individuals lacking in sufficient skeletal elements to assess overall health. Particular attention was paid to burials containing skeletal elements that would manifest tubercular pathological modifications. This required a complete or nearly complete skeleton, containing ribs, vertebral column, skull (cranium + mandible), lower long bones, and pelvic girdle. Individuals also possessing complete hand and foot bones, upper limb bones, clavicle, and sternum were ideal. Individuals possessing an intact set of paired bones were ideal; however this was not considered a factor for inclusion or exclusion. It is pertinent to emphasise that the more complete the individual skeleton, the better the ability to assess overall health.

Observation and recording of all skeletal modifications indicative of pathological or degenerative conditions was required as part of the initial assessment of each individual. This initial evaluation documented the presence of lesions on a skeletal element only, indicated the overall level of health for each individual and specified whether or not the individual would be a satisfactory candidate for molecular analysis. This final stage required an overall assessment of preservation and tubercular lesions. A secondary inventory was implemented to record and assess the exact location and condition of any pathological modification previously encountered. This allowed for a better assessment of the disease that may have affected the individual during life, including the initial determination of tubercular candidate. Each individual was appraised for modification on the characteristic tubercular skeletal elements; observation of a traditional and typical pathological modification on a single element necessitated further investigation of related

elements. However, there are a number of chronic conditions that produce alterations in the skeletal system that are not distinct enough to define the specific condition and the observation of a single lesion does not generally result in a positive inference of a disease. In cases where a single lesion was observed, the location and a description of any modifications to the element were recorded (porosity, bone deposition, type of lesions) and the condition was listed as a "general infection". In some cases a suite of modifications and conditions were observed in a single individual, for example the presence of osteoarthritis in the spinal column, and evidence of infection in the pelvic girdle. In general these two conditions are not related to a single pathological state, however their presence in the individual does relate important information about the health condition of the individual, and a qualification based on the type of alterations and severity of the modification was created to better demarcate the individual's health. A three part classificatory system was created to better demarcate individuals lacking in a typical disease condition such as tuberculosis, osteosarcoma, rickets or anaemia. Instead individuals were labelled as having minor, moderate or major modifications; these rankings were assigned based on the number of lesions, the number of affected skeletal elements and the severity of the modification.

## Differential Diagnosis of Osteological Modification

### Specific Assessment: Tuberculosis

The most common forms of tubercular infection are pulmonary and lymphatic (Steinbock 1976), with skeletal tuberculosis occurring as a secondary infection spread from the two primary forms except in rare cases. Account was taken of the diagnostic criteria presented by Ortner and Putschar (1985), Steinbock (1976), Aufderheide and

Rodriguez-Martin (1998), and Roberts and Buikstra (2003). In summary the basic criteria for a diagnosis of tuberculosis were: the common presence of lesions within the vertebral column, in particular the anterior of lumbar and thoracic vertebral bodies with involvement of one to four adjacent vertebrae; vertebral collapse and anterior concavity of several adjacent vertebrae to those with destructive lesions; cold abscess with limited posterior vertebral body involvement (Aufderheide and Rodrigues-Martin 1998; Ortner 2003; Mann and Hunt 2005); the common presence of lesions in the larger lower limb joints (hip and knee); destructive lesions, which produce cavitation through the cortical bone and into the underlying cancellous bone of the long-bone ends or vertebral bodies in conjunction with limited new bone formation; sequestra are characteristically absent; frequent vertebral body collapse with anterior kyphosis; and spinal involvement is generally limited to a central locus rather than in multiple segments.

The evidence of rib lesions, in particular lesions to the vertebral articulation ends and to the visceral surface with bone enlargement, destructive lesions or evidence of new bone growth was also an important factor. However this diagnostic criterion can also be observed in individuals suffering from severe forms of pulmonary infection and was used as a discriminatory factor only not as a pathognomic indicator for tuberculosis (Roberts and Buikstra 2003). Individuals who showed high amounts of arthritic degeneration (ostephytosis, intervertebral disk displacement) were examined closely and individuals with alterations to the spinal and transverse processes, pedicles and lamina were removed from the tubercular category and classified as non-specific, arthritic or other as they are never or rarely indicated in tubercular pathology.

The vertebral column was the major and primary diagnostic criterion because more than forty percent of all skeletal lesions associated with TB involve some aspect of the spine (Aufderheide and Rodriguez-Martin 1998), conceivably because this section of the human skeletal system has the greatest amount of trabecular bone and has significant haematopoiesis function throughout life (Aufderheide and Rodriguez-Martin 1998). The lower thoracic and lumbar vertebral bodies are the most commonly affected, but cervical and sacral vertebrae can be minimally involved as well (Roberts and Buikstra 2003). Abscesses form within the disk space between two vertebral bodies and disseminate outwards from this point. Both bone formation and destruction are seen in TB lesions; however destructive lesions are more common. Focal resorptive lesions dominate vertebral bodies and joints (Aufderheide and Rodriguez-Martin 1998), while proliferation is more commonly observed on the internal/visceral aspect of the ribs (Roberts 1999; Roberts and Buikstra 2003). The presence of woven bone indicates active lesions at the time of death, whereas sclerotic changes signal a healed inactive disease process.

Other associated pathological conditions can be included, and were used where necessary. These include: clustered pits and superficial cavitation of the flat bones, perforation of the skull (Hackett 1976); abscess or periostitis on the visceral side of ribs; expansion or enlargement of ribs; erosive lesions of the head and neck of ribs; presence of tuberculous dactylitis (spina ventosa), a thickening of the periosteum, followed by destructive lesions and eventual new bone formation under the periosteum resulting in a fusiform appearance (Adams 1986).

### Non-Specific Infection

Non-specific infection is a general term describing infection by a variety of different microorganisms. It can refer to inflammation of the marrow cavity, but also inflammation of the periosteum (periostitis) and of the bone (osteitis). Any part of the skeleton may be involved in this form of pathological infection, but most commonly the long bone metaphases is affected, with the growth plate preventing the spread of infection to the epiphysis or joint (Steinbock 1976). Identification of non-specific forms of infection can follow a number of interpretations based on the origins, location and cause of infection. In general a subperiosteal abscess forms and raises the periosteum over large areas of the cortex, resulting in inflammation and necrosis of the bone tissue and absorption of the trabaculae (Waldvogel et al. 1971; Steinbock 1976). This results in a high degree of bone destruction and subperiosteal new bone formation. Gross morphological indicators include cloaca, irregular bone formation composed of woven or primitive bone, and in the case of long bones a possible separation of the metaphysis from the epiphysis (Steinbock 1976). In short, the long bones appear swollen and rough, with an irregular surface composed of extremely porous bone growth with a "swiss-cheese" appearance. While disseminated skeletal TB can sometimes appear similar to pyogenic osteomyelitis, there are differences in the appearance of the lesions. Diaphyseal involvement in TB is limited, lesions appear more commonly in the epiphysis, or elements directly involved in a joint (Aufderheide and Rodriguez-Martin 1998; Roberts and Buikstra 2003; Steinbock 1976). TB rarely involves the cortex of the bone and lesions are generally smaller. The typical "swelling" of the bone rarely occurs in disseminated skeletal TB.

General or non-specific infection was also the category that isolated lesions, those that were atypical, occurred in the singular, or could not be assigned a specific diagnosis due to taphonomic destruction or incomplete preservation. Lesions involved in this classification occurred throughout the skeletal system, and multiple elements may have been involved, such as in the case of those classified as "moderate" or "major", but lacked any definitive connection to each other.

### Arthritis

Arthritis, particularly degenerative arthritis, can result from a number of pathological conditions including trauma and infectious diseases. It is also one of the most common skeletal pathologies observed in pre-historic and historic populations. It can take many forms: septic, resulting from infection of one or more joints; rheumatoid, inflammation of the connective tissue and resulting deformation of the surrounding skeletal elements; juvenile rheumatoid (Still's Disease); psoriatic which is similar to rheumatoid but is commonly restricted to the hands and feet; ankylosing spondylitis, causing progressive inflammation of the spinal column and the associated diarthrodial elements; metabolic, such as gout; and degenerative (Aufderheide and Rodriguez-Martin 1998; Ortner and Putschar 1981). Degenerative arthritis is the most common form of arthritis (Ortner and Putschar 1981), and this form does not involve inflammation, but instead is linked to activity and aging in an individual. The most common areas of degenerative arthritis include, in decreasing order of frequency, the knee, first metatasophalangeal joint, the hip, the shoulder, the elbow, the acromioclavicular joint, and the sternoclavicular joint (Aufderheide and Rodriguez-Martin 1998; Ortner and Putschar

1981). Initial stages of degenerative arthritis appear at the margins of an articulation and develop into bone spurs commonly referred to as "lipping" and exostoses. Erosion of the cartilage results in articular joint friction causing polished areas known as eburnations, which are surrounded by areas of large and small pits. In ball and socket joints these pits can lead to an enlargement of the socket, irregular shape, altered curvature, and deformation.

Osteophytes may also occur at the area of insertion for tendons, ligaments and muscles affected by the changes occurring at the site of articulation, such as the bony spurs and ridges that occur on the neck and head of the femur when degenerative arthritis is present in the hip joint (Ortner and Putschar 1981). Arthritic changes can co-occur with a number of other conditions, may be the causative agent or a resulting factor of said condition. In many cases, it can be difficult to separate the normal degenerative processes of aging from the arthritic changes, and observation of other factors associated with aging should be recorded.

### Enthesopathies

The enthesis or insertion site of a muscle, ligament or tendon is commonly referred to as a musculoskeletal stress marker or MSM (Chapman 1997). These refer to the distinct skeletal markings that occur where muscle, tendon, or ligament inserts into the periosteum and the underlying bony cortex (Chapman 1997). When subjected to a repetitive stress, the number of capillaries in the periosteum will increase, thereby stimulating osteonal remodelling at the point of greatest stress, resulting in hypertrophy of the bone, and the formation of rugose bony eminences on the exterior (Chapman 1997). A heavily used joint,

limb, muscle, or tendon carries certain signs of behaviour, exhibiting traits such as enthesopathies, bone imprints and modifications, or supernumerary articular surfaces, for example (Boulle, 2001). Enthesopathies are the traumatic insertion or attachment sites for muscles, ligaments and tendons that have been forced beyond natural plasticity and often result in painful and pathological indicators of repetitive behaviour (Kennedy 1989). Enthesopathic lesions were recorded by skeletal element, site of modification and type of modification. Osteophytosis included a general assessment of the size of the osteophyte in comparison to the affected vertebra or vertebrae. No scoring was recorded for enthesopathies, just a present/absent assessment.

### Fusion/Cartilization

Fusion and cartilization were observed during the analyses of other pathological conditions. The site, orientation and degree were recorded for each affected element. Note was taken of fracture and enthesopathic modification in conjunction to the site. Subsequent linkages to other pathological indicators were recorded after the initial examination.

### Fractures

Fracture occurs due to tension, compression, torsion/twisting, flexion/bending, or shearing (Ortner and Putschar 1981) and each causative method can result in a distinct form of fracture. A fracture severs the blood supply in the bone and the muscle tissue surrounding the area of trauma, resulting in a blood clot at the site and eventual vascularisation of a bone callus within days of the fracture event. The callus forms rapidly and can be quite large, the over-abundance of protective new bone is eventually resorbed

by the bone, and the remaining woven bone is eventually transformed into the classic compact cortical bone common to healthy bone. In some cases, especially in contemporary cultures or those with advanced medical care, evidence of a fracture may be completely obliterated. However, if the bone is improperly set, the callus is quite obvious, even after years. The rate of fracture repair is offset by a number of factors including age, the form of fracture, the degree of vascularisation, presence of infection and the degree of stability in the setting of the fractured ends (Ortner and Putschar 1981; Steinbock 1976). Fracture can sometimes result in arthritic changes to the bone, either directly or indirectly, and in the case of compression fracture in the spine, traumatic fracture and arthritic degeneration can sometimes be misdiagnosed (Ortner and Putschar 1981). Arthritic compression fracture is generally accompanied by the formation of osteophytes and arthritic lipping, and is more common in the areas of flexion in the spine (Merbs 1969).

### Neoplastic Conditions

Pathologies related to cancerous conditions are difficult to assess in palaeopathological and archaeological specimens and are often unrecorded or under-recorded in assessments (Brothwell 1967). Many different forms of neoplasm can occur throughout the skeleton, and careful consideration was given to the location of the lesion and the condition of the affected element. The preservation of the element, including its exposure to possible taphonomic damage (i.e. wind, water, sun exposure, root or pest damage) was assessed, if the element was in good condition and lacked indicators of taphonomic damage in the affected area, as well as other associated elements, taphonomic damage and pseudopathology could be excluded as possible diagnoses (Cassidy 1977).

Lacking radiographic and thin section analysis, macroscopic and gross analysis must be relied on for assessment. To exclude the possibility of infection, the bone in the affected area must not present evidence of swelling; the infectious agent would have to produce a single isolated lesion, and in most cases, a high degree of bone loss. There should be no evidence of trauma or any other form of pathology in association with the area. Included in this assessment category are individuals who manifest tumour growth, as the nature of the growth, either benign or malignant, cannot be assessed.

### Dental Pathologies

Two dental pathologies were included in the palaeopathological inventory: abscesses and pre-mortem tooth loss (PMTL). When maxillary and/or mandibular dentitions were present, a record of the affected teeth and the associated quadrant were recorded for either dental pathology. Resorbtion was noted when it had occurred significantly. No disarticulated teeth were recorded, nor were sockets lacking in any indication of remodelling, because this may indicate a post mortem loss. Similarly, abscessing was recorded for quadrant (e.g. Left maxillary) and by tooth or teeth where possible. Size of abscess was recorded only in individuals where a significant loss of bone or teeth had occurred. No cause for either abscessing or PMTL was recorded at the time of observation, unless other pathological indicators occurred in the same area or in conjunction with the dental pathology.

### Rickets

Rickets is a systemic disease of early childhood that greatly affects the skeleton (Ortner and Putschar 1981). It results from inadequate osteoid mineralisation and causes a disturbance in the formation of bone in the growing skeleton. The most common cause of rickets is vitamin D deficiency (Steinbock 1976). Diagnosis of rickets requires the observation of a number of criteria. In general the bone feels extremely light and may be of brittle texture; commonly the cortical layer of bone is thickened while the marrow cavity is thinned. In the long bones, particularly in the femur, the normal curvature becomes exaggerated accompanied by a lateral bowing, the neck of the femur may become bent. The cortex along the concave portion of the bow becomes thickened to reinforce the changed tension and weight distribution. This is often referred to as the classical indicator of rickets infection (Ortner and Putschar 1981; Steinbock 1976).

The variation and frequency of pathological conditions represented by the pre and post contact populations was utilized to assess changes in disease load, behaviour patterns affecting health, and the impact of European contact on Canadian Inuit populations. The examination of skeletal pathologies between two temporal populations produces a longitudinal survey of health fluctuations that can be compared to contemporary Inuit health conditions.

# Chapter 5 Molecular Analysis

Three forms of DNA were examined in the course of this study: mitochondrial (mtNDA), nuclear (nDNA) and microbial. Mitochondrial DNA was analysed as a method for determining haplogroup and haplotype, this enabled the establishment of Inuit ancestry for the molecular samples. Individuals that demonstrated sequences associated with haplogroups not previously established as Inuit or published as "non-Inuit" haplogroups were excluded from overall discussion and analysis of possible population conclusions. Nuclear DNA analyses were conducted in two ways, to confirm osteological sex and to assess immunogenetics through SNaPshot analysis. The nuclear DNA analysis provided the molecular information to examine the Inuit immunogenetic profile, specifically what phenotypes were present across the territory and through time. This established whether Inuit experienced a changing immune profile (e.g. Th1 vs. Th2) and provided information about population susceptibility to certain infectious conditions. Such information might inform our understanding of the determinants of TB and other conditions in contemporary Inuit populations. Microbial DNA was utilised to establish the presence of *M. tuberculosis* within the sample population, in an attempt to establish the presence of TB in pre-contact Inuit.

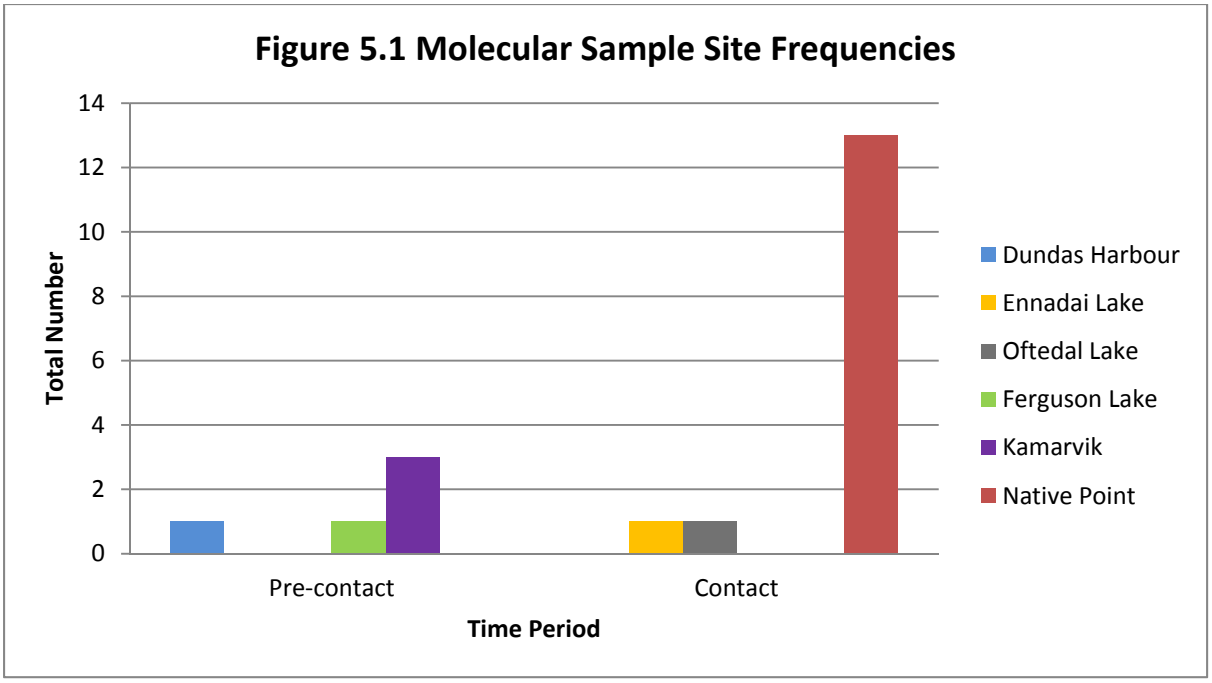
The cohort of individuals used for the immunogenetic analysis consisted of a group of 20 individuals chosen for their status as possible tuberculosis candidates, as well as for their high preservation quality. Of the 20 individuals, eleven (n=11) had manifestations of tubercular pathologies and had sufficient preservation. An additional nine individuals

without tubercular pathologies were included in the analysis because they had excellent preservation.

Either a rib with possible tubercular pathologies or a molar with no visible cracks or caries were selected from each individual. Where possible, both a rib and a molar were chosen to aid in replicating and confirming results from either element. The ribs were chosen not only because they exist in multiples in each individual but also because they are also one of the most likely skeletal elements to develop tubercular pathologies given their proximity to the lungs.

The molecular sample included individuals from six different archaeological sites: thirteen Sadlermiut individuals from the Native Point site on Southampton Island, three individuals from Kamarvik on the Western shore of Hudson Bay, and a single individual from each of Oftedal Lake, Ennadai Lake, Ferguson Lake, and Dundas Harbour (Figure 5.1 and Table 5.1). While osteological sex was assigned, discussion and admission of individual sex determination will be discussed in the results chapter for this portion of the sample. A review of the overall demographics of the sample as an entirety can be found in Chapter Four. Three adolescents and 17 adults were included; individuals were neither included nor excluded based on osteological sex, nor was the burial site or population important for inclusion. Contact status was essential, and an effort was made to include individuals from both the pre-contact and contact eras: 15 individuals from three sites were from the contact era and 5 individuals from three sites were from the pre-contact era (Table 5.1).

| Table 5.1 Molecular Sample Demographics | | | | |
|---|---|---|---|---|
| **Individual** | **Site** | **Temporal Period** | **Sex** | **Age** |
| XIV-C: 246 | Sadlermiut/Native Point | contact | M | Adult |
| XIV-C: 744 | Sadlermiut/Native Point | contact | F | Adult |
| XIV-C: 316 | Oftedal Lake | contact | F | Adolescent |
| XIV-C: 164 | Sadlermiut/Native Point | contact | M | Adult |
| XIV-C: 607 | Kamarvik | pre-contact | M | Adult |
| XIV-C: 221 | Sadlermiut/Native Point | contact | F | Adult |
| XIV-C: 149 | Sadlermiut/Native Point | contact | F | Adult |
| XIV-C: 179 | Sadlermiut/Native Point | contact | F | Adult |
| XIV-C: 328 | Ennadai Lake | contact | M | Adult |
| XIV-C: 191 | Sadlermiut/Native Point | contact | M | Adolescent |
| XIV-C: 197 | Sadlermiut/Native Point | contact | F | Adult |
| XIV-C: 217 | Sadlermiut/Native Point | contact | M | Adult |
| XIV-C: 152 | Sadlermiut/Native Point | contact | M | Adult |
| XIV-C: 183 | Sadlermiut/Native Point | contact | F | Adult |
| XIV-C: 216 | Sadlermiut/Native Point | contact | M | Adult |
| XIV-C: 111 | Sadlermiut/Native Point | contact | M | Adult |
| XIV-C: 636-1 | Kamarvik | pre-contact | I | Adolescent |
| XIV-C: 041 | Ferguson Lake | pre-contact | F | Adult |
| XIV-C: 621 | Kamarvik | pre-contact | F | Adult |
| XIV-H: 015 | Dundas Harbour | pre-contact | F | Adult |



## Molecular Methods

There were many varieties of protocols implemented during the course of these analyses; however, the initial stages of sample preparation, sterilisation, extraction and pre-PCR purification were identical regardless of DNA type or final analysis method. Three forms of DNA were analysed: nuclear, mitochondrial, and microbial DNA; and while only a single analysis method was used for the final analysis of the mtDNA, depending on the final amplicon required, two different protocols were utilised during the course of DNA analysis. Nuclear DNA was analysed for sex identification through analysis of amelogenin, as well as for immunogenetic profiling. Both of these analyses required different protocols to ensure their specific outcome. The specific methodology for the immunogenetic analysis required the implementation of a special form of nuclear DNA analysis minisequencing called SNaPshot analysis (Turner et al. 2002). The mitochondrial analysis implemented a sequencing reaction and analysis that resulted in an mtDNA profile which was used to assign a haplogroup, and thus estimate the ancestry of the individual. Final analysis included the amplification of microbial DNA through PCR amplification and sequencing to determine the specific strain of MTB present in archaeological Inuit populations.

### Primer Design and Optimization

Primer design and optimization for immunogenetic analysis was carried out at the Clinical Investigations Laboratory of the University of Manitoba prior to the commencement of all aDNA analyses at a geographically isolated secondary laboratory, the Paleo-DNA laboratory at Lakehead University, Thundery Bay, Ontario. Cytokine SNP primers were tested on modern DNA samples with successively reduced DNA

concentrations meant to mimic the degraded and fragmented nature of aDNA samples. Primers were designed to amplify the promoter regions surrounding the single nucleotide polymorphism locus of four specific cytokines. Cytokines included IL-6 (-174), Il-10 (-1082), IFNγ (+874) and TNFα (-308) and primers were designed to amplify an amplicon of between a 75 to 330 base pairs; the smaller the amplicon, the more easily, successfully and accurately amplifiable the product. Forward and reverse primers for each SNP were designed, as well as an extension primer for use during the final SNaPshot analysis (Table 5.2 )(Turner et al. 2002). The extension primers consisted of a single primer (for this research a forward primer) that amplified a fragment within one base pair of the targeted polymorphism and was limited to primers of between 19 and 22 base pairs in length. For each cytokine, the extension primer extended base pairs adjacent to the polymorphic site without incorporating the SNP itself.

***Table 5.2 Cytokine Genotyping Primers***

| | IL- 6 -174 | IL- 10 -1082 | IFN gamma +874 | TNF alpha -308 |
|---|---|---|---|---|
| ***Forward Primer Sequence 3'- 5'*** | CTT CGT GCA TGA CTT CAG C | ATC CAA GAC AAC ACT ACT AA | GCT GTC ATA ATA ATA TTC AG | CCA AAA GAA ATG GAG GCA ATAG |
| ***Reverse Primer Sequence 3' to 5'*** | CAC CCT CAC CCT CCA AC | CTC CTA TCC AGC CTC CAT GG | GAA ACC TGT ACC ATT GGG | CGG AAT CGG AGC AGG GAG GA |
| ***Extension Primer*** | AGC TGC ACT TTT CCC CCT AGT TGT GTC TTG C<br>TTC CCC CTA GTT GTG TCT TGC | CTA CTA AGG CTT CTT TGG GAA | TTA CAA CAC AAA ATC AAA TC | AAT AGG TTT TGA GGG GCC ATG |

## aDNA Extraction and Purification

### Sample Preparation

Sample preparation, including pre-analysis and pre-extraction sterilization, was utilized to offset potentail sources of contamination during curation (for museum storage conditions please see pg. 76). The molecular analysis was performed at a geographically separated and dedicated laboratory, the Paleo-DNA Laboratory, Lakehead University, Thunder Bay, Ontario. All rib and molar samples were examined prior to arrival at the Paleo-DNA laboratory with gloved hands on a sterile surface; individuals were not examined together, and were packaged separately in sealed sterile bags labelled with individual identification information in preparation for transportation from the museum. The samples were stored in a cool, dry place away from direct sunlight prior to genetic analysis. Upon arrival to the Paleo-DNA laboratory, the containers for each sample were surface sterilised with a 10% bleach solution, then wrapped in tinfoil and surface irradiated with UV light, before entering the ancient DNA clean lab. Separation of extraction and pre and post PCR procedure rooms, as well as a system of air locks, UV irradiated pass-thrus, and a one way hallway between the clean lab and the post PCR imaging and sequencing labs ensured that modern DNA was not brought into the dedicated aDNA portion of the laboratory. Standard DNA "uniforms" were used to control the possibility of technician contamination: hairnet, nitrile gloves, Tyvek suits, masks, lab glasses, sleeves, booties, and an additional set of gloves are worn at all times inside the clean lab. The use of an air shower was also mandatory before entrance to the lab itself, and then again for movement between rooms. The fume hoods, benches, pipettes and all

surfaces were sterilised before and after each experiment, and the lab spaces and equipment were wholly sterilised several times in a week.

Before extraction protocols could be implemented, it was necessary for the samples to be prepared as required for each analysis protocol. The twenty individuals that compose the genetic sample of this study are individuals who were excavated prior to the 1980s, and therefore contamination protocols at excavation were unlikely to have been implemented. Contamination with exogenous DNA, either at the time of excavation or during museum curation was highly likely, therefore it was necessary for surface sterilization to be carried out prior to the commencement of the extraction procedures. Two forms of skeletal elements were utilised as samples in this study, seventeen ribs and ten intact molars from 20 individuals. The ribs required the use of a Dremmel drill and sterile blade to remove approximately 1 to 2 inches of the sternal end of the rib. After each use a new blade was installed and the drill and work surface were sterilised. Molars were powdered whole and therefore required no preparation at this stage.

Once the sample had been excised from the larger specimen, the portion to be powdered was placed in a sterile, labelled container. To ensure that all surface contaminants were removed a series of chemical baths were employed to remove exogamous DNA and dry the cortical bone. A 10% bleach solution, followed by scrubbing with a sterile cloth and a second wash and soak in sterilised DNA free water, ending with a 30 second soak and scrub in 70% ethanol. The bone was left to air-dry in a fume hood overnight.

## Protocols Common to all Methods

### Extraction

After sample preparation the samples were powdered whole and extracted utilising the total demineralisation protocol described in Edson et al. (2004) followed by a Proteinase K extraction; this method is preferable for both sample media as it ensures the highest possible amount of DNA template is available for extraction. Approximately 200 mg of sample was added to 0 1.3mL of 0.5M of EDTA, 75µl of 20% sodium lauroyl sarcosinate (sarkosyl )and between 40 and 100 µL of Proteinase K (amount dependent upon the amount of powdered sample used). The reaction was sealed with parafilm and incubated overnight at 56ºCelsius at 1000 rpms.

### Purification

A two stage purification protocol followed extraction to remove chemical inhibitors, beginning with a silica bead purification (Boom et al. 1990; Hoss et al. 1993; Hoss and Pääbo 1993). This method employs silica to bind the DNA molecules and a series of washes to remove proteins and contaminants, which would otherwise interfere with the isolation of purified DNA. The high salt concentrations found within the reaction enables the DNA to bind to the silica, while contaminants are discarded with the supernatant. 3mL of guanidine thioisocyanate and 15 µL of silica beads are suspended with extracted supernatant and refrigerated for at least one hour. A working wash buffer is added and agitated to resuspend the silica and the supernatant is discarded. If the required clarity is not yet achieved additional washes may be performed. A final wash of 200 µL of 100% ethanol was employed and repeated if the sample appeared cloudy or opaque or if particles

of silica were still visible in the liquid. The ethanol was aspirated and the sample left to air-dry overnight, desiccating the sample and removing any liquid residue that may inhibit or reduce DNA concentration. To resuspend the purified silica sample, 50-100 µL of sterile water was added to the dried reaction and incubated on a thermomixer at 56 ºCelsius for at least one hour at between 300-400rpm and the silica and purified sample separated. An additional purification, Micro Bio-Spin Columns with Bio-Gel P-30 in Tris Buffer, was utilised to ensure lower risk of inhibition during amplification, particularly to ensure that no silica remained within the template and carried out according to the manufacturer's recommendations (BioRad, Hercules, CA, USA).

### Amplification

Amplification was achieved through polymerase chain reactions (PCR). PCR reactions have certain chemicals which have fixed ratios within each reaction, while the DNA template and dd$H_2O$ amounts varied. Platinum Taq DNA polymerase was used in all initial PCR amplifications, and the following protocol (Table 5.3) is the foundational protocol for all other amplifications.The amounts of buffer, dNTP, primers, $MgCl_2$, BSA and polymerase remain constant. BSA was added in reactions to counteract humic acid inhibition that is a frequent problem in ancient DNA PCR reactions (Tuross 1994; Cheng et al. 2003). Other additives include Q solution, DMSO, SybrGreen, and Formamide, which can be added to counteract a variety of problems encountered during the PCR process.

| Table 5.3 Platinum Taq Polymerase Basic Protocol | |
|---|---|
| **10X PCR Buffer** | 2.5 µl |
| **10 mM dNTP Mix** | 0.5 µl |
| **Forward Primer** | 0.25 µl |
| **Reverse Primer** | 0.25 µl |
| **50 mM $MgCl_2$** | 1.0 µl |
| **Platinum Taq Polymerase** | 0.1 µl |
| **BSA additive** | 0.5 µl |
| **DNA Template** | Varies (2-20 µl) |
| **$ddH_2O$** | Up to 25 µl* |
| **Total Amount of Reaction** | **25 µl** |

*$ddH_20$ amounts vary in conjunction with the amount of DNA template used

A master mix was utilised to ensure that equal amounts of all chemicals were aliqouted into each reaction. The final volume of each chemical was derived by multiplying the above amounts by the number of samples being analysed. The DNA template and $ddH_2O$ were adjusted on the basis of prior reaction outcomes. For example, the lack of a visible band after electrophoresis resulted in the increase of DNA template and a reduction in $ddH_2O$ amount. The DNA template was added to the individual reactions after the master mix had been aliquoted to reduce the possibility of contamination. This process is performed at a dedicated bench separate from the pre-PCR bench.

For each PCR a negative extraction control, a PCR negative control and a diluted normal control of modern DNA reduced to an approximate aDNA concentration were included to ensure the accuracy of each stage of the experiment. The modern normal control was added in the thermal cycling room after exiting the dedicated aDNA portion of the lab, to ensure no contamination by ancient and modern DNA occurred. Additionally, the researcher's mitochondrial sequence was amplified and analysed as a further control, resulting in the assignment of the N haplogroup. Thermal cycling parameters were also variable dependent upon the type of reaction being conducted, as well as the primers being

utilised. The initial parameters used for PCR amplification included a hot start at 94ºC for 1 minute, followed by a denature cycle at 94ºC for 30 seconds, an annealing cycle at 55ºC for 30 seconds, and an extension cycle at 72ºC for 1 minute, and a final extension cycle of 72°C for 2 minutes for 50 cycles (49 repeats) before being held at 4ºC. For a full accounting of the cycling parameters used during research, please see Appendix 2.

### Visualization- Gel Electrophoresis

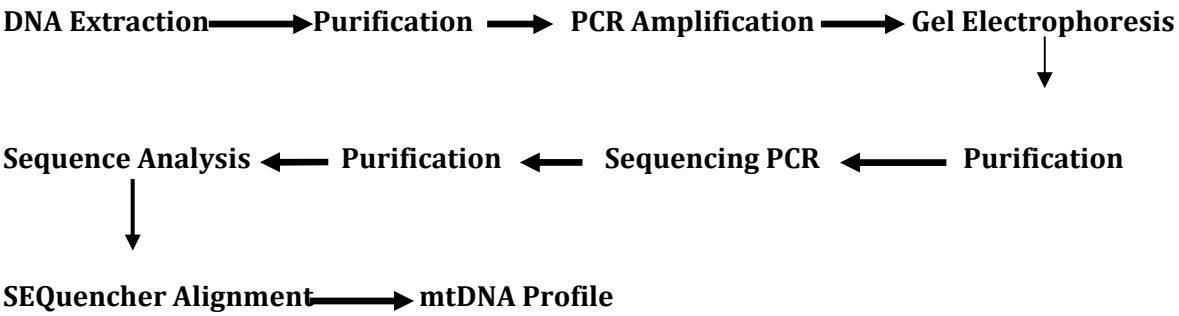
Following PCR amplification, products were run on 6% polyacrylamide gels (PAGE) at 118 volts for 45 minutes. Five (5) µl of product were mixed with three (3) µl of 6X loading dye and loaded into a gel lane and run along with two (2) µl of ultra low range ladder that measured up to 300 base pairs. After electrophoresis the gels were placed in an ethidium bromide stain for 20 minutes and then placed on the transilluminator, viewed on wavelength B UV light and digitally photographed. Electrophoresis was carried out in the sequencing/Post PCR laboratory, and visualisation occurred in a specialised room off the post-PCR laboratory. PCR reactions were repeated if the amplicon failed to visualize or was not in the target region.

## Mitochondrial DNA Protocols and Analysis (Haplogroup determination)

With aDNA it is necessary to reduce the amplicon size and use multiple smaller target regions to ensure amplification. For this research, mitochondrial sequencing (protocol summarized in Figure 5.2) utilized , a set of four primers (See Appendix 1) was utilised during the PCR stage, two resulting in overlapping amplicons in HVR I of the mitochondrial genome, and two resulting in amplicons in HVR II of the mitochondrial

genome. Each of these primer sets employed a forward and reverse primer, and required a separate PCR resulting in four amplifications and four amplicons While the HVR I is sufficient for confirming ancestry, the amplification and sequencing of HVR II acts as confirmation and can be employed to more minutely differentiate haplogroups and haplotypes.

**Figure 5.2 Mitochondrial DNA Analysis Protocol Summary (Sequencing)**




PCR chemicals and primers were removed from the samples with a post-PCR purification method not employed in the sex determination analysis and different from the one employed in the SNaPshot analysis. The QIAcube (QIAgen Inc. Toronto, Canada) automated purification system employs the same protocols as QIAquick spin column (QIAgen Inc. Toronto, Canada) purification. The automated system was used following manufacturers protocols and suggested guidelines.

### Sequencing reaction

The forward primer from each primer set was implemented in conjunction with the Big Dye Terminator Cycle Sequencing protocol to sequence the four mtDNA amplicons necessary for establishing a haplogroup from an mtDNA profile. The sequencing reaction was carried according to the supplied Big Dye Terminator sequencing protocol (Applied Biosystems, Foster City, CA), with a single exception. The amount of purified PCR product

utilised was determined based on the strength of the visualised band; the brighter the band the higher DNA concentration and therefore lower amount of purified PCR product needed. Two different concentrations were used during sequencing: for bright bands, a standard amount of 3 µL of product was used, and for samples with a faint band indicating lower concentrations of amplified product, 5 µL were used. For each sample two different sequencing reactions were employed, one each for the forward and reverse primers, resulting in two overlapping amplicons of the same target region. This allowed for a greater degree of flexibility in determining the represented peaks, especially if resulting sequences contain damaged regions. The reactions were run on the thermal cycler with Paleo-DNA lab specific protocols (Appendix 2 PDLSEQ). After sequencing purification protocols were employed to remove sequencing chemicals prior to loading on the ABI 3100.

### Ethanol sodium acetate precipitation and Dye-Ex Purification

Ethanol sodium acetate precipitation was employed to remove the dyes and primers used during the sequencing process and desiccate the samples so that no water remains to inhibit the capillary electrophoresis process. Three µL of 3M sodium acetate at a pH of 5.4, along with 62.5 µL of 95% ethanol were combined with 20 µL sample reactions, and let sit at room temperature for 20 minutes. The tubes were then placed in a microcentrifuge, with their orientations very carefully marked, and spun at 13,000 rpms for twenty minutes. The supernatant was then carefully aspirated; the pellet resulting from the solution will be resting on the tube side orientated to the outside of the microcentrifuge, pellets are commonly not visible. 250 µL of 70% ethanol was added and

once again spun at 13 000 rpms for five minutes. After aspirating the supernatant, the mostly liquid free tubes are then placed in a vacuum centrifuge for ten minute intervals on medium heat, until no moisture is evident. Moisture inhibits the capillary electrophoresis, so the desiccation is performed in stages to ensure the complete removal of moisture without over drying. The Dye Ex methodology utilises spin column chromatography (Sambrook et al. 1989) and was performed according to manufacturer's specifications (QIAGEN Inc, Mississauga, Canada).

### ABI 3100 Sequence Analysis

Each purified and desiccated sample was suspended in 15 µL of Hi-Di Formamide and vortexed for one minute, ensuring that the formamide was kept near the bottom of the tube. The samples were denatured at 95ºC for three minutes, and then chilled on ice or in the freezer for two minutes. Fifteen (15) µL of the sample was loaded in the ABI plate in series of 16 sample runs and run on the ABI 3100 with run parameters set according to the analysis process and capillary type. Mitochondrial sequences were analysed twice to ensure accuracy of results. The initial analysis was performed with Sequencher DNA sequence analysis software (Gene Codes Corp, Ann Arbor, MI) and compared to existing mitochondrial sequences (www.mitosearch.org). Mitochondrial sequences were aligned by hand and mutations analysed using Bioedit v7.1.3. (Hall 2011) and compared to existing databases to verify haplogroup assignment (www.mitosearch.org). Further differentiation of haplotypes was performed for the A haplogroup results with existing literature (Helgason et al. 2006).

## Nuclear DNA Analyses

Two methods of nuclear DNA analysis were utilised in this research. The initial method utilised the analysis of the amelogenin gene for sex determination. This form of analysis was the preliminary step to confirm the viability of nuclear DNA from the different samples by confirming a sufficient amount of amplified product. Two individuals (XIV-H:015 and XIV-H:041) resulted in no readable or determinable sex and were deemed too damaged and fragmented to proceed with further analysis. The second method of nuclear DNA analysis was utilised to assess single nucleotide polymorphisms of the immune system, in particular those related to tuberculosis immune response. SNaPshot analysis (Applied Biosystems, Foster City, CA) is a commercial minisequencing kit that uses extension primers and fluorescently labelled ddNTPs to tag target polymorphic sites.

### Sex Determination

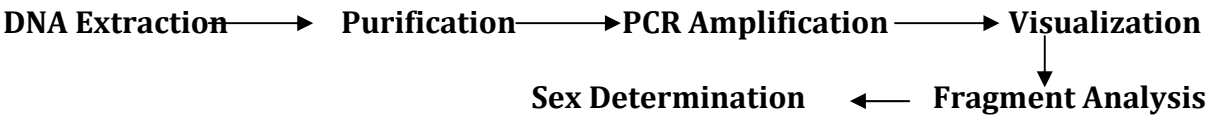
To determine sex a DNA fragment was amplified with a forward primer (AMEL 1F) and a reverse primer (AMEL 2R). This resulted in a small amplicon which demonstrated either two peaks for female or one peak for male. A homozygous X genotype, which illustrates female, peaked only once at 109nt, whereas the heterozygous male determination peaked twice, at 111nt and 103nt. Three rounds of amplification for sex determination were performed. In each round, samples that failed to visualize were re-amplified using a progressively larger amount of DNA template (5, 10 and finally 15 µL). Cycling parameters remained constant through each round of PCR amplification (Appendix 2). Following amplification, products were run on 6% PAGE gels at 118 volts for 45 minutes. Five (5) µl of product were mixed with three (3) µl of 6X loading dye and loaded

into a gel lane. The fourteen or less individual products were run along with two (2) µl of ultra low range ladder that measured up to 300 base pairs. The gels were then stained with ethidium bromide for twenty minutes before being visualised.

### Fragment Analysis

For sex determination, fragment analysis is carried out directly after visualisation (Figure 5.3). One µL of the samples with a visualised band were loaded with 0.2 µL of size standard and 9 µL of Hi-Di Formamide. The reactions were then denatured at 95°C for three minutes and cooled for two minutes before being loaded and run according to ABI 3100 run parameters. The chromatographs were then analysed using the Genemapper program v.4.0 (Applied BioSystems, Foster City, CA). Controls were analysed along with amplified product to ensure the accuracy of results. Analysis protocols are summarized for this method in Figure 5.3.

**Figure 5.3 Sexing Analysis Protocol Summary**

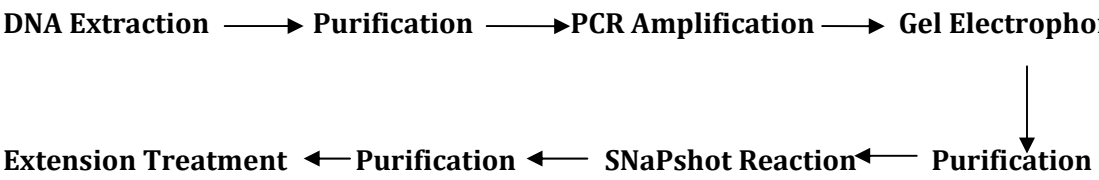



### Cytokine Genotyping with SNaPshot Analysis

The second form of nuclear DNA assessment was SNaPshot microsatellite analysis, sometimes referred to as minisequencing (Figure 5.4). Prior to SNaPshot analysis it was necessary to determine the concentration of the DNA template to ensure that a sufficient

concentration was present and to correct the reaction aliquots accordingly. This was done through the employment of a flurometer and quantification. Quantification is carried out after the initial PCR and prior to SNaPshot analysis and was employed to ensure that the ratio of chemicals utilised during the SNaPshot protocol were optimized for readable and accurate results. A final concentration between 0.01 μL/mg and 0.40μL/mg was optimal.

**Figure 5.4 SNaPshot Analysis Protocol Summary**




SNaPshot analysis uses two distinct forms of amplification to analyze the target polymorphism. The initial standard PCR reaction utilised specially designed primers (Table 5.2) to amplify a target region around the polymorphic site and utilised cycling parameters specially designed for optimal outcome. Cycling parameters (Appendix 2) for IL-10 (-1082), IL-6 (-174) and TNFα (-308) were the same, but INFγ (+874) required a higher annealing temperature (58°C) to accurately amplify. Similar to other protocols a visualization step was needed to ensure that each sample resulted in the required amplicon. Initial PCR products were run on 6% PAGE gels for 45 minutes at 118 volts, then bathed in ethidium bromide for 20 minutes before being imaged under a UV light and digitally recorded. Samples with a clear band were then purified.

The SNaPshot protocol utilised its own distinct purification processes for both pre-SNaPshot and post-SNaPshot products. Post PCR purification required treatment with an ExoI and Shrimp Alkaline Phosphate (SAP) blend and then activation at 37°C for 60

minutes and deactivation at 75°C for 15 minutes prior to the SNaPshot protocol. A single extension primer was used to detect the target single polymorphic nucleotide position from the first PCR amplicon. SNaPshot reactions amplify 17 to 22 base pairs fragments and fluorescently label the polymorphic site, which is recognised as the first base pair immediately adjacent (prior) to the extension primer. A post reaction purification treatment was required to remove chemical residues of the reaction and all non-specific DNA such as primer residues.

The SNaPshot reactions require a combination of the provided SNaPshot Ready Reaction Mix (Applied Biosystems, Foster City, CA), depending upon the concentration of the samples, a 3-5 μL PCR product, the larger amount for smaller concentration and vice versa and the designed extension primer. This mixture was briefly vortexed, spun down and immediately placed on ice. The reactions were then placed in the thermal cycler (Tetrad A1), cycled according to product protocols (Applied Biosystems, Foster City, CA: Appendix 2). Cycling parameters can be adjusted as needed for individual primers, or to troubleshoot for reactions resulting in non-definable peaks.

After the SNaPshot reaction, the extension primers and chemicals used must be removed prior to analysis with the ABI 3100. A treatment with either Shrimp Alkaline Phosphate (SAP) or Bovine Serum (BSA) is recommended by the manufacturer. Because an Exo I and SAP pre-treatment is required, SAP was available and efficient for this purpose. The kit extension treatment and analysis protocols (SNaPshot analysis, Applied BioSystems, Foster City, CA) were followed.

The resulting chromatographs were labelled as follows: A = green peaks, C = Black peaks, G = Blue peaks, and T = Red peaks. Results were manually assessed and assigned a genotype based on the demonstrated peaks.

## Detection of Mycobacterium tuberculosis

Microbial analysis was carried out on samples identified as having pathological lesions associated with tuberculosis (n=9) as previously described on pages 88-90. For a description of individuals with observed TB lesions please refer to Appendix 3. As per agreement with the Canadian Museum of Civilization and Inuit Heritage Trust, samples consisted of a molar or rib fragment obtained from the proximal shaft. Therefore, samples were not obtained at the sight of the lesion, but instead were a general sample from an individual who manifested pathological modifications either on the sampled rib, or on the remains in the case of molars. Microbial DNA protocols follow the same protocols common to all analyses for this project. Tuberculosis primers IS1F and IS2R (Appendix 1) provided by the Paleo-DNA Laboratory were utilised to amplify a 123bp product. . Analysis generally requires a much more conservative amplicon length, somewhere between 70 to 100 base pairs (Donoghue 2010 personal communication). Thermal cycling protocols followed a standard employed by the laboratory for aDNA samples (Appendix 2). Gel electrophoresis was performed to verify the presence of microbial DNA. Those that amplified a band within the expected range were recorded as a positive for pathogenic DNA and were re-amplified to verify results with the same abovementioned protocols. To verify the "positive" results and attempt to identify the strain of MTB, sequence analysis was performed on the amplified product. The chromatographs were then analysed using the

Genemapper program v.4.0 (Applied BioSystems, Foster City, CA) and verified with a BLAST analysis.

# Chapter 6 Results

## Osteological and Palaeopathological Results

The sample was assessed for ten pathological conditions consisting mainly of chronic diseases, as acute conditions are rarely manifested in the skeleton. Acute conditions generally resolve themselves before bone involvement or result in a quick death inhibiting dissemination throughout the skeletal system. Chronic conditions, while generally not lethal, can commonly affect skeletal elements due to the duration of pathogenesis. The ten conditions assessed include: two forms of dental pathology, 1) pre-mortem tooth loss and 2) mandibular or maxillary abscessing 3) tuberculosis (TB), 4) arthritis, 5) enthesopathic musculoskeletal markers, 6) fusion of bone or cartilage, 7) fractures, 8) neoplastic conditions, 9) rickets and 10) general infection (non-specific) for pathological modifications of a non-specific variety. The final category is neither chronic nor acute, and contains individuals manifesting pathological modifications that could not be assigned to a specific condition due to preservation, including damaged or missing elements and a lack of sufficient pathological criteria.

### General or non-specific Infections

In total 88 individuals from the osteological sample manifest a form of general infection. Of these, 61 (69%) are from the post-contact population and nine different population subsets and 27 (31%) from the pre-contact period and eight different population subsets (Figure 6.1). Notably, in the pre-contact period 17 (63%) of these

individuals were from the Kamarvik subset. Five individuals, four males from the post-contact period and one pre-contact female manifest what are described as "major" non-specific infections. These major infections occur in five skeletal elements; one individual manifests a major infection in the tibia, another on the pubis and pubis symphysis; the third and fourth individuals have lesions on the cranium and major infection and inflammation of the fibula; the final individual shows extreme inflammation and infection of the fibula alone. Nineteen individuals manifest "moderate" general infections, three pre-contact male and females each, 13 post-contact of which six are males, five are females and two are of indeterminate sex. The pelvis is the most common area of moderate non-specific infection (Table 6.1 and Figure 6.1), and pathological modification includes lesions of the ilium, auricular surface and pubis; bone deposition of the auricular surface, and woven bone in the acetabulum. The humerus and scapula are also common areas of moderate infection with 8 and 7 individuals respectively, manifesting pathological modification of these elements. By far the largest category in general infections is the "minor" non-specific category. Sixty-six individuals manifest minor non-specific infections, 44 post-contact individuals and 22 pre-contact individuals. The post-contact population is evenly divided between males (n= 20) and females (n= 20) with the remaining individuals being indeterminate. The pre-contact population is slightly more heavily weighted by males (n= 13) than females (n= 9). The post-contact population has two areas that are commonly affected by minor non-specific infections, the pelvis (n=22) and the humerus (n=14), both of which have equal expression in both males (n=11 and n= 7) and females (n= 11 and n= 7) respectively. In the pre-contact period the pelvis (n=10) is again one of the more commonly affected areas, but the second most common is the femur (n=6).

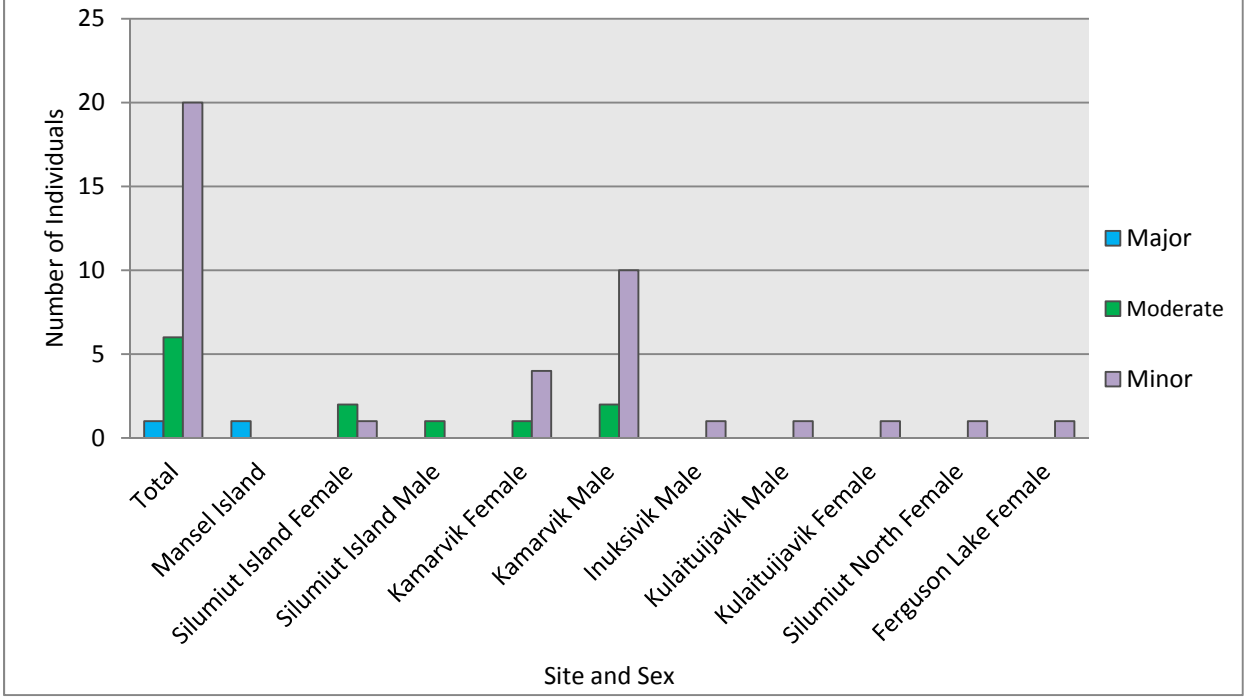
Within the study population males and females appear equally likely to manifest infections of the pelvis, whereas the pre-contact males are more likely to develop infections in the femur (n= 4) compared to pre-contact females (n= 2).

Overall 14 skeletal elements demonstrated pathological modification in the "general infections" category (Table 6.1 and Figure 6.3). The pelvic girdle, including the innominate, pubis and ischium, was the most commonly affected element. Fifty-five individuals (61%) demonstrated lesions in one of the three areas of the pelvic girdle. The next most common skeletal element is the humerus, which shows a dramatic decrease in occurrence compared to the pelvic girdle, with only 32 (36%) individuals with general infections manifesting lesions on this element. The scapula is the next most common, with 21 (23%) individuals demonstrating involvement, and infection co-occurs between the scapula and the humerus of the same side, 9 times out of 21 individuals (43%) affected. The sacrum exhibits lesions in 19 individuals, with the sacro-iliac articulation and first sacral vertebrae body being the most common area affected. The sacrum and the pelvic girdle manifest co-infections in twelve individuals, 22% of pelvic infections and 62% of sacral infections. Table 6.1 presents the frequencies for all skeletal elements.

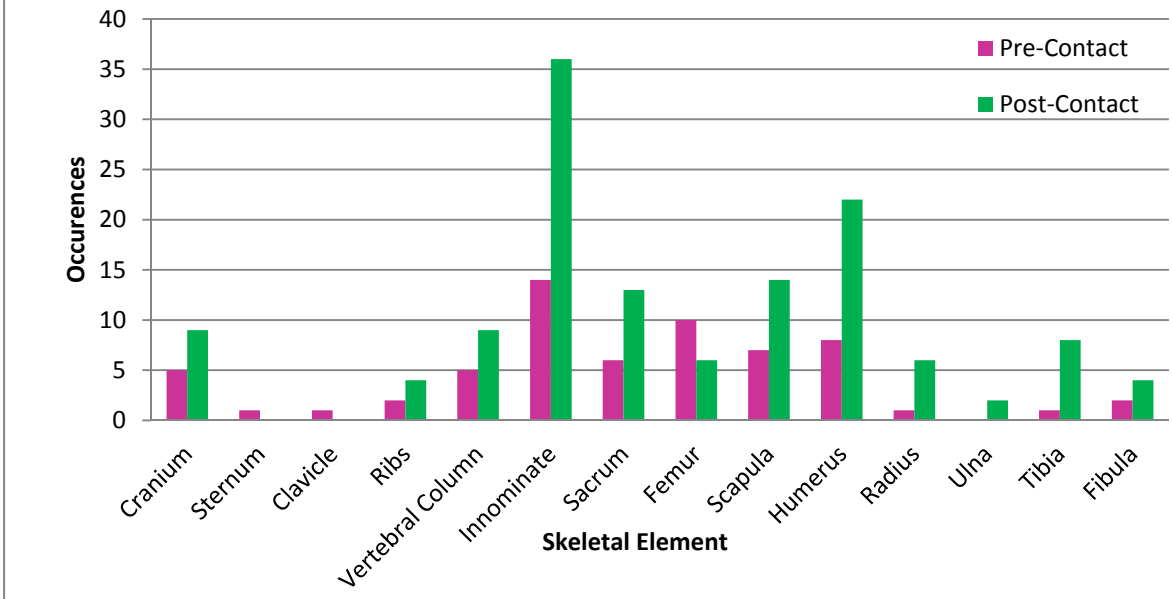
| Table 6.1 General Infection and Co-involvement Frequencies | | |
|---|---|---|
| ***Skeletal Element*** | **Number of Occurrences** | **Notes** |
| ***Pelvis*** | **55*** | |
| ***Humerus*** | **32** | |
| ***Scapula*** | **21** | ***9/21 co-involvement with same side humerus*** |
| ***Sacrum*** | **19** | ***12/19 co-involvement with element of the pelvic girdle*** |
| ***Femora*** | **16** | |
| ***Vertebral Column*** | **14** | ***Isolated lesions*** |
| ***Cranium*** | **13** | ***Includes calvaria and facial bones*** |
| ***Tibia*** | **9** | |
| ***Radius*** | **6** | |
| ***Ribs*** | **6** | |
| ***Fibula*** | **6** | |
| ***Ulna*** | **2** | |
| ***Sternum*** | **1** | |
| ***Clavicle*** | **1** | |

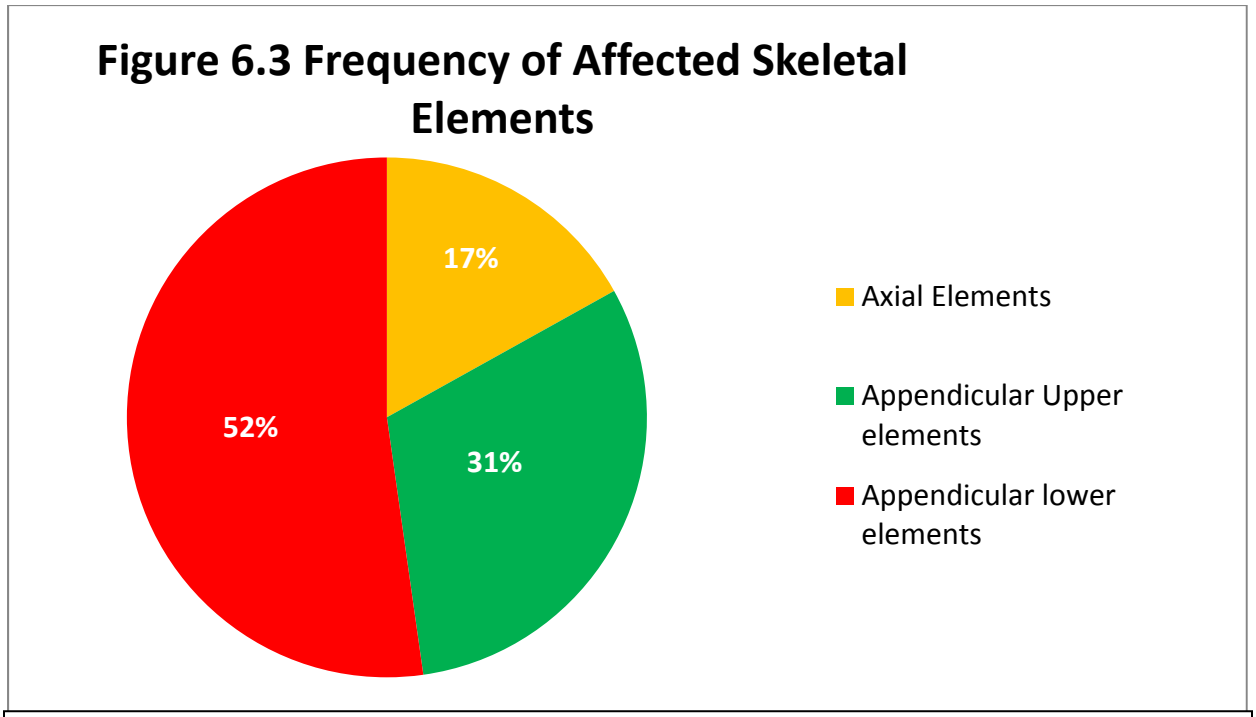
***This refers to the number of recorded pathological modifications. For simplicity modifications of the pubis, ischium and ilium are grouped under the pelvis. The total number therefore may reflect a different frequency than that seen in actual individuals.**

## Figure 6.1 Pre-Contact General Infection Distribution




## Figure 6.2 Frequency of Affected Elements Pre and Post Contact






Axial Elements: cranium, vertebral column, ribs and sternum
Appendicular, Upper elements: scapula, humerus, ulna, radius, clavicle
Appendicular, Lower elements: innominate, sacrum, femur, tibia, fibula

## Co-occurrences

No individual experienced all of the pathological conditions assessed (Tables 6.2 and 6.3). However a number of individuals manifested co-occurrences of one or more pathological conditions. Of the total 152 individuals, 14 individuals suffer from a single isolated condition, 33 individuals suffer from two pathological conditions, 42 individuals manifested three pathological conditions, 22 individuals exhibited four pathological conditions, 19 individuals demonstrated five pathological conditions, six individuals manifested 6 pathological conditions, three individuals demonstrated seven pathological conditions, and a single individual manifested 8 of the 11 pathological conditions observed. Four displayed none of the pathological conditions observed in the rest of the population. When the results are charted for the number of co-occurrences of pathological conditions, a

noticeable shift in health is observed between the pre-contact and post-contact periods (Figure 6.4 and Table 6.2). Pre-contact individuals experienced co-occurrences the same as post-contact populations, however, the pre-contact population peaked at the co-occurrence of two pathological conditions, and no sub-set of the population experienced the highest levels of co-occurrences. In contrast, the post-contact period peaked at the co-occurrence of four pathological conditions and has individuals in population sub-sets that experienced the highest categories of co-occurrence.

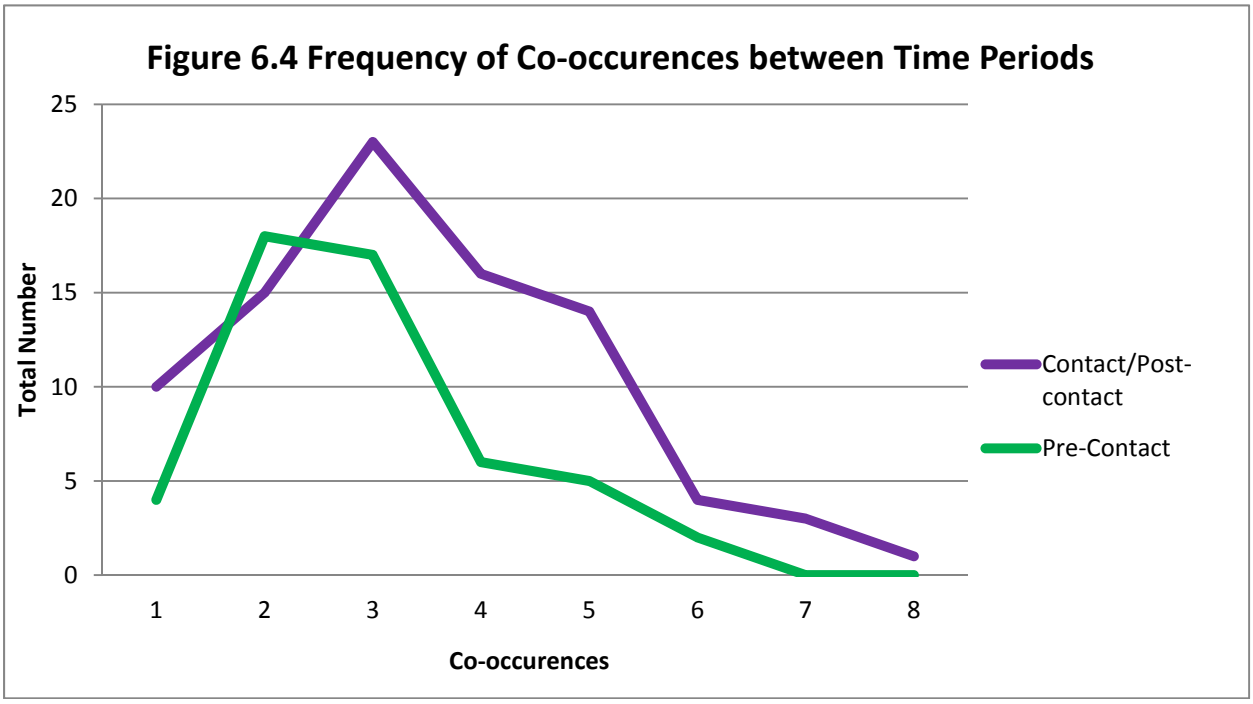



Table 6.2 Pathological Condition Co-Occurrences by Contact Period

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Total population |
|---|---|---|---|---|---|---|---|---|---|
| Pre- contact | 4 | 18 | 17 | 6 | 5 | 2 | 0 | 0 | 57 |
| Contact/ Post-contact | 10 | 15 | 23 | 16 | 14 | 4 | 3 | 1 | 89 |

*The bracketed number indicates the percentage of population while the number outside the brackets indicates the actual number of individuals.

| Table 6.3 Distribution of Pathological Conditions within Population sub-sets | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Number of Pathological Conditions | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Total Population |
| Arctic Bay | | | | 1(100)* | | | | | 1 |
| Bathurst Island | | | 1(100) | | | | | | 1 |
| Caribou Point 1 | | | | | 1(100) | | | | 1 |
| Crossing Point Loc 2 | 1(100) | | | | | | | | 1 |
| Dundas Harbour | 1(20) | 2(40) | 1(20) | | 1(20) | | | | 5 |
| Ennadai Lake | | | | | 1(100) | | | | 1 |
| Ferguson Lake | | | | 1(100) | | | | | 1 |
| Griffith Lake | 1(100) | | | | | | | | 1 |
| Inuksivik | | | 3(75) | | | | | | 4 |
| Inuksivik North | | | 1(50) | 1(50) | | | | | 2 |
| Kamarvik | 1(3) | 12(40) | 11(37) | 2(7) | 2(7) | 1(3) | | | 30 |
| Kulaituijavik | 1(33) | 1(33) | | 1(33) | | | | | 3 |
| Mansel Island | | | | | 1(100) | | | | 1 |
| Mittimatalik/Mittima's Grave | | 1(100) | | | | | | | 1 |
| Oftedal Lake | | 1(20) | 3(60) | | | 1(20) | | | 5 |
| Sadlermiut/ Native Point | 8(11) | 11 (16) | 18(25) | 14(20) | 11(16) | 3(4) | 3(4) | 1(1) | 71 |
| Sadlermiut/ Prairie Point | | | 1(100) | | | | | | 1 |
| Seven Pound Island | | 1(100) | | | | | | | 1 |
| Silumiut Island | | 2(18) | 2(18) | 2(18) | 2(18) | | | | 11 |
| Silumiut North | 1(25) | 1(25) | | | | 1(25) | | | 4 |
| Victory Lake | | 1(100) | | | | | | | 1 |

## Tuberculosis

A total of 27 individuals manifested tubercular pathologies, 21 from the post-contact period and 6 from the pre-contact period. The post-contact population with tubercular lesions consisted of 12 males, 7 females, and 2 individuals of indeterminate sex. The pre-contact subset consists of 3 females and 3 males. There is a substantial difference in the occurrence of TB lesions between the two temporal periods. Overall the post-contact period consists of 89 individuals from various geographic locations, of these, 21 individuals or roughly 24%, exhibited tubercular pathologies. In contrast the pre-contact period consists of 59 individuals from various geographic locations of whom only 6, or roughly 10%, manifest tubercular pathologies. Therefore, in this sample population the post-contact period had a 2.3 times higher rate of pathological modification based on skeletal evidence of tubercular infection. The population, both the pre and post contact cohorts, exhibited pathological modification of the cervical, thoracic and lumbar vertebrae, ribs, and some isolated lesions found on other skeletal elements including the pelvic girdle, especially the hip articulations, knee joints and a single individual evidenced modification of the metatarsals and metacarpals. The most commonly affected area was the vertebral column, with 17 individuals (63%) exhibiting pathological modification. T8 (n= 7) and T9 (n=7) were the most commonly affected vertebrae, however T6 through L3 all show a substantial degree of pathological modification in comparison to the upper thoracic, entire cervical and lower lumbar vertebrae. The cervical vertebrae showed involvement in two individuals, while the upper thoracic (T1-T5) showed pathological modification in six individuals. The lower lumbar (L4 & L5) were involved in a single case. The ribs were the second most common area of involvement with 14 individuals exhibiting modification; four

out of six pre-contact individuals manifested these lesions, while ten out of twenty-one demonstrated them from the post-contact population. The pelvic girdle, knee joint, and feet and hand bones combined for a total of involvement of six times. Possibly, localised tubercular infection within the Inuit population is more common than disseminated infection.

It is important to note that the following pathological conditions co-occur in individuals with characteristic TB. Tuberculosis and indications for dental abscess and pre-mortem tooth loss are the pathological conditions that co-occur most frequently. TB and dental abscess occurs in 20 individuals and TB and pre-mortem tooth loss also co-occurs in 20 individuals, although not the exact same 20. The third most common co-occurrence is between TB and general infections which manifest in 19 individuals, while it is possible that tuberculosis may have disseminated in these individuals, many of the areas observed are rarely affected by tuberculosis. TB and arthritis, particularly arthritis of the vertebral column co-occur in 16 individuals, while enthesopathies co-occur with TB thirteen times. Fusion and fractures co-occur three times each.

## Arthritis

The most common form of degenerative change noted in the population was that associated with various forms of arthropathy. While a proportion of this may have been the result of advancing age changes, the population exhibited a high degree of arthritic change throughout. In total, 52 individuals (35%) exhibit arthritic modification, ranging from minor osteoarthritic changes of the joints to highly traumatic arthritic changes to the vertebral column. Arthritic modification of the spinal column was both extensive and

widespread. The lumbar region of the spine was more commonly affected than the cervical or thoracic, and was the most common area of arthritic modification in the population. Alterations to this area ranged from minor lipping along the margin of the vertebral body to the development of osteophytosis in the form of large parrot beak osteophytes, and in rare cases partial fusion to adjoining vertebrae or the sacrum. Osteophytosis occurred in 10 individuals, and in a small percent of the population was severe enough to limit mobility. The basic alterations included minor lipping along the vertebral body margins, more common in the thoracic and lumbar vertebrae, but also observed within the cervical vertebrae. The cervical vertebrae also exhibited the porous quality of osteoarthritic bone in isolated cases. There is a high correlation between arthritic modification and enthesopathies within the overall population, particularly when pathological modification of the spine is observed. Over 40% of individuals who exhibited arthritis somewhere in the skeletal system also manifested an enthesopathic musculoskeletal marker. Of the enthesopathies that occur, over 50% occur in the spinal column, generally in the thoracic or lumbar sections, with anterior collapse and compression the most common manifestation.

A comparison of arthritic modification between pre- and post-contact cohorts reveals an observable difference in frequency in the post-contact cohort compared to the pre-contact. In the pre-contact cohort (n=16), 8 females from 5 population subsets and 8 males from 4 different population subsets (50% Kamarvik), manifest arthritic pathologies. The most common areas affected indicate a considerable difference between the male and female behaviour patterns; 38% of pre-contact females exhibit arthritic modification of the lumber and thoracic vertebrae, whereas 38% of males indicate modification of the ulna, the

humerus and the lumbar vertebrae. In 67% of cases the ulna and humerus modification co-occur, with 33% manifesting modification of either the ulna or the humerus. The post-contact period includes more than twice the amount (n= 36) of individuals demonstrating arthritic alterations from 8 different population subsets. This time period also exhibits a marked difference between the sexes with 64% (n. 23) of individuals who exhibit arthritic changes being males from 7 different populations and only 25% (n.9) being females from only three different population subsets and a significant proportion (67%) from the Sadlermiut cohort. The sexes also display a difference in the areas affected by arthritic pathology; a total of 44% of the females in the post-contact period manifest modification of the cervical vertebrae, with an additional 44% also exhibiting thoracic collapse. In contrast, the most affected area in post-contact males is the lumbar vertebrae. Fourteen males manifest arthritic changes in this area of the vertebral column, with six of these individuals exhibiting osteophytosis and six also exhibiting lumbar compression. The thoracic section of the vertebral column and the ulna were the next most common areas affected in post-contact males, with each having respectively eight individuals presenting arthritic modification.

## Enthesopathies

Human bone has a great deal of plasticity and is dynamic in its ability to adapt to various stresses manifesting changes observable on the skeletal system which can indicate the degree of usage and indicate long term repetitive behaviour. The Inuit population samples in this research are from a historical and pre-historical period; they lived a non-mechanised life and the amount of physical labour and repetitive activity would have

created observable markers for many muscles, ligaments and tendons. However, a part of the population displays the highest grade of musculoskeletal markers in the form of enthesopathic muscle insertions and attachment sites. Enthesopathies are pathological lesions at loci of muscular insertion or attachment resulting in traumatic markers on an element (Kennedy 1989) and can take many forms. These sites can become infected and inflamed, resulting in erosive lesions or stress fractures and in some cases osteophytes (Kennedy 1989). Forty individuals within the overall population (n=40) present enthesopathic musculoskeletal stress markers (MSM's) on a diverse assortment of skeletal elements. However, incidents within the population are slightly more frequent, with fifty recorded incidences occurring within the forty individuals. The highest rate of occurrence (59%) is localised to the vertebral column. In some cases the majority of the spine showed dramatic alterations, in the form of compression of the entire vertebral body of several adjacent vertebrae, or through Schmorl's nodes resulting in traumatic disk displacement. Of the vertebrae exhibiting enthesopathic alteration, the thoracic vertebrae were the most commonly affected. Eighteen thoracic vertebrae from T5 to T12 manifest pathological modifications. Vertebrae were included in this category when pathological modifications indicating infection, arthritic changes such as lipping, and osteophytosis were absent. Compression of the vertebral body or anterior collapse of the body, sometimes accompanied by angular kyphosis, were the most common forms of enthesopathic modification. Nine lumbar vertebrae exhibited modification similar to that manifested by the thoracic section. The second most common area of enthesopathic modification was the humerus, in particular the insertion sites for the teres minor/latissiums dorsi/ pectoralis major muscles, tendons and ligaments. This occurred 13 times in the population, eight

times unilaterally in the right humerus and five times bilaterally in both the left and the right humerus. The remaining enthesopathic lesions occurred in less than three individuals each. A MSM in the iliac crest of an innominate occurred in an isolated individual; the tibia manifested two different pathologies, one an attachment site along the shaft, the second at the tibial tuberosity; the radii and ulnas manifested eburnations at the distal and proximal ends, at the articulation facets respectively. While the final two MSM's can also be classified as osteoarthritic modification, a lack of sufficient skeletal alteration indicating arthritis was observed.

When examining the demographic frequencies, patterns appear in the distribution of enthesopathies across space and time. Twenty-seven post-contact individuals manifest enthesopathic modifications, approximately 30% of the total affected population, compared to the pre-contact population in which only 13 individuals displayed enthesopathic modifications, which is roughly 23%. In the post-contact population only women (n=6) of the Sadlermiut population subset exhibit enthesopathic modification and these are only manifested in the vertebral column. Compared to the post-contact females, the 16 post-contact males, 12 from the Sadlermiut, three from Oftedal Lake and one from the Ennadai Lake subset, have a much more varied set of enthesopathic lesions. Ten of the overall vertebral enthesopathic modifications occur in this subset, all in individuals from the Sadlermiut population, as well as the single modification to the innominate, both modifications of the ulna and one each of the tibia and radius modifications. Only five of the 13 occurrences of enthesopathic modification of the humerus occur in the post-contact period, one occurring in a male from Ennadai Lake, the other two in Sadlermiut males.

There are six pre-contact females from four different population subsets, Kamarvik (n=3), Silumiut North (n=1), Mittimatalik/Mittima's Grave (n=1), and Silumiut Island (n=1). There were no significant patterns in the occurrences between the subsets, nor any significant trend in the distribution of enthesopathic lesions. The pre-contact female (n= 6) population exhibits enthesopathies of the spine, humerus and radius. There are seven pre-contact males, six from the Kamarvik subset and one from Silumiut North subset. Five individuals manifest the enthesopathic proximal MSM of the humerus out of the total pre-contact male subset; this is roughly 71% of this population subset. Unlike pre-contact females, males show a definite trend in enthesopathic distribution, specifically for modification of the humerus. There is also an overall difference between males and females. In general 46% more males suffer from enthesopathies than females (22 compared to 12). Males experienced enthesopathic modification more commonly in the humerus (10:1) compared to females. Females exhibited an over-representation of enthesopathic modification of the spine.

## Fusion and Cartilization

Eighteen incidents of fusion occur in the total population, manifesting in 16 individuals. The spinal column was the most common skeletal element resulting in fusion, either with an additional vertebra or with the sacrum. The most common area of fusion within the vertebral column is the twelve thoracic vertebrae, approximately 33 percent of the incidences occurred within this area. Two other areas comprise the second most common areas of fusion; the cervical vertebra and the lumbar-sacral articulation, each encompass 18 percent of the fusion for the total population. The remaining incidents of

fusion include the following skeletal elements: sacro-iliac joint, ribs, sternum, and tibia. Of the sixteen individuals twelve are from the post-contact Sadlermiut subset, seven are males, three females and two indeterminate. The remaining four individuals are pre-contact individuals from four different population subgroups and evenly divided between males and females. Overall, the differences between the sexes suggest that this pathological condition occurs more frequently in males (50%), compared to females (31%). Fusion also appears to occur more frequently in post-contact populations, and in particular in the Sadlermiut population.

## Fractures

Only eight percent of the analysed population showed evidence of fractures. These twelve individuals represent a small proportion of the sample, and each fracture shows evidence of reactive bone and manifests the presence of a bone callous in various stages. The most common area of fracture was the ribcage with six individuals exhibiting fracture in this area. Four other individuals exhibit fracture of the pelvic girdle; the most catastrophic fracture occurred at the margin of the pubis and the ischium of the right innominate. This individual displayed a complete trans-section of the area, and a nearly complete remodelling of the surface of the fracture. The surrounding areas of the pelvic girdle demonstrated some resulting irregularities, which can be presumed to be effects of the fracture not being set and healing abnormally. The final two fractures are a possible incomplete fracture of the tibia and an incomplete fracture of the atlas. The observed fractures are split almost evenly between the two temporal populations, seven occurring in the post-contact period and five in the pre-contact period. Similarly, there is little

difference between male and female expression with six females, five males, and one indeterminate possessing indicators of a fracture. The population subsets also show no pattern of occurrence, with fractures occurring in eight different geographic locations.

## Neoplastic Conditions

Roughly one percent (n= 2) of the total population demonstrates characteristics indicative of neoplastic conditions. The first individual, a post-contact male from the Sadlermiut population, manifests a bony growth on the frontal bone. The growth is roughly the size of a transected golf ball (43mm). There is little evidence of endocranial involvement; however the protuberance itself shows active bone changes. The second case is more typical of osteosarcoma expressed in a post-contact female from the Sadlermiut cohort. The affected area presents a thinning of the cortical bone and an almost complete degradation of the mandibular cortical and trabecular bone. With such a small sample it is impossible to say if any patterns are represented by these two cases.

## Dental Pathologies

Dental pathologies are the most common specific pathological modification within the population. The two health indicators for teeth that were observed were pre-mortem tooth loss (PMTL) and dental abscess. Just over two thirds of the sample (n= 97) manifest PMTL of at least a single tooth from the mandibular or maxillary dentition (n= 946). Fourteen percent (n= 21) manifest at least a single pre-mortem tooth loss in the mandible only and thirteen percent (n=20)only in the maxillary. However an additional 34% (n= 52)

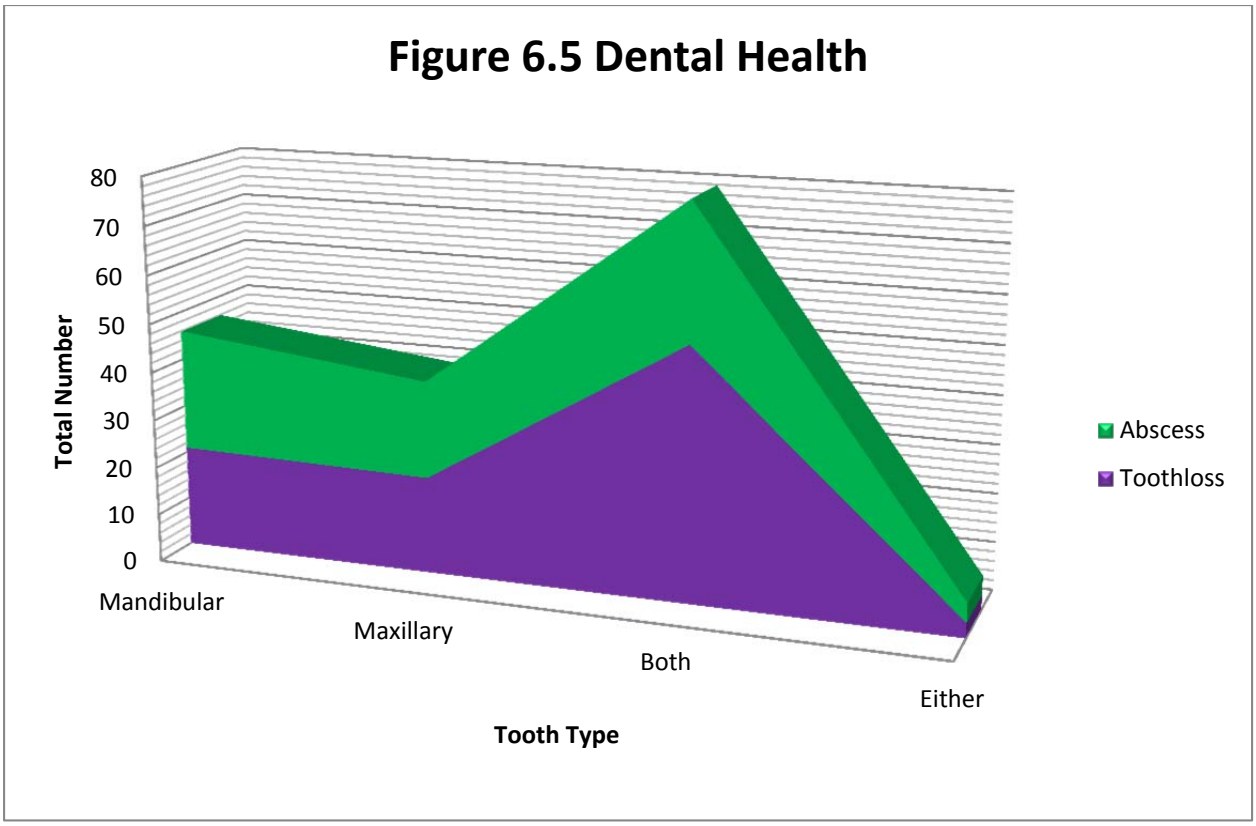
present with both mandibular and maxillary pre-mortem tooth loss of a single tooth in each area. A total of 946 teeth were recorded as lost antemortem from a total possible amount of 3104[7]. The most common form of tooth loss on average was the molar, followed closely by the anterior incisor (Table 6.4 and Figure 6.6). Within the population the single most common tooth to be lost was the right mandibular first incisor (n= 49). The least commonly lost tooth and dental type was the canine and the maxillary canine specifically.

**Table 6.4 Pre-Mortem Tooth Loss by Tooth Type**

| | Incisors | Canine | Premolars | Molars |
|---|---|---|---|---|
| 1 | 149 | 65 | 84 | 130 |
| 2 | 93 | | 103 | 164 |
| 3 | | | | 184 |
| Total | 242 | 65 | 187 | 478 |
| Average | 121 | 65 | 93.5 | 159 |

Overall, abscessing is slightly less common within the population than tooth loss, 50% (n= 76) individuals present with abscessing of at least a single tooth (Figure 6.5). Seventeen percent manifest abscessing of the mandibular teeth and thirteen percent manifest abscessing of the maxillary. An additional 18% (n= 28) of individuals present with abscessing of one or more teeth in both the mandibular and maxillary areas.

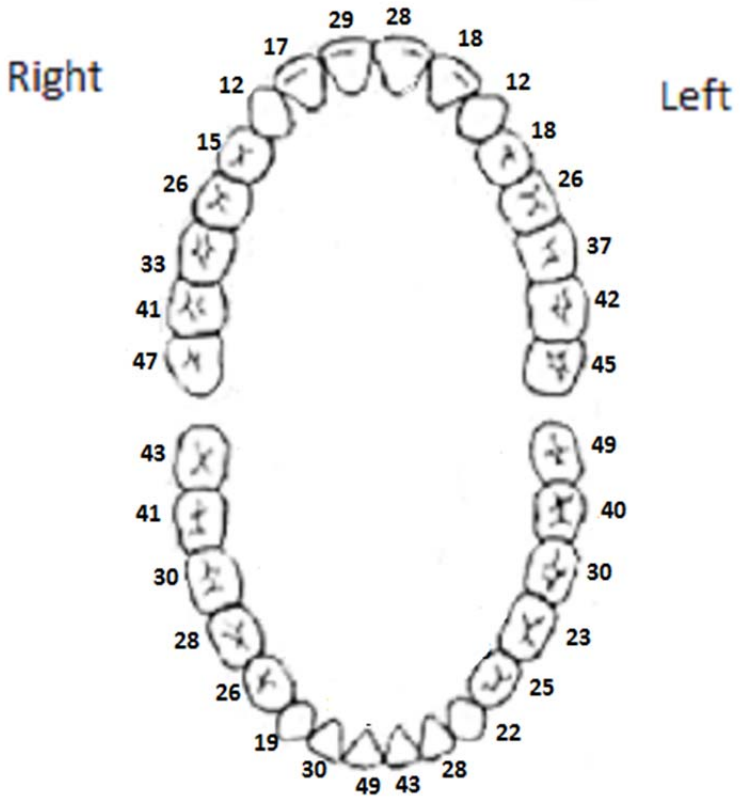
[7] Human dental formula 2.1.2.3 per quadrant= 8 incisors, 4 canines, 8 premolars and 12 molars, for a total of 32 teeth per individual. (2+1+2+3)4 x n= 3104 teeth, when n=97 indivduals.




A total of 53 individuals presenting pre-mortem tooth loss are from the post-contact period and different population sub-sets, the largest proportion coming from the Sadlermiut population. Of the post-contact period, 19 individuals are female, 95% of them from the Sadlermiut subset; 26 individuals are male, and 73% of them are from the Sadlermiut subset; and eight individuals are of indeterminate sex all from the Sadlermiut subset. A total of 42 individuals are from the pre-contact period and seven different population subsets; a total of 20 females in the pre-contact period present with tooth loss, 21 males and one indeterminate. A total of 46 individuals from the post-contact period exhibit abscessing of either the mandible or maxillary from 8 different population subsets; 17 (37%) are females, sixteen of which are from the Sadlermiut subset; 21 (47%) are males from 8 different population subsets; and 8 are of indeterminate sex all from the Sadlermiut

population subset. A total of 28 individuals from the pre-contact period present with dental abscessing; 13 (46%) are females from 6 different subsets and 15 (54%) are males from 7 different population subsets.

**Figure 6.6 Frequency of Individual Ante Mortem Tooth Loss in Skeletal Sample**




## Rickets

A single male individual suffered from rickets, with the symptomatic curvature of the lower limbs. This post-contact individual was from the Native Point population of Sadlermiut Inuit on Southampton Island and manifested two additional pathological conditions, dental abscess and pre-mortem tooth loss, as well as the presence of at least one discontinuous trait.

## Molecular Results

### Mitochondrial Results

Positive mitochondrial results were obtained for eighteen individuals (Tables 6.5, 6.6 and 6.9) and haplogroups were assigned to 17, the remaining individual, XIV-C: 111's mitochondrial sequence for both the HVR I and HVR II were too fragmented to accurately assign a haplogroup. Both the Hyper-variable Region I (HVRI) (15971 to 16410) and Hyper-variable Region II (HVRII) (1-389) of the D-loop were amplified, with verification of haplogroups primarily using polymorphisms from the HVRI. No other haplogroup was expected to appear in pre-contact Inuit populations, and small percentages of European haplogroups may occur in more contemporary post-contact populations. However maternal admixture was extremely rare in early contact populations, and therefore European haplogroups should be almost non-existent.

The Sadlermiut molecular subset (n=13) consisted of mitochondrial results for the post-contact period. Individuals XIV-C: 246 and 149 from this site present with haplogroup A ; XIV-C: 179 and 221 were further defined as haplotype A2. Of the A2 haplogroup, two haplotypes are represented within the overall population, however only haplotype A2b contains post-contact Sadlermiut individuals (XIV-C: 744, 217and 152). Three individuals were assigned the A2b haplotypes, which is marked by the possession of the key polymorphism 16265G (Helgason et al. 2006). Four other individuals presented with the polymorphisms 16223 and 16362, but lacked the two additional A polymorphisms at 16290 and 16319, marking them members of haplogroup D/D3. Additional polymorphisms in the HVRII and HVRI that lack definitive haplogroup membership are also present in many sequences. Sixty-three percent of this population were determined as

members of haplogroup A, making this haplogroup 2.01 times more common in the Sadlermiut population from the Native Point, Southampton Island site compared to Hg D/D3.

| | Table 6.5 mtDNA Mutations of the HVR I | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 16093 T | 16111 C | 16173 C | 16192 C | 16223 C | 16265 A | 16278 C | 16290 C | 16294 C | 16309 A | 16311 T | 16319 G | 16362 T | 16390 G |
| XIV-C: 111 | | | | | T | | | | | | | | | |
| XIV-C: 149 | | | | | T | | | T | | | | A | | |
| XIV-C: 152 | | | | | T | G | | T | | | | A | C | |
| XIV-C: 164 | | T | | | T | | | | | | | | | |
| XIV-C: 179 | | T | | | T | | | T | | | | A | C | |
| XIV-C: 183 | C | | T | | T | | | | | | | A | C | |
| XIV-C: 191 | | | T | | T | | | | | | | A | C | |
| XIV-C: 197 | C | | T | | T | | | | | | | A | C | |
| XIV-C: 216 | | | T | | T | | | | | | | A | C | |
| XIV-C: 217 | | | | | T | G | | T | | | | A | C | |
| XIV-C: 221 | | T | | | T | | | T | | | | A | C | |
| XIV-C: 246 | | | | | | | | T | | | | A | C | |
| **XIV-C: 316** | | **T** | | | **T** | **G** | | **T** | | | | **A** | **C** | |
| **XIV-C: 328** | | **T** | | | **T** | **G** | | **T** | | | | **A** | **C** | |
| XIV-C: 607 | | | | | | | T | | T | G | | | | A |
| XIV-C: 621 | | T | | T | T | | | T | | | C | | C | |
| XIV-C: 744 | | T | | | T | G | | T | | | C | | C | |
| XIV-C: 636-1 | | T | | T | T | | | T | | | C | | C | |
| **Note: The two light gray shaded and dark gray shaded rows represent possible family groups based on the available mitochondrial** data | | | | | | | | | | | | | | |

| Table 6.6 mtDNA mutations of the HVR II | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 73 A | 146 T | 153 A | 235 A | 263 A | 309.1* | 315.1* |
| **XIV-C:111** | | | | | | C | C |
| **XIV-C:149** | G | C | | G | | | |
| **XIV-C:152** | G | | | | | C | C |
| **XIV-C:164** | G | C | G | G | | C | C |
| **XIV-C:179** | G | C | G | G | | | |
| **XIV-C:183** | G | | | | | C | |
| **XIV-C:191** | G | | | | | | C |
| **XIV-C:197** | | | | | | C | C |
| **XIV-C:216** | G | | | | | C | C |
| **XIV-C:217** | G | C | G | G | G | C | C |
| **XIV-C:221** | G | C | G | G | G | C | C |
| **XIV-C:246** | G | | | | | C | C |
| **XIV-C:316** | G | C | G | G | G | C | C |
| **XIV-C:328** | G | C | G | G | G | C | C |
| **XIV-C:607** | G | C | G | G | G | C | C |
| **XIV-C:621** | G | C | G | G | G | C | C |
| **XIV-C:636-1** | G | C | G | G | G | C | C |

The remaining two post-contact individuals (XIV-C:316 and 328), to whom haplogroups could be assigned, are from two different archaeological sites. A single individual from Ennadai Lake(XIV-C: 328) bordering the south-western border of Nunavut and Northwest Territories resulted in membership in haplogroup A2b, with polymorphisms at 16223, 16290, 16319, and 16362 and the defining haplotype polymorphism is 16265. The final individual from Oftedal Lake (XIV-C: 316) in the central portion of southern Nunavut also presented with membership in haplogroup A2b with polymorphisms at 16223, 16290, 16319, and 16362 and the defining polymorphism at 16265. The final post-contact individual (XIV-C: 111) resulted in highly fragmented mitochondrial sequences. The contig obtained from this individual had large gaps in both the HVR I and HVR II sequences. No definable haplogroup was assigned, however the individual did express polymorphisms at 16223 and the insertions at 309.1 and 315.1 common to this cohort.

Three mitochondrial sequences for individuals from the pre-contact period produced results (XIV-C: 621, 636-1, and 607). Two fell into the expected haplogroups while one did not fall into an Inuit haplogroup or even an Amerindian haplogroup for North or South America. Two individuals from the Kamarvik site on the western shores of Hudson Bay north of Chesterfield Inlet (XIV-C: 621 and 636-1) presented with polymorphisms at 16223, 16290, 16319, and 16362, indicating membership to haplogroup A, while also presenting polymorphisms at 16311, 16111 and 16192 indicating haplotypes A2a.

The final individual (XIV-C: 607), dated archaeologically to the pre-contact period, was sequenced as a member of haplogroup U or H which are haplogroups common to Europe. The sequence was highly degraded and lacked sufficient fidelity to differentiate between these two haplogroups. Because the individual is from the pre-contact period, admixture is unlikely, and as the researcher (M.L. Campbell) is a member of haplogroup N, contamination during research was not possible. However contamination during excavation and curation remains a possibility.

### Insertion Sites

Two insertion sites in the HVRII were observed in 14 individuals from the overall molecular sample (Table 6.6). The two insertions occurred at 309.1 and 315.1. The "C" base inserted in a repetitive "C" sequence in both regions. From the cohort twelve individuals exhibited insertion of both "C" bases (Table 6.6). A single individual exhibited insertion for each "C" base at 309.1 (XIV-C: 183) and 315.1 (XIV-C: 191).

### Family Groups

Mitochondrial DNA analysis resulted in the discovery of two possibly distinct family groups from within the molecular cohort. Individuals XIV-C: 636-1 and XIV-C: 621 presented with mutations at 16111, 16192, 16223, 16290, 16311, 16362 of HVR I and 73, 146, 153, 235, and 263, with insertions at 309.1 and 315.1 of HVR II. Both individuals were recovered from the Late Prehistoric (pre-contact) Kamarvik site. XIV-C: 621 is described as an adult (>19 years) female, while XIV-C: 636-1 is described as an indeterminate adolescent between the ages of 13 and 18 years of age. Both hold a unique mutation at nt position 16192 which distinguished them from other members of the cohort. This suite of mutations and the possession of a unique mutation are suggestive of familial kinship. In conjunction with this unique mitochondrial sequence and their discovery at the pre-contact Kamarvik archaeological area, it is possible to suggest a descendent relationship.

The second familial association is less clear. Individual XIV-C: 316 is from the contact Oftedal Lake site, while XIV-C:328 is from the contact Ennadai Lake site. While recovery from two different sites does somewhat detract from the possibility of kinship, it does not preclude the possibility. Both of these individuals present with mutations at nt positions 16111, 16223, 16265, 16290, 16319 and 16362 of the HVR I and 073, 146, 153, 235, 263, with insertions at 309.1 and 315.1 of the HVR II (Appendix 1, tables 6.6 and 6.7).

## Sex Determination

Eighteen out of 20 samples resulted in positive sex determination (Table 6.9). Two samples were determined to have been too highly degraded to achieve results from nuclear DNA. These samples were excluded from further analysis based on their degraded sample

quality. Fifteen of the eighteen results obtained correspond with osteological assignment of sex (Table 6.7). One individual XIV-C:636-1 is assigned the osteological designation of indeterminate, which is assigned to adolescents whose morphological characteristics are as of yet not discrete enough to characterise sex or to individuals whose traits are obscured or undefined for various reasons. XIV-C: 636-1 is an adolescent between the ages of thirteen and eighteen and therefore falls into the former category. Sex assignment was possible and XIV-C: 636-1 was determined to be genetically male. The final two individuals resulted in contrasting osteological and molecular results. Individuals XIV-C: 246 and XIV-C: 328 osteologically assessed as males were genetically determined as females.

**Table 6.7 Comparison of Osteological and Molecular Determination of Biological Sex**

| Individual | Osteological Determination | Molecular Determination |
|---|---|---|
| XIV-H:041 | FEMALE | FEMALE |
| XIV-C:149 | FEMALE | FEMALE |
| XIV-C:152 | MALE | MALE |
| XIV-C:164 | MALE | MALE |
| XIV-C:179 | MALE | MALE |
| XIV-C:183 | FEMALE | FEMALE |
| XIV-C:191 | MALE | MALE |
| XIV-C:197 | MALE | MALE |
| XIV-C:216 | MALE | MALE |
| XIV-C:217 | MALE | MALE |
| XIV-C:221 | FEMALE | FEMALE |
| XIV-C:246 | MALE | FEMALE |
| XIV-C:316 | FEMALE | FEMALE |
| XIV-C:328 | MALE | FEMALE |
| XIV-C:607 | MALE | MALE |
| XIV-C:621 | FEMALE | FEMALE |
| XIV-C:636-1 | INDETERMINATE* | MALE |
| XIV-C744 | FEMALE | FEMALE |

## Cytokine Genotype Profiles

Where possible, cytokine genotype profiles were obtained from individuals with template extracted from molar samples. Cytokine genotype profiles from rib samples of individuals from whom molar samples were unavailable resulted in unreadable chromatographs and the inability to assign either genotype or phenotype. The molar samples that did not yield results most likely experienced post-mortem loci specific damage for the desired gene/allele. Results for IL-6 (-174) were obtained from eight individuals. All of these individuals were characterised by the homozygous genotype of G/G. Multiple attempts were conducted to assess the genotypes for the remaining nine individuals. Four separate extractions and more than five SNaPshot analyses were carried out, but these did not yield results. Part of the difficulty with this cytokine may have been due to the large fragment amplified by the primers, IL-6 forward and reverse primers resulted in a 332bp fragment, which is quite large in terms of expected aDNA fragments.

IFNγ was successfully amplified in nine samples. Eight individuals were detected as homozygous A/A, and a single individual was typed as heterozygous A/T. The detection of both alleles is important as it demonstrated the detection of both alleles suggesting that allelic dropout was not an issue.

Genotypes for IL-10 (-1082) were detected in twelve samples. Homozygous G/G genotypes were detected in 10 individuals and heterozygous genotypes were detected in two individuals. The detection of both alleles for the IL-10 (-1082) loci reinforced the efficacy of the primers and protocols, as well as proving allelic drop-out did not occur for this SNP.

TNFα primers and analyses produced the most results (n=15) of all the cytokine analyses with fifteen of the sixteen individuals analysed resulting in readable peaks. Fourteen of these individuals were detected as homozygous G/G genotypes, and a single individual was detected as a heterozygous G/A. This heterozygous individual proved that the analyses were detecting both alleles.

Individuals who expressed a heterozygous genotype are considered intermediate producers of all four cytokines. Individuals XIV-C: 217, 246, 316 and 607 were heterozygous for only a single cytokine. Each of their additional cytokine genotypes were homozygous. Individual XIV-C: 217, who was heterozygous for TNFα, is a male from the post-contact Sadlermiut population; XIV-C: 246, who was heterozygous for IFNγ, is a female from the post-contact Sadlermiut population; XIV-C: 316 and 607 were both heterozygous for IL-10, and both are from the pre-contact period, but 316 is from the Oftedal Lake subset and 607 is from the Kamarvik subset. Given the small sample size little can be suggested about pattern of heterozygosity between the the pre-contact and post contact populations. Likewise, the two post contact individuals are from the same archaeological site, however, the two pre contact individuals are from different geographical areas.. Results obtained from individual XIV-C: 607's rib sample was considered contaminated; molar samples resulted in indefinite Inuit ancestry.

### Allele and Genotype Frequencies

Genotype frequencies, the classification of an individual as either a low, high or intermediate producer, and the specific allele frequencies for each cytokine polymorphism are listed in Table 6.8. For three of the four cytokine polymorphisms analysed, two

different genotypes and both alleles were detected. However, the IL-6 (-174) SNP only ever managed to amplify a single genotype and only the G allele was detected.

| Table 6.8 Allele and Genotype Frequencies | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Alleles | | | Genotypes | | | |
| | **G** | **A** | **Total Alleles** | **Low** | **Int.** | **High** | **Total** |
| TNFα(-308) | 29 (97%) | 1 (3%) | n=30 | 14 (93%) | 1 (7%) | 0 (0%) | n=15 |
| | **T** | **A** | | | | | |
| IFNγ (+874) | 1 (6%) | 17 (94%) | n=18 | 8 (89%) | 1 (11%) | 0 (0%) | n=9 |
| | **G** | **A** | | | | | |
| IL-6 (-174) | 28 (93%) | 2 (7%) | n=30 | 0 (0%) | 2 (13%) | 13 (87%) | n=15 |
| | **G** | **C** | | | | | |
| IL-10 (-1082) | 16 (100%) | 0 0%) | n=16 | 0 (0%) | 0 (0%) | 8 (100%) | n=8 |

## Amplification and Sequencing of *Mycobacterium tuberculosis* DNA

Analysis of microbial DNA was performed to investigate the presence of MTB in the archaeological Inuit population from pre-contact and post-contact periods. Positive results were obtained from the first PCR amplification and visualisation for eight individuals. The remaining amplifications resulted in the absence of a band. The lack of a band during visualization for these individuals was interpreted as a lack of *Mycobacterium tuberculosis* in the samples. Two additional amplifications resulted in repeatable results for only two individuals, XIV-C: 246 and XIV-C: 607. Comparing these results to the osteological assessment (for summary of pathological conditions see Appendix 3), individual 246 did not exhibit any typical lesions for TB pathogenesis; however individual 607 manifested

cranial lesions on one parietal bone that could indicate TB pathogenesis. Individual 607 lacked any of the typical TB lesions such as modification of the major joints, visceral surface of the ribs or thoracic vertebrae. Tuberculosis can be detected in skeletal remains lacking definitive lesions (Baron et al. 1996); individuals were included, where needed, who had pathological modification and good preservation. The two individuals were also from two different demographic and temporal populations: XIV-C: 246 is from the Sadlermiut Native Point post-contact period population and XIV-C: 607 is indicated as from a Kamarvik pre-contact population. To reinforce the positive PCR results, sequencing was attempted for the nuclear DNA of the *M. tuberculosis* genome. The two repeatable results, along with an additional three other positive results from the initial PCR were sequenced. While positive results were obtained via amplification, direct sequencing of these samples resulted in sequences too highly degraded to align and read. Therefore no readable results or definable TB sequences were obtained from the original PCR positive amplifications. Further analysis via other methodologies is necessary to support definitive classification of microbial amplification from these samples.

Of the twenty individuals sampled for molecular analyses, results were successfully obtained from 17 (Table 6.9). Complete genetic profiles including haplogroup assignment, sex determination and genotypes for all four cytokines were obtained from only seven of the seventeen individuals. Of the remaining ten, one resulted in sex determination alone, three in the attainment of all results but a single cytokine, and the remaining five a mixture. Fifteen of the seventeen individuals resulted in both sex and haplogroup determination, and these fifteen individuals became the foundation sample for the SNaPshot analyses to assess for cytokine genotype profiles.

| Table 6.9 Summary of Palaeogenetic Results | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Site | Sex | Hg/ Haplotype | TNFα | IFNγ | IL-10 | IL-6 |
| **XIV-C 149** | Sadlermiut | F | A | G/G LOW | | G/G HIGH | G/G HIGH |
| **XIV-C 152** | Sadlermiut | M | A2b | | | | |
| **XIV-C 164** | Sadlermiut | M | A | | | G/G HIGH | |
| **XIV-C 179** | Sadlermiut | M | A2 | G/G LOW | A/A LOW | G/G HIGH | G/G HIGH |
| **XIV-C 183** | Sadlermiut | F | D3 | G/G LOW | | G/G HIGH | |
| **XIV-C 191** | Sadlermiut | M | D | G/G LOW | | G/G HIGH | |
| **XIV-C 197** | Sadlermiut | M | D3 | G/G LOW | | G/G HIGH | |
| **XIV-C 216** | Sadlermiut | M | D | G/G LOW | | G/G HIGH | |
| **XIV-C 217** | Sadlermiut | M | A2b | G/A INT. | | | |
| **XIV-C 221** | Sadlermiut | F | A2 | G/G LOW | A/A LOW | G/G HIGH | G/G HIGH |
| **XIV-C 246** | Sadlermiutt | F | A | G/G LOW | T/A INT. | G/G HIGH | G/G HIGH |
| **XIV-C 316** | Oftedal Lake | F | A2b | G/G LOW | A/A LOW | G/A INT. | G/G HIGH |
| **XIV-C 328** | Ennadai Lake | F | A2b | G/G LOW | A/A LOW | G/G HIGH | |
| **XIV-C 607** | Kamarvik | M | contaminated U/H | G/G LOW | A/A LOW | G/A INT. | G/G HIGH |
| **XIV-C 621** | Kamarvik | F | A2a | G/G LOW | A/A LOW | G/G HIGH | G/G HIGH |
| **XIV-C 636-1** | Kamarvik | M | A2a | G/G LOW | A/A LOW | G/G HIGH | G/G HIGH |
| **XIV-C 744** | Sadlermiut | F | A2b | G/G LOW | A/A LOW | G/G HIGH | |
| **XIV-H 015** | Dundas Harbour | | Amplified product, no readable sequences or peaks in any analysis | | | | |
| **XIV-H 041** | Ferguson Lake | F | No amplified product for mtDNA, nor readable results for SNaPshot | | | | |
| **XIV-C 111** | Sadlermiut | Amplified product, no readable sequences or peaks in any analysis | | | | | |

# Chapter 7 Discussion

The diversity of North American Aboriginal cultures can be observed at both the cultural and genetic levels. Selective pressures such as environmental fluctuations, pathogen load, nutritional deficiencies, cultural practices and war, have created circumstances that mold populations into heterogeneous groups. The objectiveof this thesis was to understand how the health conditions and immunogenetic profiles of an archaeological Inuit population may have impacted the current Inuit population's ability to effectively combat infection by tuberculosis. There are historical data noting that human populations vary in their abilities to effectively combat tuberculosis infection (Matthews 1886, 1888; Koenig 1921; Hrdlicka 1909). Various studies attest to longstanding discrepancies in susceptibility and resistance based on morbidity and mortality rates between different population groups (Ferguson 1950; Davies 1967; Bjerregaard and Young 2008). The following sections explore the possible explanations for the differences in rates for TB between Inuit, First Nations' and Canadian non-Aboriginal populations. This vulnerability is based on discovered patterns in palaeopathology and cytokine SNPs, and how the obtained cytokine genotype profile fits with previous assumptions concerning Canadian Aboriginal people's moving from a Th2 based immune response in a pre-contact world towards a Th1 immune response in post-contact. Both the Inuit and First Nations' populations maintain a Th2 immunogenetic profile with variability observed within the frequency of genotypes for specific SNPs.

## New World Health Burdens

The observed vulnerability of Canadian Aboriginal populations is in large part due to the particular circumstances experienced by each individual comprising the overall population. Variations occur because of gender and biological sex, age, occupation, habitual activities, environmental factors, genetics and in some cases chance. Each age group is vulnerable to different health issues; the sexes have developmental, genetic, and biological differences that impact their health. Different genders experience vulnerabilites based on cultural and social inequalities and cultural expecatations toward gender roles. What an individual does in everyday life and over a lifetime impacts the type of pathologies to which they experience; spontaneous mutation and inherited genes can cause individual disease or affect the immune system in different ways. All of these factors create a unique health situation for each individual within a population and the patterns that emerge from these individuals collectively create a population health profile that can be compared to other groups, similar and disparate from the sample population. Among the Inuit, there is a wide degree of diversity in cultural practices between populations and a diverse set of health conditions affecting different individuals. The Inuit represent a unique segment of the Aboriginal population of Canada in their environment, culture, pathogen exposure and delayed European contact, all of which have resulted in a pattern of health different from more southern populations including First Nations living below the tree line.

In recent decades studies have demonstrated the presence of a robust diversity in pathogens and pathogen load between North American Aboriginal populations (Verano and Ubelaker, 1992; Larsen and Milner, 1994; Larsen, 1994) and have demonstrated the considerable variability in disease patterns in response to varying cultural factors within

and between different geographic regions (Aufderheide, 1992; Ubelaker and Verano 1992). With a few exceptions, there is a lack of palaeopathological analyses of Arctic populations (Keenleyside 1998) in general and specifically for more recent Arctic populations such as the Inuit. Most studies have focused on the Aleut in various forms (Zimmerman et al.., 1971, 1981, Keenleyside 1998), or comparisons of the Eskimo and Aleut (Zimmerman and Smith 1975; Stewart 1979; Lobdell, 1980 Salter, 1984; Zimmerman and Aufderheide 1984). Most examine the presence of a pathological indicator or attempt to assign a specific condition to a presented lesion; very few have sought to demonstrate patterns of disease in Arctic populations prior to European contact, or compare differences in prevalence between regions and between time periods. Studies of degenerative and traumatic changes in specific populations of the Canadian Arctic do exist (Merbs, 1968, 1969, 1983; Cassidy 1977; Keenleyside 1998), but rarely are conditions outside of degenerative conditions or those caused by nutrient deficiencies discussed.

There is also very little information available concerning the health of the Sadlermiut. Much of the osteological information relayed has concerned the patterns of activity induced pathologies and degenerative disorders such as arthritis and severe musculoskeletal stress markers. The foremost source of this information comes from a single researcher Charles F. Merbs (1968, 1969, and 1983). From this collection of research it is possible to compare some of the degenerative conditions discussed in the results and somewhat deduce the condition of health in individuals who perhaps do not suffer from an infection but rather a chronic degenerative condition.

A basic overview of infectious diseases, those that manifest in the skeleton and degenerative changes provide insight into aspects of health in the archaeological

population. By describing pathological modifications within the sample population estimations concerning the severity of a condition based on both morbidity (those who present with healing or healed modifications) and mortality (those who died with active lesions) can be achieved. In reference to TB, the observation of TB pathologies in the skeletal sample provided a basis for the molecular analysis, provided candidates for immunogenetic profiling and TB analysis. The observation of co-occurring pathological conditions provided one of the greatest indicators of the overall health condition, as well as changes in health with contact.

## Osteological and Palaeopathological Discussion

Arthritic modifications were highly common within the sample cohort. Arthritic changes have been noted to frequently appear in Canadian Inuit populations in a number of articular units, and presented itself in some combination of lipping, eburnation (polishing) and/or porosity. Affected elements previously reported include: temporomandibular surfaces; shoulder (scapula, clavicle and humerus); elbow (distal humerus, radius, and ulna); wrist and hand; vertebral column; ribs; hip (acetabulum and femoral head); knee, ankle and foot (Merbs 1969, 1983). In general arthritic modification, commonly referred to as osteoarthritis, is more common in the upper limbs compared to the lower regardless of sex (Merbs 1983). In this skeletal population the vertebral column was more commonly affected and the observed frequency of involvement is slightly different from those previously published. Merbs (1983) states that in males the order of involvement is elbow, shoulder, wrist, hip, knee and ankle, while in females the same order is followed with the exception that the knee precedes the hip. Side dominance is observed in a minute number

of articular units; in both sexes the elbow and shoulder show a minor dominance and in only males in the wrist. In contrast this study saw a significant amount of arthritic modification in the vertebral column of both sexes. In females the cervical vertebrae were the most frequently affected, followed by the thoracic vertebrae. In contrast males demonstrated a high degree of modification in the lumbar area as well as the articular units of the humerus, both shoulder and elbow. This suggests a differentiation in activity patterns, and possibly habitual posture. Given that Inuit behaviour is often categorised as sexually dichotomous (Merbs 1983,) it is quite possible that differences in posture and behaviour patterns could explain the differential observation of arthritic changes. A number of cultural behaviours and biomechanical influences have been indicated as having an impact on Inuit populations in general and the Sadlermiut population in particular. While some are characteristics of humankind as a whole (posture, locomotive pattern, side dominance, pregnancy) others are related to Inuit cultural components (driving dog sleds, scraping skins, softening skins and boots, paddling kayaks) (Merbs 1983).

Enthesopathies, particularly those observed in the Sadlermiut subset, are one of the more highly discussed skeletal modifications (Merbs 1969, 1983). They are discussed here in context of arthritic changes because the two occur in a high degree of frequency and are often caused by similar behaviours. The population overall shows a high degree of enthesopathic modification of the spinal column. This is manifested through the development of degenerative disk disease (osteophytosis) which causes the growth of osteophytes, bony growths along the margin of the vertebrae caused by the disk becoming compressed, reducing the space and stimulating the periosteum to produce new bone. This form of degeneration, sometimes referred to as marginal spondylosis, as well as other

forms, are common in Inuit communities (Merbs 1995, 2002). The severity of the osteophytes followed similar patterns to arthritic changes, in that they occurred more commonly in the lower thoracic of women and upper lumbar of men, suggesting that degenerative disk diseases as an enthesopathic condition could be caused by gender-specific behavioural practices. Also similar to arthritic modifications is the high frequency of enthesopathic MSM's of the humerus in both men and women. Insertion sites for the *latissimus dorsi, teres minor and pectoralis major,* all of which insert on the proximal portion of the diaphysis, were the most common form of appendicular enthesopathy in both males and females, however as discussed above the division of labour in most Canadian Inuit populations means that the behaviour responsible is different for males and females.

In terms of fusion and cartilisation, the reason for the high frequency of spinal involvement can most likely be linked to degeneration of the intervertebral disk or arthritic irritation. Osteophytosis, also known as marginal spondylosis, is caused by a thinning of the intervertebral disk which allows bone margins to come into contact. Irritation of the periosteum triggers the growth of bony nodules called osteophytes. Continued irritation can result in osteophytes of great size and eventual fusion of the adjoining vertebrae. This occurs in areas of high flexion, the lower cervical, lower thoracic and lower lumbar vertebrae (Aufderheide and Rodrigues-Martin 2006; Duthiee and Bentley 1987). Costal fusion, or fusion of the ribs, is considered a congenital malformation, and the pathology generally occurs in the first two ribs on an individual (Aufderheide and Rodriguez-Martin 1998). However, individual XIV-C:179, a Sadlermiut post contact male, exhibits costal fusion to the left 5$^{th}$ and 6$^{th}$ rib, as well as a visceral surface lesion to the L1 rib, which may

indicate modification in response to infection rather than a congenital condition as the reason for costal fusion.

Injuries sustained through trauma were a considerable problem in many pre-contact populations. Breaks, fractures and wounds could lead to disability, infection and eventual death (Fortuine 1989). Vertebral and thoracic fractures, sometimes referred to as the telescoped type, are a form of compression fracture extremely common in Canadian Inuit populations (Merbs 1969, 1983). Vertebral trauma was encapsulated in both the arthritic modifications observed and in the angular compression and whole body compression described in the enthesopathic section of this thesis, and was commonly observed throughout the population. Merbs (1969, 1983) attributed the high frequency of this pathological condition to cultural practices, particularly the use of dog sleds. The condition of the snowpack and ice and the speed and force that the sled travelled could lead to traumatic injury to the weight bearing vertebrae and may account for a proportion of the arthritic as well as traumatic fractures and modifications seen in the vertebral column of the population. Given that dog sledding is a common mode of transportation, which in more technologically dependent populations has been replaced to some degree by ski-doo use, continued trauma to the spine could be observed for both the pre-contact and post-contact populations, without a high amount of discrepancy between the two groups. Trauma as part of everyday life takes many forms, and is a commonly reported and investigated occurrence. While luxation (dislocation of the joint) was not readily observed in the osteological sample, it has been reported in Arctic communities (Keenleyside 2003) but is difficult to detect in archaeological samples unless it remains unreduced for a significant period of time, allowing for deviations in normal bone growth and the

development of new articular surfaces (Aufderheide and Rodriguez-Martin 1998; Keenleyside 2003).

Traumatic pathologies are often of more interest to palaeopathologists when the individual demonstrates evidence of life after the trauma (Aufderheide and Rodriguez-Martin 1998), an excellent example of this is individual XIV-C: 149 from both the osteological and the molecular cohorts. This female adult exhibits a complete fracture of the pelvis, resulting in abnormal development of the pelvic girdle and the deviation of the pubis and the ischium. The surface of the break (both sides) demonstrates a significant amount of remodeling, with a smooth appearance across its surface. While the individual would undoubtedly have been in pain, and her ability to walk would have been highly impacted, to the point where she may have been disabled, it is evident she lived for a significant time after her trauma occurred. In a highly mobile and nomadic people, who rely on hunting as a main source of subsistence, this demonstrates a high degree of cooperative living and palliative care.

Tooth loss is common in many cultures which lack advanced forms of dentistry. Unlike modern Western populations, high sucrose and acid levels in diet played little part in the degradation and eventual loss of teeth in pre-contact and early contact Inuit populations. Instead, heavy usage of teeth in everyday life outside of digestion result in higher levels of damage, such as cracking, chipping or knocking out, all of which can lead to infection and eventual loss of the teeth. It has been suggested that the high levels of tooth loss in the Arctic Inuit result from such heavy tooth use, particularly of the anterior dentition (Merbs 1969). Similar levels of pre-mortem tooth loss (PMTL) are seen in other Arctic populations such as the Aleut (Keenleyside 1998). The Aleut express a high level of

PMTL (74% or 42/57) and Eskimo populations have similar levels of PMTL (65% or 77/118) (Keenleyside 1998) to that of the Inuit and the Aleut. Early research (Hrdlicka 1940; Merbs 1968, 1969) suggests that the Sadlermiut suffered from one of the highest rates of anterior tooth loss in comparison to most other cultures. Later populations of Inuit and Eskimo across the Arctic have demonstrated a decrease in the frequency (e.g. 1 tooth compared to 5 teeth) of loss the closer to contemporary times the population existed (Pedersen 1949; Moorrees 1957; Merbs 1969; Mayhall 1979). Suggested causes of tooth loss in the Sadlermiut populations is supposed by behaviours observed from other Inuit populations, so data can be extrapolated to suggest that these causes are valid for the entire osteological sample not just those from the Sadlermiut sites. Suggested causes for tooth loss include wrestling (Comer 1910; Birket-Smith 1928), wrenching of the teeth by sled traces while untangling (Merbs 1969) and by fishing lines while towing large fish behind a kayak (Nansen 1893). Early studies suggested ritual ablation (Hrdlicka 1940) as the main cause of PMTL but this has since been disproved (Merbs 1968). Trauma, and indirectly infection caused by trauma, appears to be the leading cause of tooth loss (Merbs 1968). . However, most studies discuss the "third hand" cause in relation to anterior PMTL. This behaviour would explain the high levels of anterior tooth loss within the sample, however, the most commonly lost tooth type in this skeletal population are molars. The third hand hypothesis, therefore, does not explain the equally high loss of molars. Grinding, such as that performed during hide preparation, has been posited as one explanation for molar loss. Small micro fractures have been observed in Eskimo populations (Pedersen 1949), dental wear is common throughout Inuit and Eskimo dentition (Merbs 1968), but these micro fractures appear only in the molar crowns (Pedersen 1949; Merbs 1968). A

combination of explanations, such as micro-wear caused by hide preparation and the third hand model would explain the high level of PMTL in both the anterior dentition and molars for both the pre and post contact populations. A similar trend in PMTL between more historic populations and more contemporary populations can be observed across the Arctic. With the advent of European colonists and European culture, changes in behaviour and activities have resulted in a decrease in PMTL (Merbs 1968).

As discussed above, common to most Arctic populations are pathologies related to nutrient deficiencies. The single isolated case of rickets or osteomalacia (Aufderheide and Rodriguez-Martin 1998) is completely in keeping with expected observation, though it is somewhat extraordinary considering the rate of vitamin and mineral deficiency observed in other Arctic populations (Keenleyside 1998). Neoplasms related to cancer are under reported, and except for a single report of possible metastatic cancerous lesions (Cassidy 1977) in a post-contact Sadlermiut individual, there is very little information concerning cancer incidence rates in archaeological populations. The two possible cases observed in this study (XIV-C:179 and XIV-C:149) represent two disparate forms. The first (XIV-C:179) is an osteoma or neoplasma, most likely benign, on the frontal bone, and most likely benign. Osteomas in this region are not uncommon (Brothwell 1967) and they can occur fora number of reasons. The second form (XIV-C:149) found in the mandible is more suggestive ofa malignant form of neoplasm. Due to the small sample size and the varying forms, little can be inferred about neoplastic conditions based on observation from this sample.

These results establish health burdens for both pre and post-contact Inuit individuals. The increase in observed TB lesions in post-contact individuals may be suggestive of an increased exposure to Mtb, as well as an increase in susceptibility to new

or more virulent strains. Many of the other pathological conditions that co-occurred with TB, or increased from pre-contact to post-contact periods are conditions controlled by the innate immune system. These observations, in conjunction with the observed immunogenetic profiles for both time periods, are suggestive of a stronger Th2 immune response in both time periods. The increase in degenerative and occupational stress markers may also be linked to changes in cultural and socio-economic behaviours, whereby the Aboriginal populations experienced a shift in nutritional habits, housing, and even environment (traditional lands to reserves or hamlet communities).

## General Molecular Trends

Two forms of skeletal elements were taken for molecular sampling, ribs and molars. In the process of preparing for nuclear analysis it was necessary to determine DNA concentration to ensure a sufficient amount was present for detectable and readable results. Molar samples consistently resulted in a higher DNA concentration than that of rib samples; in many instances the molar samples contained upwards of ten times the amount of DNA compared to rib samples from the same individual. When mitochondrial sequences were compared between the two different sample materials, DNA extracted and sequenced from molar samples consistently had less static, less contamination and more definitive peaks and overall sequences. DNA extracted and sequenced from powdered bone resulted in more failures to sequence, more highly degraded and fragmented sequences and a higher amount of basepair misread. Similar results have been recorded for amplification success using teeth versus compact bone (Meyer et al. 2000; O'Rourke et al. 2000). The two "contaminated" samples obtained during mtDNA analysis, were both extracted from

rib specimens.Additional amplification of both the previously amplified rib extractions, as well as, amplification from molar extractions for individuals XIC-C:152 and 607 resulted in incongruent mitochondrial sequences. This confirmed that the DNA from the rib samples was contaminated and all further analysis was performed using molar extractions.

### Haplogroups and Family Groups

Haplogroup analysis was performed to ensure membership in the Inuit ethnicity. . Similar to other studies of Canadian Inuit (Lorenz and Smith 1996), the only two haplogroups exhibited by this study's population are haplogroup A and haplogroup D (Hayes 2002; Helgason et al. 2006). Haplogroup A maintains the dominant position in Eastern and Central Inuit populations with a substantial portion of ancient and contemporary populations demonstrating haplotypes associated to this haplogroup (Lorenz and Smith, 1996; Starikovskaya et al.. 1998; Schurr et al. 1999; Marchani et al. 2007). Mitochondrial haplogroup monomorphism, the overwhelming expression of a single haplogroup, is considered common in the Thule populations for these territories, as well as extending into the Greenlandic Inuit population (Saillard et al. 2000; Hayes et al. 2003; Helgason et al. 2006). The pre-contact population of the Kamarvik site represented by three individuals tentatively dated to approximately 800 years before present demonstrates and supports the theory of mitochondrial haplogroup monomorphism for haplogroup A. Two individuals present with mutations common to haplotypes A2a and the third individual, while possessing an incomplete sequence, displayed polymorphisms in the HVRII common to the A2a individuals. The remainder of the cohort, dated to the contact period, had more mitochondrial sequence variation than that of its pre-contact neighbours.

As shown in Table 6.9, the fourteen individuals that comprise this group consisted of four individuals from haplogroup D (29%) and ten individuals from haplogroup A (71%), suggesting a decrease in the genetic isolation of the population. These findings coincide with similar haplogroup frequencies in other studies, where Arctic communities, particularly Inuit populations in the central and eastern Arctic, express almost complete association with haplogroup A (Lorenz and Smith 1996; Hayes 2002; Helgason 2006) with a small proportion expressing haplogroup frequencies in either C (SubArctic Dogrib, Lorenz and Smith 1996; Merriwhether et al. 1995; Torroni et al. 1992) or D (Inuit, Lorenz and Smith 1996; Haida, Lorenz and Smith 1996; Torroni et al. 1993). Thirteen contact individuals are from the Sadlermiut Inuit culture from Southampton Island. Hayes (2003) found that the Sadlermiut expressed haplogroup frequencies dissimilar to their contemporary neighbours and ascendants; the Thule demonstrated monomorphism for haplogroup A (n= 17, 100%), the Dorset expressed monomorphism for Haplogroup D (n=2, 100%), while the Sadlermiut demonstrated frequencies split between haplogroups A (n=10, 55.6%) and D (n.8, 44.4%). The one similarity that all four populations, Inuit, Thule, Dorset, and Sadlermiut share is the lack of any expression of haplogroups B or C at any frequency in their populations (Lorenz and Smith 1996; Hayes 2002; this study). The lack of diversity found within these populations, particularly pre-contact populations, is suggestive of small groups living in relative isolation from other Arctic populations, who may have experienced some form of genetic bottleneck and a distinct lack of gene flow (Marchani et al. 2007) prior to contact.

Two individuals expressed mitochondrial sequences outside of the expected haplogroups for the population; this can be attributed to exogamous DNA contamination.

All archaeological remains for this study were excavated between 1930 and 1980, before any widespread knowledge of DNA contamination. Contamination protocols then were not in place, and the majority of the skeletal elements were handled by a wide variety of individuals before being housed in various research institutions. The possibility of contamination by exogamous DNA is very high.

The first individual (XIV- C:152) was contaminated by a non-human source whose DNA sequence had a 40% match to bacterial sewer sludge (*Clostridium saccharolyticum)*. Manual alignment of the sequences obtained from this individual illustrated the amplification of two contigs, one of the human variety and one of non-human nature; three separate extractions/amplifications/sequencing reactions resulted in the same two contigs. The second individual's (XIV-C:607) mitochondrial sequence obtained from a rib sample had mutations that indicated either a U or an H haplogroup. This individual is from a pre-contact population and no other research has assigned these haplogroups to an Inuit population, logically the individual was contaminated by an outside source. Mitochondrial sequences from the HVRII were obtained using sample extracted from the molar of the same individual. Sequence analysis from the molar resulted in readable sequences in the HVRII but no haplogroup could be assigned based on these results. As the researcher, I am the logical source for contamination, however my mitochondrial sequence is assigned the N haplogroup, and I therefore cannot possibly be the source of contamination. Thus, it is most likely that the remains were contaminated at some point in the course of excavation or curation.

The two family groups represented within the molecular cohort exemplify the continued occupation of the Inuit in the western Hudson Bay area and also illustrate the

highly mobile nature of smaller family groups, characteristic of many historic Inuit populations (McGhee 1996; Helgason 2006). Individuals XIV-C: 636-1 and 621 were recovered from the Kamarvik site, which is thought to represent classical Thule culture and date to approximately 800 years before present (McCartney, 1971, 1977; Morrison, 1989; Wilmeth, 1978). Individual XIV-C: 636-1 represents an adolescent male (determined through molecular analysis) and XIV-C: 621 an adult female both dated to the pre-contact period. XIV-C: 636-1 was recovered along with an adult possibly female individual (636-2, not examined in this study) with neonate remains, so a parental relationship between XIV-C:621 and XIV-C:636-1may be unlikely. However, given that both individuals were recovered in proximity to each other, from the same time period, and both possess a mitochondrial sequence containing mutations not present in the other individuals recovered and sequenced from the same site, a family relationship can be inferred. Both individuals express substitutions common to what has been referred to as the root A2a haplogroup (16111c-t, 16192c-t, 16223c-t, 16290c-t, 16319g-a, 16362t-c) (Helgason et al. 2006; Gilbert et al. 2007). A2a is common across both Greenland and the Eastern and Central Canadian Arctic (Helgason et al. 2006). These individuals, unique within the population (16192c-t), share an affinity with Greenlandic Inuit mitochondrial sequences (Gilbert et al. 2007) and may exemplify a migration route across the Canadian Arctic to the western coast of Greenland. The exact nature of their familial relationship cannot be assessed. Less can be said about the second family group who express substitutions common to haplogroup A2b, particularly the A-G substitution at nt 16265 (Helgason et al. 2006). Other individuals within the population express the same substitution but lack mutations at other shared sites.

## Cytokine Immune Profiles

The anaylsis of cytokine SNPs resulted in immunogenetic profiles that may provide some insight into the vulnerability of contemporary Inuit populations to TB. While complete profiles were not obtained for the entire molecular cohort, it was possible to compare the archaeologicall Inuit profiles from this study to published First Nations' profiles. In one study (Larcombe et al. 2008) two First Nations populations from Manitoba with high rates of tuberculosis were analysed for a suite of cytokine and immune system genes that may impact the outcome of tuberculosis infection. The Dené were found to have an high frequency of homozygous G phenotype or a G/G genotype (97%) for TNFα -308; an high frequency of homozygous A genotype (93%) of INFγ +874 SNP; a high frequency of homozygous G/G genotype of IL-6 -174 SNP (95%); and a slightly more diverse demonstration of the IL-10 -1082 SNP, with the heterozygous genotype of G/A occurring in 20% of individuals and the homozygous A/A genotype occurring in 78% of individuals (Larcombe et al. 2008). In the same study, a Cree population had a similar expression of cytokine genotype frequencies for TNF alpha (-308), but slightly more variation of frequencies in the remaining three cytokine SNPs (Table 7.1).

In contrast the Inuit population of this study demonstrated a high frequency of the the high producing phenotype for IL-10 (-1082); 87% (n=13 see Table 7.1 and 7.2 for individual results) of the population expressed the homozygous G/G genotype typical to a high producer. The two First Nation's populations discussed above exhibit a more diverse expression of genotypes. The results are not due to allelic drop out as two individuals were genotyped as intermediate producers with a heterozygous genotype G/A. No Inuit

| Table 7.1 Cytokine SNP Frequencies for Three Cohorts | | | | | |
|---|---|---|---|---|---|
| | Allele Frequency | | Genotype Frequency | | |
| TNFα ( -308) | A | G | High A/A | Int. A/G | Low G/G |
| Dené | n=2 | n=98 | 0 | 2(3%) | 59(97%) |
| Cree | n=2 | n=98 | 0 | 1(3%) | 44(98%) |
| Canadian Inuit | n=3 | n=97 | 0 | 1(7%) | 14 (93%) |
| IFNγ ( +874) | T | A | T/T | T/A | A/A |
| Dené | n=3 | n=97 | 0 | 4(7%) | 57(93%) |
| Cree | n=26 | n=74 | 4(11%) | 14(30%) | 27(59%) |
| Canadian Inuit | n=6 | n=94 | 0 | 1(11%) | 8(89%) |
| IL-6 (-174) | G | C | G/G | G/C | C/C |
| Dené | n=98 | n=2 | 58(95%) | 3(5%) | 0 |
| Cree | n=90 | n=10 | 38(84%) | 6(14%) | 1(2%) |
| Canadian Inuit | n=100 | n=0 | 8 (100%) | 0 | 0 |
| IL-10 (- 1082) | G | A | G/G | G/A | A/A |
| Dené | 12 | 88 | 1(2%) | 12(20%) | 48(78%) |
| Cree | 20 | 80 | 2(5%) | 11(25%) | 31(70%) |
| Canadian Inuit | 93 | 7 | 13(87%) | 2(13%) | 0 |

Dené and Cree data adapted from Larcombe et al. 2008

individuals expressed the genotype of A/A, a marked difference from both the Cree and the Dené populations. The cytokine IL-10 is associated with a Th2 immune response, and as a down regulator of Th1 immune responses (Candelaria et al. 2007). While polymorphisms in IL-10 (-1082) have been linked to hepatitis C resistance and progression (Minuk and Uhanova 2003; Minuk et al. 2003; Grebely et al. 2007) the association has usually been in relation to allelic variation in IL-10 polymorphisms being linked with the disease progression of chronic HBV infection. Heterogeneity in the promoter region of the IL-10 gene has been reported to have a role in determining the initial and sustained response of chronic hepatitis C to IFN-α therapy. High production of IL-10 results in the interference with a number of Th1 cytokines like IFN and TNF through down regulation (Edwards et al.

1999; Miyazoe et al. 2002; Cheong et al. 2006). While Canadian Aboriginal peoples show a unique resistance to chronic hepatitis C infection (Minuk and Uhanova 2003), this would appear to be in contrast to the archaeological Inuit results in this study. A clear explanation of why IL-10 (-1082) high producer phenotypes are so common in Inuit populations is not apparent. It could be suggested that IL-10 (-1082) and other associated IL-10 polymorphisms as immune regulators have a stronger association to Th2 immune responses than that of Th1 immune responses. Given that it has been previously established that Amerindians favour a Th2 immune response there could be an association between this immune characteristic and the high producer phenotype in Inuit. The other genotype frequencies and their associated phenotypes fall within the expected ranges for Canadian Aboriginal populations. The high producer phenotypes for IL-6 and the low producer phenotypes for TNF alpha (G/G), and IFN gamma (A/A) are associated with Th2 and Th1 immune responses respectively. IFN gamma and TNF alpha are linked to the host's ability to produce Th1 immune responses that combat TB, while IL-6 and IL-10 are known to adversely affect and down regulate the production of IFN gamma, which works synergistically with TNF alpha (Romagnani 1994, 1996, 1999; Flynn and Chan 2001; North and Jung 2004). A previous study suggests that the low producer genotype and phenotype of A/A for IFNγ (+874) is more common in individuals with tuberculosis (Tso et al. 2005), suggesting that the low producer phenotype increases an indvidual's susceptibility to TB infection.

| Table 7.2 Cytokine Profiles for Individuals with Observed TB Pathologies | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Site | Sexing | Haplogroup/ Haplotype | TNFα | IFNγ | IL-10 | IL-6 |
| **XIV-C 149** | Sadlermiut/ Native Point | FEMALE | A | G/G LOW | | G/G HIGH | G/G HIGH |
| **XIV-C 164** | Sadlermiut/ Native Point | MALE | A | | | G/G HIGH | |
| **XIV-C 179** | Sadlermiut/ Native Point | MALE | A2 | G/G LOW | A/A LOW | G/G HIGH | G/G HIGH |
| **XIV-C 197** | Sadlermiut/ Native Point | MALE | D3 | G/G LOW | | G/G HIGH | |
| **XIV-C 217** | Sadlermiut/ Native Point | MALE | A2b | G/A INT. | | | |
| **XIV-C 221** | Sadlermiut/ Native Point | FEMALE | A2 | G/G LOW | A/A LOW | G/G HIGH | G/G HIGH |
| **XIV-C 316** | Oftedal Lake | FEMALE | A2b | G/G LOW | A/A LOW | G/A INT. | G/G HIGH |
| **XIV-C 328** | Ennadai Lake | FEMALE | A2b | G/G LOW | A/A LOW | G/G HIGH | |

Cytokine immune profiles for the analysed SNPs were obtained for eight of the individuals from whom tuberculosis typical lesions were recorded (n=11). Complete immunogenetic profiles were obtained for three individuals (XIV-C: 221, 316,179) and for only two was a single SNP obtained (XIV-C: 164, 217). Of the four genotypes obtained for IFNγ (+874) in individuals with observed TB pathologies all four demonstrate a low producer phenotype (homozygous A/A), which is linked to tuberculosis infection (Tso et al. 2005). Six of the individuals demonstrated the high producer genotype for the SNP of IL-10 (-1082), and one individual (XIV-C: 316) demonstrated the intermediate genotype. Six individuals also presented a homozygous expression of the G allele (G/G) indicative of the

low producer genotype for TNFα (-308) SNP. These results suggest that the Inuit of this study most likely favoured a Th2 immune response as opposed to a Th1 immune response.

## Tuberculosis Results

### Palaeopathological Discussion

Tuberculosis was originally considered absent or at the very least rare amongst pre-contact Amerindian populations (Hrdlidka 1909), but substantial evidence of tubercular modification of human remains has been discovered in the last few decades (Roberts and Buikstra 2003). In Canada, several osteological collections have yielded evidence of pre-contact TB infection: Woodlawn, Farity Ossuary, Bennett site, and Uxbridge (Roberts and Buikstra 2003). However, concrete evidence of TB in pre-contact and early contact Inuit populations remains elusive. While the sample distribution between the two time periods is skewed in favour of the post-contact period, which may affect the interpretation of the distribution of lesions, the data are suggestive of a rise in the number of infections through time. The sample analyzed demonstrates osteological evidence of a higher presence of lesions associated with TB in individuals from the contact period (n= 21) compared to the pre-contact (n= 6) cohort. This could support research suggesting an increase from endemic to epidemic levels post-contact (Fortuine 2005). Poor preservation of skeletal elements can obstruct pathological lesions and affect the ability of a researcher to accurately assess a specific pathological condition. Likewise, a lack of burials restricts the sample selection pool and may not accurately represent the living population, or even the archaeological population. While DNA amplification resulted in "positive" TB results for two individuals, confirmed identification of TB through sequencing of the obtained

pathogenic amplification products was not possible. The sequences were unreadable or in fragments that could not be uniquely identified as *M. tuberculosis.*

### Microbial DNA Discussion

A number of mammalian species are capable of infecting humans with mycobacterium, including: goat, pig, sheep, horse, cat (feral and domestic), elk (Tessaro 1986), dog (feral and domestic), fennec fox, deer, bison, buffalo, badger, possum, cattle, hare, ferret, antelope, Arabian oryx, camel, llama, alpaca, primates including other humans, and the lechwe (Gallagher et al. 1972; Clancy 1977; O'Reilly and Daborn 1995). The exact method in which animal to human transmission occurs in many zoonotic forms is unclear (Kaevska et al. 2010). However the most common form in domesticated animals is through ingestion of contaminated milk (Thoen and Barletta 2004), inhalation of infected animal cough aspirations (Cosivi et al. 1998) and possibly ingestion of contaminated raw animal tissue (Kaevska et al. 2010). In wild animal populations inhalation and ingestion of milk is rare, but consumption of raw tissue could possibly lead to infection. The Inuit population of this study maintained no domesticated animals in which to obtain infected milk from or inhale bacilli. Ingestion of raw meat from caribou or birds species is a possible mode of transmission, but occurs in rare cases; therefore the Inuit were highly unlikely to contract *M. bovis*. Pre-contact TB could have been contracted from low level endemic TB within the environment. The slight increase in TB pathologies observed in this study may be indicative of increased infection through contact with other human populations.

An effort to confirm the presence of TB in archaeological Inuit populations in Canada was undertaken through the amplification of pathogenic DNA from individuals in

this study. However, results indicated that the primers, designed to amplify a target region of *Mycobacterium tuberculosis*, were nonspecific and could result in the amplification of other varieties of Mycobacterium species including *bovis, microti* and *africanum* (O'Reilly and Daborn 1995). Therefore, while amplification indicated a "positive" result for two individuals, further sequencing is suggestive of infection with another form of mycobacterium. Additional investigation would be required to rule out those other possible forms of mycobacterium. Lack of skeletal lesions does not mean the absence of *Mycobacterium tuberculosis*; however, diagnosis of tuberculosis could not be confirmed through aDNA analyses for this study.

## Conclusions

This research has determined that the cytokine genotype frequencies for TNFα (-308) IFNγ (+874), IL-6 (-174) expressed by archaeological Inuit populations are similar to contemporary First Nation's populations in Manitoba but with slight fluctuations in allele frequencies. Similar to research comparing cytokine genotype frequencies between First Nations populations and contemporary Canadian Caucasian populations (Larcombe 2005; Larcombe et al. 2005; Larcombe et al. 2008), Inuit genotype frequencies for the aforementioned cytokines are substantially different from those expressed in Caucasian populations. As discussed this may be a result of the historical and evolutionary history of the descendant population. Amerindian populations in the New World appear to favour a Th2 immune response based not only on cytokine genotype frequencies, but also on KIR haplotypes frequencies and the distribution of HLA haplotypes (Tsuneto et al. 2003; Rempel 2011), whereas Caucasians appear to favour a Th1 immune response. Studies of

African Americans have shown statistically higher production levels of Th1 associated cytokines (IL-2 and TNF alpha) when healthy compared to healthy Caucasians (Kimball et al. 2001).

The analysis of cytokine immune profiles in past populations may contribute to the understanding of current issues in contemporary populations. It may contribute to a more detailed understanding of the specific relationship between cytokine genotypes and disease. A comparison of both pre-contact and contact individuals from across the circumpolar region, including Aleuts to Siberian Yupiks would more effectively demonstrate the relationship between the Arctic environment and the retention of the pre-dominantly Th2 immune response discussed.

This research demonstrates that archaeological Inuit samples of several hundred years will yield reliable results in both mtDNA studies and nuclear DNA studies. A reliable method was developed from sample preparation to analysis for the determination of cytokine SNPs associated with the ability to combat tuberculosis. Fine tuning primer design at the PCR and SNaPshot levels for IL-6 (-174) and IFN gamma (+874) would result in more reliable SNaPshot results. Individual optimization for cycling parameters for each cytokine SNP's primer sets could produce superior amplification results, especially with regard to IFN gamma (+874). Regardless of technologic difficulties, preliminary results presented in this study suggest that this form of research may provide interesting results and avenues for exploring relationships between Canadian Aboriginal populations. This form of research also represents a unique method for understanding migration and evolutionary origins, as well as determining environmental and selective pressures related to host-pathogen interactions in archaeological populations.

Every year new methods of analysis related to aDNA research are developed. Immunogenetic research, particularly analysis of ancient populations is still in the foundational stage, and access to individuals, particularly those of Canadian Aboriginal descent, is limited. Nevertheless, the analysis of immunogenetic profiles may contribute to understanding potential biologic determinants of diseases including hepatitis, HIV/AIDS and tuberculosis. Recent research into KIR and HLA haplotypes in Amerindian populations provides a wide geographical understanding of immunogenetics, but population specific studies are still needed. Analysis of distinct cultural or geographical groups would address the gaps in understanding differences within the homogenous "Amerindian" groupings. This research attempted to address the Inuit cohort from a historical perspective. By providing an analysis of not only the immunogenetic profiles of individuals from a pre-contact and contact period, but also addressing the basic health status and fluctuations in pathological conditions experienced by both populations. Immunological responses develop, in part, in response to pathological stimuli, by understanding the pathogen load and other health conditions within the Inuit it was possible to suggest that the "pathological" history of the Inuit was a contributing factor to current disparities in TB rates observed in contemporary Canadian Inuit communities. However, given the plethora of determinants that affects TB disease in Canadian Aborginal and non-Aboriginal populations, it is currently unclear how important genetic variables are to explaining observed disparities in health. Improved understanding of the variation in genetic response to Mtb may assist in the development of improved methods of prevention and treatment.

In conclusion the following points summarize the impact of this study on the three questions that arose from the observed discrepancies in tuberculosis rates among Aboriginal and non-aboriginal Canadian populations:

- Osteological health indicators demonstrate that chronic conditions were a significant burden in both pre-contact and post-contact cultures
    - Infectious diseases, while present, were observed at lower frequency within the skeletal population
- TB pathologies were observed in both pre and post contact populations, however, molecular analyses failed to confirm its pre contact presence
- Both mtDNA and nuclear DNA were successfully amplified and analyzed from archaeological Inuit remains
- The pre and post contact Inuit maintained a cytokine SNP profile suited to combating parasitic, fungal and environmental conditions, favoured by the production of Th2 immune cells
    - Immunogenetic profiles were maintained across pre- and post contact Inuit populations
- Variation in immunogenetic profiles was observed between the Inuit study samples and previously reported studies of First Nations and Caucasian populations
    - With the exception of IL-10 (-1082), Inuit express cytokine SNP frequencies similar to their Southern counterparts in the First Nations

Despite the maintenance of a Th2 immune response in both First Nations and Inuit populations, variation in genotype frequencies suggests differences in immune response. Further investigation into the difference in genotype frequency of IL-10 (-1082) in other Inuit subpopulations may reveal specific selective pressures in Inuit pathological history. A comparison to contemporary Inuit populations may also reveal changes in the immunogenetic profiles compared to the skeletal sample in this study, and a closer

genotype frequency to contemporary First Nations' populations. The inclusion of analysis of more cytokine SNPs associated with immune response to TB infection, such as IL-12 and IL-18, could lead to a further understanding of the continued vulneralbity of both the Inuit and First Nations.

# Appendix 1 Primers

## Mitochondrial Analysis Primers

| | |
|---|---|
| 15971 F | TTA ACT CCA CCA TTA GCA CC |
| 16258 R | TGG CTT TGG AGT TGC AGT TG |
| 16191 F | CCC ATG CTT ACA AGC AAG TA |
| 16410 R | GAG GAT GGT GGT CAA GGG AC |
| 1 F | GAT CAC AGG TCT ATC ACC C |
| 280 R | GAT GTC TGT GTG GAA AGT GG |
| 155 F | TAT TTA TCG CAC CTA CGT TC |
| 389 R | CTG GTT AGG CTG GTG TTA GG |

## *Mycobacterium tuberculosis* Amplification

| | |
|---|---|
| IS 1 | CCT GCG AGC GTA GGC GTC GG |
| IS 2 | CTC GTC CAG CGC CGC TTC GG (123 bp product) |

## Sex Determination

| | |
|---|---|
| AMEL 1 F | (6FAM) CCC TGG GCT CTG TAA AGA ATA GTG |
| AMEL 1 R | ATC AGA GCT TAA ACT GGG AAG CTG |

## Cytokine Genotyping

### Il-6 (-174)

Forward: CTT CGT GCA TGA CTT CAG C

Reverse: CAC CCT CAC CCT CCA AC

Extension: AGC TGC ACT TTT CCC CCT AGT TGT GTC TTG C

Extension 2: TTC CCC CTA GTT GTG TCT TGC

### IL-10 (-1082)

Forward: ATC CAA GAC AAC ACT ACT AA

Reverse: CTC CTA TCC AGC CTC CAT GG

Extension: CTA CTA AGG CTT CTT TGG GAA

### IFN gamma (+874)

Forward: GCT GTC ATA ATA ATA TTC AG

Reverse: GAA ACC TGT ACC ATT GGG

Extension: TTA CAA CAC AAA ATC AAA TC

### TNF alpha (-308)

Forward: CCA AAA GAA ATG GAG GCA ATAG

Reverse: CGG AAT CGG AGC AGG GAG GA

Extension: AAT AGG TTT TGA GGG GCC

# Appendix 2 Cycling Parameters

## SNaPshot Cycling Parameters

**Denature: 96°C for 10 seconds**

**Anneal: 50°C for 5 seconds**

**Extend: 60°C for 30 seconds**

**Hold: 4°C until ready for post extension**

**Repeat: 25 cycles (1 hour and 10 minutes)**

## Post SNaPshot Extension

**Incubate: 37°C for 60 minutes**

**Anneal: 75°C for 15 minutes**

## TB PCR

**Hot Start: 94°C for 10 seconds**

**Denature: 94°C for 30 seconds**

**Anneal: 60°C for 1 minute**

**Extend: 72°C for 2 minutes**

**Hold: 4°C until ready for post extension**

**Repeat: 50 cycles**

## Sex Determination PCR #1

Hot Start: **94°C for 2 minutes**

**Denature: 94°C for 30 seconds**

**Anneal: 60°C for 1 minute**

**Extend: 72°C for 2 minutes**

**Hold: 4°C until ready for post extension**

**Repeat: 50 cycles**

## Sex Determination PCR #2

Hot Start: **94°C for 2 minutes**

**Denature: 94°C for 30 seconds**

**Anneal: 50°C for 1 minute**

**Extend: 68°C for 2 minutes**

**Final Extension: 72°C for 2 minutes**

**Hold: 4°C until ready for post extension**

**Repeat: 50 cycles**

## Immunogenetic PCR

Hot Start: **94°C for 2 minutes**

**Denature: 94°C for 30 seconds**

**Anneal: 50°C for 1 minute**

**Extend: 72°C for 2 minutes**

**Final Extension: 72°C for 2 minutes**

**Hold: 4°C until ready for post extension**

**Repeat: 50 cycles**

## IFNγ PCR

Hot Start: **94°C for 2 minutes**

**Denature: 94°C for 30 seconds**

**Anneal: 58°C for 1 minute**

**Extend: 72°C for 2 minutes**

**Final Extension: 72°C for 2 minutes**

**Hold: 4°C until ready for post extension**

**Repeat: 50 cycles**

## Appendix 3: Contact Cohort Osteological Summary

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| Caribou Point 1 | C:329 | F | 19+ | Almost complete skull and mandible, nearly complete skeleton | ribs lesions (bilateral), R innominate abscess, R femur, L Radius , arthritic lipping on lower thoracic, PMTL with abscessing and high degree of remodeling, modification of zygoma | rib lesions | | |
| Crossing Point Loc 2 | C:307 | I | 13-18 | Complete skull and mandible, nearly complete skeleton | Left scapula; R humerus 2 enthesopathic lesions at proximal MSM site | | | |
| Ennadai Lake | C:328 | M | 19+ | Complete skull and mandible, nearly complete skeleton | rib lesion, arthritic lipping L5 and L4, lesions on L1, T6, T2, enthesopathy R humerus, possible modification of R distal Tibia (damaged), PMTL with abscessing and remodeling | rib and vertebral lesions | Yes | |
| Griffith Lake | C:320 | M | 19+ | Complete skull and mandible, nearly complete skeleton | right radius | | | |
| Inuksivik | C:664 | M | 19+ | Almost complete skull, mandible, nearly complete skeleton (2 sets of ribs unclear) | Spondylolysis of L4 and L5, arthritic lipping on lower thoracic, T2 lesion, MSMs R/L radius, abscessing with minor PMTL | | | |
| Inuksivik North | C:679 | M | 19+ | Almost complete skull, mandible, some postcranial remains | R/L pubic symphysis lesions, PMTL with abscessing, isolated lesion in thoracic, arthritic lipping of lumbar | | | |
| Kamarvik | C:531 | F | 19+ | Mandible, nearly complete skeleton | | | | |
| | C:611 | M | 19+ | Skull frags, mandible, some postcranial remains | PMTL with abscessing | | | |
| Kulaituijavik | C:694 | M | 19+ | Complete skull and mandible, nearly complete skeleton | L rib lesions, L/R humerus head, extranumerary foramen superior to auditory canal, minor PMTL with minor abscessing and remodeling, incomplete fusion of sacrum & R innominate, arthritic lipping on L vertebrae, lesion on pubic symphysis | possible, rib lesions | | |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| Oftedal Lake | C:316 | F | 13-18 | Complete skull and mandible, nearly complete skeleton | possible involvement of sacrum (damaged); lesions, T3, 8, 9, L1, 2, 3, 4; lesion/abscess R/L ribs; R femur; R scapula arthritis with lesion b/w glenoid and coracoid ; large abscess R/L innominate with remodelled bone; R mastoid possible mastoiditis, slight abnormality to morphology of L side mental eminence | lesions in ribs and T/L vertebrae | Yes | |
| | C:317 | M | 19+ | Almost complete skull and mandible, nearly complete skeleton | arthritis L ulna and radius; R humerus MSM, fracture of R fibula; R pubic symphysis abscess; PMTL with abscessing and remodelling; fracture at L sacrum; L5 collapse; fracture to zygoma with signs of infection | | | |
| | C:319 | M | 19+ | Almost complete skull, mandible, some postcranial remains | L occipital condyle arthritis, cranial lesions, caseation on R parietal, PMTL with large abscess and remodelling, R fibula inflammation, R tibia arthritis, R pubic symphysis abscess | cranial lesions | | |
| | C:323 | M | 13-18 | Complete skull and mandible, nearly complete skeleton | R scapula involvement; Left innnominate obturator foramen, ischium, pubis and symphysis; R innominate abscess; lesions on L (3/4) and R (2/3) ribs (badly damaged); possible wolf's tooth (r maxillary); fracture of cervical vertebrae; Lesions T7-11, L3, Tibial MSM, R humerus lesion shaft | | | |
| | C:324 | M | 19+ | Complete skull and mandible, nearly complete skeleton | R/L humerus MSM | | | |
| Sadlermiut | C:073, 264 | F | 19+ | Some postcranial remains, see comments | lesion on right rib | | | |
| | C:096 | F | 19+ | Skull frags, some postcranial remains | minor abscess with PMTL, thoracic anterior collapse and compression | | | |
| | C:098 | F | 19+ | Almost complete skull, mandible, nearly complete skeleton | lesion on left side rib and right pubic symphysis | | No | radiocarbon range **1521-1683** Coltrain et al 2003 |
| | C:099, 93 | I | I | Some postcranial remains | L glenoid osteoarthritis, L5, lesions L1-3, T12 severe collapse with lesion, fusion of T10/11 with lesions | thoracic lesions | | |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| | | | | | lumbar osteoarthritis | | | |
| | C:102, 258 | M | 19+ | Some postcranial remains | | | | |
| | C:103 | F | 19+ | Almost complete skull, mandible, nearly complete skeleton | some arthritic modification of lumbar vertebrae, alterations of 5th left rib sternal end | | No | |
| | C:104, 89 | I | I | Complete skull and mandible, some postcranial remains | L humerus lesions, PMTL abscessing , L2 anterior compression, L1 lesion, T5-12 lesions and caseation with anterior collapse | lesions throughout thoracic | | |
| | C:105, 275 | F | 19+ | Almost complete skull, mandible, some postcranial remains | arthritis of the vertebral column, lumbar lesions, scattered thoracic lesions with anterior collapse, abscessing, OPMTL R/L innominate, L parietal lesion | lesions in vertebral column | No | |
| | C:111 | M | 19+ | Complete skull and mandible, nearly complete skeleton | mandibular abscessing, right rib visceral surface modification and enlargement | Lesions on visceral ribs, right side | Yes (molar and rib) | |
| | C:112 | F | 19+ | Complete skull and mandible, nearly complete skeleton | lumbar disk displacement, 2nd rib modification | | No | radiocarbon range **1485-1661** Coltrain et al 2003 |
| | C:117 | M | 19+ | Complete skull and mandible, nearly complete skeleton | anterior mandibular abscessing, minor involvement of innominate, sacrum, pelvis and patella | | No | |
| | C:126 | M | 19+ | Complete skull and mandible, nearly complete skeleton | arthritis of Lumbar vertebrae, mastoiditis, mandibular abscessing, lesions on the pubic symphisis, minor modification of sacrum and scapula | | No | |
| | C:145, 263 | F | 19+ | Partial skull, mandible, nearly complete skeleton, lichen covered | compression and collapse of T12, arthritis sternal ribs, arthritis in lumbar | | No | radiocarbon range **1436-1573** Coltrain et al 2003 |
| | C:146, 271 | M | 13-18 | Almost complete skull, mandible, nearly complete skeleton | lesions on the Right and Left Humeri, Right fibula, manubrium, and modification of the ribs, PMTL | modification of ribs and manubrium | No | radiocarbon range **1488-1673** Coltrain et al 2003 |
| | C:147, 288 | F | 19+ | Many postcranial remains | lesion L1, schmorl's nodes in Lumbar, arthritic lipping throughout thoracic, microporosity R parietal, large mandibular abscessing, PMTL with major remodelling, L scapula, L tibia | | | |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| | C:149 | F | 19+ | Complete skull and mandible, nearly complete skeleton | severe pelvic fracture with remodelling, ribs lesions with remodelling | rib lesions | Yes | |
| | C:152 | M | 19+ | Most postcranial remains | L innominate, heavy arthritic modification R/L "elbow", eburnation on distal radius, arthritic modification of all L and lower T vertebra, L2 lesion, T12 & T8 anterior collapse, T8 lesion, porosity | | Yes | |
| | C:153, 95 | I | N/A | Nearly complete skull, mandible, postcranial remains, see comments | Lesions on sacrum, PMTL with abscessing and remodelling,T11-12 lesions, T8 caseation, T6,8,9 anterior collapse, L2-3 anterior collapse and lesion | thoracic lesions | | |
| | C:155, 115 | I | N/A | Complete skull and mandible, postcranial remains, see comments | osteophytosis L3-4, L innominate perforated ilium, abscess on syhmphysis, R innominate abscess on symphysis multiple small perforations, porosity of sacro-iliac, R/L glenoid osteoarthritis, PMTL with abscessing and remodelling | | | |
| | C:156, 113 | I | N/A | Almost complete skull and mandible, postcranial remains, see comments | complete PMTL with remodelling, L glenoid osteoarthritis, R/L pubic symphysis lesions, PMTL with abscessing and remodelling, cartilization of xyphoid and manubrium. C2-4 porosity, anterior collapse T12, arthritic lipping thoracic and lumbar vertebrae, L3 lesion, osteoarthritis of R/L ulna | | | |
| | C:157 | F | 19+ | Complete skull and mandible, postcranial remains, see comments | R/L humerus lesions, L acromion osteoarthritis, L innominate perforating lesions, R innominate perforating lesions, PMTL with abscessing and remodelling | | | |
| | C:158 | F | 13-18 | Complete skull and mandible, nearly complete skeleton | possible mastoiditis, modification near coronal-sagittal suture junction, minor dental abscessing, minor modification of 2nd ribs, macroporosity on 3rd and 4th ribs | | | |
| | C:164 | M | 19+ | Skull frags, mandible, some postcranial remains | T8 complete perforation of body with anterior collapse, T5 complete perforation, T7 lesions, PMTL | thoracic lesions | Yes | |
| | C:165 | M | 19+ | Most postcranial remains | L4 lesion, L tibia lesion on medial malleous | | | |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| | C:166, 114 | I | N/A | Most postcranial remains, see comments | L tibia highly arthritic, L femur distal end arthritic modification, spurring of lateral aspect, L humerus, arthritic modification of Lumbar and thoracic, compression of T8 & 11, fused sacrum and L5 | | | |
| | C:167 | M | 19+ | Mandible, most postcranial remains | L ulna lesion and macropososity (osteoarthritis), PMTL with major remodelling, osteoarthritis of R/L ulna, fusion C2-3, C4-5 caseation, severe lipping of lower lumbar, sacral fracture, R/L tibial enthesopathy | | | |
| | C:170 | M | 19+ | Almost complete skull, mandible, some postcranial remains | major infection of the R innominate with several localised areas of lesions, R radius arthritis, eburnation on L ulna and radius at articular site, PMTL with abscessing and remodel, T10 lesion, T5 slightly compressed | | | |
| | C:174 | M | 19+ | Mandible, some postcranial remains | PMTL with abscessing and minor resorbtion, arthritis of cervical vertebrae, perforations T10-L2, R/L proximal humerus osteoarthritis, R ulna lipping, L tibia severe inflammatory response | | | |
| | C:175 | F | 19+ | Almost complete skull, some postcranial remains | several wormian bones, R/L innominate lesions, L/R glenoid osteoarthritis | | | |
| | C:178 | F | 19+ | Almost complete skull, mandible, some postcranial remains | R/L innominate, minor PMTL with abscessing, T5 perforated lesion and caseation of body with minor anterior collapse, R/L septal aperture, osteoarthritis R/L "elbow" | | | radiocarbon range 1510- 1688 Coltrain et al 2003 |
| | C:179 | M | 19+ | Complete skull and mandible, nearly complete skeleton | PMTL with abscessing and major resorbtion, R/L innominate minor modification, major modification of frontal bone (large osteoma), abscessing pubic symphysis, fusion L rib 5-6, lesion in 1st rib, T1-2 macroporosity, lumbar lipping | macroporosity of ariticular aarea of T1-2, lesions in ribs | Yes | |
| | C:181 | M | 19+ | Almost complete skull, mandible, nearly complete skeleton | moderate infection of the Right humerus (proximal shaft), PMTL with remodelling and abscessing, lumbar arthritis incl. extensive osteophytes, partial collapse of T12, arthritic lipping throughout thoracic, severe lipping mid cervical, rib enlargement and curvature, some lesions t5-t8 | | | |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| | | | | | lesions on L1, sacrum and pelvic girdle | | | |
| | C:182, 82 | I | N/A | Most postcranial remains, see comments | | | No | |
| | C:183 | F | 19+ | Complete skull, mandible, nearly complete skeleton | Ovoid lesion in ilium near iliac crest, arthritis bilaterally in humerus; severe mandibular abscessing with PMTL and remodelling, vertebral body collapse at T4, 6-9, 11-12 | | Yes | |
| | | | | | lumbar lipping | | | |
| | C:190 | M | 19+ | Mandible and most postcranial remains | | | No | |
| | C:191 | M | 13-18 | Skull frag, mandible, some postcranial remains | R upper limb osteoarthritis, osteophytosis L2-3, anterior collapse of L1-2, lesions T6-12, anterior collapse T9-12 with lipping | lesions in thoracic vertebrae | Yes | |
| | C:192 | F | 19+ | Complete skull and mandible, nearly complete skeleton | bilateral arthritic lipping on distal radius medially; some minor modification of acetabulum; PMTL with remodelling | | | |
| | C:193 | M | 13-18 | Complete skull, mandible, most postcranial remains | R humerus MSM, T12 lesion | | | |
| | C:197 | M | 19+ | Most post cranial remains | sacro-iliac joint several lesions, L innominate perforated lesion, R pubic symphysis lesions, R glenoid osteoarthritis, modification to L4-5, L radius, R ulna arthritic R/L humerus osteoarthritis, T4-L5 highly modified | lesions in thoracic vertebrae | Yes | |
| | C:198 | M | 13-18 | Partial, mandible, most postcranial remains | osteoarthritis of humerus, several wormian bones | | | |
| | C:216 | M | 19+ | Almost complete skull, mandible, nearly complete skeleton | minor modification on visceral ribs; minor erosion of pubic symphysis bilaterally' left innominate lesion of the iliac crest, most likely enthesopathic; anterior collapse of T8 and L1; Left fibula enlargement of distal portion; bilateral arthritic modification of the "elbow"; mastoiditis; PMTL with abscessing and remodelling | | Yes | |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| Sadlermiut | C:217 | M | 19+ | Complete skull and mandible, nearly complete skeleton | evidence of inflammatory response L tibia with partial fusion, Left ribs possible healed fracture and moderate modification, 1 inca bone, several wormian bones, minor PMTL with partial remodelling and minor abscessing, R humerus, compression L1, osteophytosis L4/5 | | Yes | |
| | C:219, 273 | F | 19+ | Complete skull, mandible, postcranial remains, see comments | PMTL with resorbtion and abscessing, osteoarthritis innominate, S1 lesions, L glenoid osteoarthritis | | | |
| | C:221 | F | 19+ | Complete skull and mandible, nearly complete skeleton | R/L acromion process, L ribs cysts, healed fracture at sternal end, R rib fractured with large callus, L innominate lesion, R innominate several lesion, L sacrum lesions, R humerus lesion, PMTL with remodelling, minor maxillary abscessing, abnormal morphology L mastoid, lesion R temporal region, T5/6/9 lesions with anterior collapse | lesions in adjoining vertebra, lesions in the ribs, possible evidence of disseminated TB in cranium and innominate | Yes | |
| | C:230, 265 | M | 19+ | Complete skull, some postcranial remains | PMTL with remodelling, several wormian bones, R innominate abscess, R humerus enthesopathy | | | |
| | C:233, 269 | F | 19+ | Postcranial remains, | minor modification of R/L innominate | | | |
| | C:234 | F | 19+ | Complete skull and mandible, nearly complete skeleton | R humerus modification, L/R innominate lesions, major modification of R/L pubic symphysis, modification of cervical , large lesions and caseation of T6, 9-11, anterior collapse T11,7,4, PMTL with abscessing and remodelling | | | |
| | C:235, 730 | F | 19+ | Postcranial remains, see comments | | | | |
| | C:237, 277 | F | 19+ | Almost complete skull and mandible, some postcranial remains | cartilization of sternal rib ends, R sacro-iliac lesions, C2-6 macroporosity of bodies, T2 lesion, T6-10 lipping, parrot beak osteophytes of L4-5, perforation of ilium, PMTL with abscessing and remodel | | | |
| | C:241, 260 | F | 19+ | Some postcranial remains, see comments | R/L septal aperture, L scapula lesion, major PMTL with minor resorbtion and abscessing | | | |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| Sadlermiut | C:242 | F | 19+ | Some postcranial remains | osteoarthritis upper limb complexes, partial fusion T9-10, nearly complete fusion of T4-5, minor lipping throughout spinal column, partial fusion of L5 and sacrum | | | |
| | C:243 | M | 19+ | Complete skull and mandible, nearly complete skeleton | abscess left pubic symphysis; minor PMTL with remodelling (preservation of many bones obscures accurate assessment) | | | |
| | C:244, 177 | I | N/A | Complete skull and mandible, postcranial remains, see comments | R spetal aperture, fusion T6-7, fracture L rib 2-3, PMTL with minor abscessing, osteoarthritis L mandible | | | |
| | C:245 | F | 13-18 | Most postcranial remains | R innominate lesion | | | |
| | C:246 | M | 19+ | Almost complete skull, mandible, nearly complete skeleton | modification of R/L humerus head; arthritis of Tibia with erosive lesions of Femora medial distal condyle; partial fusion of sacrum and L5; arthritis of pubis; abscessing near nasal cavity; PMTL | | Yes | |
| | C:247 | F | 19+ | Almost complete skull, mandible, nearly complete skeleton | Thoracic disk displacement and compression; L3 severe disk displacement with anterior collapse; moderate modification of right upper limb | | | radiocarbon range 1532-1807 coltrain et al 2003 |
| | C:248, 218 | I | N/A | Complete skull, mandible, postcranial remains, see comments | osteophytes L4/%, osteoarthritis of L "elbow", PMTL minor abscessing and remodelling, R pubic symphysis lesions | | | |
| | C:261, 74 | M | 19+ | no cranium/mandible partial post cranial remains | | | | |
| | C:736, 127 | M | 19+ | Nearly complete skeleton, see comments | bilateral pitting and spurring of pubic symphysis, severe lipping of Lumbar, minor lipping throughout Thoracic, PMTL with abscessing , minor alveolar erosion, minor curvature of R/L fibula, L tibia small to midsize pit lesion | | | |
| | C:737 | M | 19+ | Nearly complete skeleton, see comments | R scapula glenoid cavity, minor lumbar lipping, minor anterior compression and collapse of T12, caseation (bilateral) of mastoid process and surrounding area | | | radiocarbon rang **1525-1723** Coltrain et al 2003 |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| | C:739 | F | 19+ | Partial skull and mandible, many postcranial remains | PMTL with major resobtion and minor abscessing, lesion R acromion process, R/L humerus lesion | | | |
| | C:743 | F | 19+ | Complete skull and mandible, nearly complete skeleton | L/R sacro-iliac lesions and pitting, L1 lesion, PMTL with abscessing and resorbtion | | | |
| | C:744 | F | 19+ | Partial skull and mandible, some postcranial remains | sternal foramen, R sacro-iliac arthritis, L auricular surface evidence of dislocation, PMTL | | Yes | |
| | C:748 | M | 19+ | Most postcranial remains, see comments | R/L radius osteoarthritis, wormian bones, PMTL with abscess, possible rickets | | | |
| | C:749 | F | 19+ | Most postcranial remains, see comments | PMTL with minor abscess, R humerus septal aperture, arthritis of L pubic symphysis, lesion L parietal with microporosity | | | |
| | C:750 | F | 19+ | Most postcranial remains, see comments | L septal aperture, inca bone, PMTL, R/L innominate perforations, R femur lesion | | | |
| | C:751 | M | 19+ | Most postcranial remains, see comments | R/L innominate lesion, minor PMTL with Minor resorbtion, isolated abscess, osteoarthritis at stern-clavicular articulation | | | |
| | C:752 | F | 19+ | most post cranial remains, mandible, skull partial dentition | PMTL with abscessing and resorbtion, minor modification of innominate | | | |
| | C:753 | M | 19+ | Complete skull and mandible, nearly complete skeleton | L 5th rib small lesion on costal groove, modification of pubic symphysis, anterior collapse and pitting of lumbar body (L1/2), minor arthritic lipping of lumbar, T12 collapse and minor osteophytes, possible incomplete fusion of T12 and L1, PMTL with abscessing and remodelling | | | |
| | C:755 | M | 19+ | Most postcranial remains, see comments | T12 lesion, arthritic lipping throughout thoracic, T6-T10 caseation, porosity of left rib articular surfaces, L innominate arthritis of symphysis, R innominate woven bone, R/L 2nd ribs partial fusion with clavicle, possible fracture (callus on ribs 4-7, sternal foramen, enthesopathic sterno-clavicular articulation, osteophytosis, severe osteoarthritis of bilateral upper limbs | Ribs and thoracic lesions | | |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| | C:756 | M | 19+ | Most postcranial remains, see comments | PMTL with abscessing, T12 perforating lesion, R glenoid osteoarthritis | | | |
| | C:757 | M | 19+ | Complete skull and mandible, nearly complete skeleton | PMTL with resorbtion minor abscessing, minor modification to L innominate, osteoarthritis of L radius, R femur lesions, R/L fibula evidence of inflammation, T12/9 lesions, R/L acromion process osteoarthritis, metopic suture | | | |
| | C:758 | F | 19+ | Most postcranial remains | PMTL with abscessing and resorbtion, wormian bones, Inca bone, R sacro-iliac lesion, R/L innominate porosity | | | |
| | C:761 | F | 19+ | Almost complete skull and mandible, nearly complete skeleton | possible incomplete fracture of L innominate, bilateral osteoarthritis of glenoid, rib fracture, cartilization of rib articular surfaces, severe lipping C vertebrae, T5 anterior collapse, sever anterior collapse with kyphosis of T6, compression T9, R lower arm osteoarthritis, PMTL with remodelling | | | |
| Sadlermiut/ Prairie Point | C:097, 81, 116 | I | N/A | Almost complete skull, mandible, some postcranial remains | R humerus MSM, L scapula, R humerus greater tuberosity, R scapula (acromian process), L innominate | | | |
| | C:229 | F | 19+ | Most postcranial remains | severe collapse/kyphosis of lumbar region, posterior fusion of L1 and T12, compression of thoracic (compression fractures), minor to moderate arthritic lipping throughout, osteoarthritis of both radii | | | |
| Seven Pound Island | C:306 | M | 19+ | Partial skull, mandible, nearly complete skeleton | arthritis R humerus; severe arthritis proximal R ulna; arthritis R/L scapula (acromion and coracoid processes); PMTL; minor arthritis L and T vertebrae | | | |
| Silumiut Island | C:518 | M | 19+ | Complete skull and mandible, nearly complete skeleton | R innominate perforating abscess, fracture of R ischium, L innominate, R sacrum lesion, R glenoid osteoarthritis, PMTL, | | | |
| Victory Lake | C:313 | F | 19+ | Complete skull and mandible, nearly complete skeleton | L Humerus minor modification; Lumbar bone deposition; T6 lesion | | | |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| | | | | | Pre-Contact Osteological Summary | | | |
| Arctic Bay | H:019-1 | M | 19+ | Complete skull and mandible, nearly complete skeleton | lumbar lipping, fusion C2-3, C1 fracture, L fibula fracture, Put with abscessing | | | |
| Dundas Harbour | H:011 | F | 19+ | Complete skull and mandible, some postcranial remains | minor modification of the sacrum, R sacro-iliac lesion, R humerus osteoarthritis, isolated vertebral lesion (lumbar), wormian bones | | No | |
| | H:012-1 | M | 19+ | Almost complete skull, mandible, few teeth, fragments of vertebral | minor modification of the tibia, R glenoid osteoarthritis, PMTL with resorbtion, several wormian bones | | No | |
| | H:013 | M | 19+ | Complete skull and mandible, most teeth, almost complete vertebral column, fragments of pelvic girdle, some post cranial remains | PMTL with abscessing and resorbtion, osteoarthritis L humerus | | No | |
| | H:014 | M | 19+ | Complete skull and mandible, some postcranial remains | L/R innominate perforations, PMTL, resorbtion, wormian bones, lesion L orbital notch | | | |
| | H:015 | F | 19+ | Complete skull, some postcranial remains | L/R sacro-iliac lesion, R innominate evidence of dislocation, L femur lesion, L innominate perforation, T6-T12 lesions with collapse (T12), L1-L5 lesions, arthritic lipping, PMTL, wormian bones | possible TB lesions in VC | Yes | |
| Ferguson Lake | H:041 | F | 19+ | Complete mandible, some postcranial remains | modification of ribs and cervical vertebrae, dental abscessing, bilateral glenoid osteoarthritis, lesions bilateral ribs, R 10(?) also has enlargement (bone deposition), 4 cervical vertebra with caseastion | modification of ribs and cervical vertebrae | Yes | |
| Inuksivik | C:661 | M | 19+ | Complete skull and mandible, some postcranial remains | R glenoid osteoarthritis, R/L septal aperture, L humerus lesion, Inca bone, several wormian bones, T12 lesion, PMTL | | | |
| | C:665 | F | 19+ | Almost complete skull, mandible, some postcranial remains | cervical vertebrae porosity, right rib fracture with callus in unaligned shaft, PMTL with abscessing and remodelling | | | |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| | C:672 | F | 19+ | Complete skull and mandible, many postcranial remains, badly damaged | too badly damaged to comment | | | |
| | C:685-2 | F | 19+ | Partial skull | maxillary abscessing | | | |
| Inuksivik North | C:681 | M | 19+ | Complete skull and mandible, nearly complete skeleton | caseation of mastoid process, minor abscessing of mandible, minor modification of R innominate, single rib lesions, osteophytes L5, pseudo-mastoid suture, minor modification of R innominate | | | |
| Kamarvik | C:532 | F | 19+ | Complete skull and mandible, damaged post cranial remains | some evidence of vertebral lesions (damaged), PMTL with abscessing and remodelling, possible lesion to R tibia (damaged) | | | |
| | C:533 | M | 19+ | Skull frag, mandible, some postcranial remains | major PMTL with abscessing and resorbtion | | | |
| | C:545 | M | 19+ | Complete skull and mandible, some postcranial remains, damaged spine | PMTL with abscessing and resorbtion, inflammatory response sagittal suture, abscess pubic symphysis | | | radiocarbon range **1449-1646** Coltrain et al 2003 |
| | C:573 | M | 19+ | Complete skull and mandible, some postcranial remains | L/R humerus MSM, distal fibula lesion, L humerus lesion, pseudo-mastoid suture, PMTL | | | |
| | C:590 | M | 19+ | Complete skull and mandible, some postcranial remains, damaged and missing vertebra | Inca bone, PMTL, L innominate perforated, osteoarthritis upper limbs, isolated lesionon rib fragment | | | |
| | C:605 | F | 19+ | Almost complete skull, some postcranial remains | PMTL, lesion on temporal bone | | | |
| | C:607 | M | 19+ | Complete skull, mandible, most postcranial remains | minor PMTL with minor abscessing and remodelling, inca bone, macroporosity L parietal near sagittal suture | macroporsity of cranium | Yes | |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| | C:610 | M | 19+ | Complete mandible, some postcranial remains | minor compression throughout Thoracic vertebrae, minor PMTL | | | |
| | C:613 | M | 19+ | Skull frags, mandible, some postcranial remains | osteoarthritis of upper arm complexes, evidence of minor infection of vertebral column | | | radiocarbon range **1447-1649** Coltrain et al 2003 |
| | C:619 | F | 19+ | Complete skull and mandible, most postcranial remains | lesion on sacrum, PMTL with major remodelling, minor osteoarthritis of R femur, moderate on L femur | | | |
| | C:621 | F | 19+ | Complete skull and mandible, many postcranial remains | minor abscess in maxillary with minor PMTL and remodelling, wormian bones, Large inca bone, pseudo-mastoid suture, bilateral septal aperture, minor anterior collapse throughout Thoracic | | Yes | radiocarbon range **1444-1636** Coltrain et al 2003 |
| | C:622 | F | 19+ | Almost complete skull, mandible, some postcranial remains | PMTL with partial remodelling, L innominate perforations, L greater trochanter caseation, almost complete fusion of C2-3 | | | |
| | C:623 | F | 19+ | Complete skull and mandible, few postcranial remains | osteoarthritis Right "elbo", osteoarthritis left glenoid, PMTL with abscessing and some remodelling | | | |
| | C:624 | M | 19+ | Almost complete skull, mandible, some postcranial remains | PMTL with abscessing and remodelling, isolated thoracic lesion | | | |
| | C:625 | M | 19+ | Almost complete skull, mandible, some postcranial remains | L/R humerus enthesopathy, dental abscess, lesion R fovea capitis | | | |
| | C:626 | F | 19+ | Skull frag, mandible, some postcranial remains | S1 lesion, PMTL with abscessing and remodelling, R radius osteoarthritis | | | |
| | C:627 | M | 19+ | Almost complete skull, mandible, many postcranial remains | fracture of the L innominate, MSM's on R humerus, minor PMTL | | | |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| | C:628 | M | 19+ | Mandible, some postcranial remains | R radius thickening of shaft, osteoarthritis of head, R ulna osteoarthritis, osteoarthritis L hyumerus, porosity of cervical vertebrae, R/L innominate lesions, parrot beak osteophytes L2-3, major PNMTL with almost complete resorbtion (mandible) and abscessing, R olecranon fossa obliterated by cartilization and bone deposition | | | |
| | C:631 | M | 19+ | na - missing | perforating lesion on L rib, sporadic lesions throughout Lumabr and Throacic, large enthesopathy on R/L humerus, R/L humerus osteoarthritis of condyles with lipping, multiple wormian bones, arthritic lipping to the olecranon process (L), minor abscessing | | | |
| | C:632 | F | 19+ | Complete skull and mandible, many postcranial remains | mastoid infection, septal aperture, minor modification of innominate | | | |
| | C:634 | F | 19+ | Almost complete skull,some postcranial remains | L1 disk dsiplacement, isolated compression in thoracic with lipping, exotosis in L innominate, osteoarthritis of L upper arm complex, PMTL with abscessing and partial remodelling, lesion near squasmoid suture | | | |
| | C:636-1 | I | 13-18 | Complete skull and mandible, some postcranial remains | PMTL, mastoiditis | | Yes | |
| | C:639 | M | 19+ | Almost complete skull, mandible, some postcranial remains, partial spine | lipping and compression of vertebrae, PMTL with abscessing and partial remodelling, large cranial lesion L parietal, | | | |
| | C:647 | M | 19+ | Almost complete skull, mandible, some postcranial remains | R/L pubic symphysis lesions, L innominate, R femur shaft lesion, PMTL with abscessing and remodelling, abnormal placement of PM2 | | | radiocarbon range **1422-1531** Coltrain et al 2003 |
| | C:648 | M | 19+ | Complete skull and mandible, some postcranial remains | L innominate perforations, several wormian bones, PMTL | | | |
| | C:649 | M | 19+ | Almost complete skull, mandible, some postcranial remains | R/L sacro-iliac lesions, PMTL | | | |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| | C:650 | F | 19+ | Almost complete skull, mandible, some postcranial remains | anterior conmpression, L radius lesion, minor PMTL with minor abscessing | | | |
| | C:652 | M | 19+ | Partial skull, mandible, many postcranial remains | r ight glenoid, L tibia lesion, mastoiditis, PMTL | | | |
| Kulaituijavik | C:685-1 | M | 19+ | Complete skull and mandible, some postcranial remains | dental abscess | | | |
| | C:686 | F | 19+ | Partial skull, mandible, some postcranial remains | 1 wormian bone, L femur shaft large lesion | | | |
| Mansel Island | C:294 | F | 19+ | Almost complete skull, nearly complete skeleton | minor modification R femur; inflammation of distal R fibula; fracture rightside mid ribs; C2 vertebral body; arthritic modification of cervical vertebrae | | | |
| Mittimatalik/ Mittima's Grave | H:033-1 | F | 19+ | Complete skull and mandible, some postcranial remains | L innominate lesion, R ilium perforated, R fibula osteoarthritis, R/L humerus osteoarthritis, T12 compression, wormian & inca bone | | | |
| Silumiut Island | C:342 | F | 19+ | Complete skull and mandible, some postcranial remains | PMTL with remodelling, several wormian bones, | | | |
| | C:364 | M | 19+ | Complete skeleton and mandible, some postcranial remains | 2 inca bones, several wormian, lesion R parietal, bilateral septal aperture, R/L humerus enthesopathy, large bony growth on L rib fragment | | | |
| | C:375 | M | 19+ | Almost complete skull, mandible, some postcranial remains | minor modifiaction L innominate | | | |
| | C:390-2 | F | 19+ | Skull frags, mandible, some postcranial remains | osteoarthritis to L upper limb complex, L pubic symphysis perforated, porosity to ilium with lesion and bone growth, R innominate macroporosity with bone growth, L innominate perforated, osteoarthritis of L lower arm, PMTL with resorbtion and minor abscessing | | | |
| | C:412 | F | 19+ | Almost complete skull, mandible, some postcranial remains | PMTL minor remodelling, L septal aperture, possible healed lesions on vertebra | | | radiocarbon range **1427-1643** Coltrain et al 2003 |

| Site | Individual | Sex | Age | Preservation | Pathological Modifications | TB Indicators | Molecular Candidate | Other |
|---|---|---|---|---|---|---|---|---|
| | C:413 | F | 19+ | Complete skull and mandible, most postcranial remains | PMTL with abscessing, 2 large lesions ar base of skull, lipping lest occipital condyle, curvature of L fibula, L femur, R/L humerus enthesopathy, R scapula glenoid and acromion head | | | |
| | C:416 | M | 19+ | Cranium, mandible and almost complete skeleton | curvature of R ulna, L tibia lesion, posterior fusionof T3/4, arthritic lipping of T8/9, T9 lesion, osteophytes L2-4, possible spondylolysis of L% with extreme osteophytosis and sacral involvment, wormian bones, PMTL | | | |
| | C:420 | F | 19+ | cranium, mandible and partial skeleton | sacro-iliac joint small lesions, PMTL with remodelling, 1 wormian bone, R innominate abscessingat symphysis, curvature of L fibula and R ulna, arthritic lipping of lumbar | | | |
| | C:425 | M | 19+ | Skull frags, mandible, some postcranial remains | / | | | |
| | C:494 | F | 19+ | Almost complete skull, mandible, some postcranial remains (remains highly damaged) | R septal aperture, L humerus shaft curvature | | | |
| Silumiut North | C:741-N | F | 19+ | Almost complete skull, mandible, some postcranial remains | compression L5, R/L acromion process osteoarthritis, R iliac crest lesion | | | |
| | C:742-N | F | 19+ | Complete skull, mandible, few postcranial remains | macroporosity of L humerus head and greater tubercle, minor modification of bilateral mastoid | | | |
| | C:745-N | F | 19+ | Partial mandible, some postcranial remains | R/L sacro-iliac , PMTL | | | |
| | C:746-N | F | 19+ | Partial mandible, some postcranial remains | fracture R rib, R radius lesion, osteophytosis L3-4, compression L1-2, 4-5, fusion T10-11, lesion T6, thoracic lipping, R ilium perforation, PMTL with abscessing and resobtion | | | |
| n/a | C:315 | n/a | n/a | cranium, mandible, partial post cranial remains | osteophytosis, wormian bones, PMTL with abscessing | | | |
| Bathurst Island | H:029 | M | 19+ | Allmost complete skull, mandible, nearly complete skeleton | R "elbow" highly arthritic, PMTL with major abscessing | | | |

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