Health Research with Manitoba First Nations.

An investigation of gene variants affecting the Th17 immune pathway and the P2RX7 receptor.

by

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Abstract

Introduction: Canadian First Nations experience a significantly higher rate of *Mycobacterium tuberculosis* (MTB) infection than non-Indigenous Canadians. Th17 cells are a subset of $CD4^+$ T cells that are distinguished by their production of Interleukin-17A (IL-17A), an important cytokine for defense against mycobacteria. IL-17 is a primary contributor to the formation and stabilization of the lung granuloma, a biological containment vessel to protect the host from tuberculosis (TB). Past research with First Nations people has identified single nucleotide polymorphisms (SNPs) in the Th1 and Th2 immune pathways may affect their disease risk. However, SNPs in key Th17 related genes and the $P2RX_7$ gene have not been explored in First Nations despite their important role against infectious diseases.

Hypothesis: This research hypothesizes that distinct First Nations groups (Dene, Cree and Saulteaux) will have a different frequencies of SNPs in the key Th17 immunity related genes (IL-17A, IL-17AR, IL-23R, and IFN-γR) and the $P2RX_7$ gene, as compared to a non-Indigenous Canadian group.

Methods: SNP profiles (IL-17A rs2275913, IL-17RA rs4819554, IL-23R rs10889677, IFN-γR rs2234711 and $P2RX_7$ rs3751143) were identified through literature research and the NCBI database was used for identifying gene motifs, primer locations and Restriction Enzyme cut sites. Polymerase Chain Reaction and Restriction Fragment Length Polymorphism analysis was performed on and visualized on agarose gel to determine specific allele frequencies. Four different Manitoba First Nations communities; the Northern Dene (Dene 1 N=69. Dene 2 N=52), Central Cree (N=46), and Southern Saulteaux (N=56), participated in this research and their SNP profiles were compared to a non-Indigenous Canadian cohort (N=99).

Results: Allele frequencies for IL-17A were statistically different for every First Nation community when compared to the non-Indigenous cohort (Dene 1 p=0.0043, Dene 2 p=0.0000, Cree p=0.0001, Saulteaux p=0.0000). Allele frequencies for IL-17RA were statistically different for every First Nation community except Saulteaux when compared to the non-Indigenous cohort (Dene 1 p=0.0000, Dene 2 p=0.0028, Cree p=0.0000). Allele frequencies for IL-23R were statistically different for Dene 1 and Saulteaux community when compared to the non-Indigenous cohort (Dene 1 p=0.0002, Saulteaux p=0.0000). Allele frequencies for IFN-γR were statistically different for Cree community when compared to the non-Indigenous cohort (Cree p=0.0026). Allele frequencies for $P2RX_7$ were statistically different for both Dene communities when compared to the non-Indigenous cohort (Dene 1 p=0.0000, Dene 2 p=0.0000).

Conclusions: An effective Th17 response is required to bring Th1 cells to infected tissues and to balance inflammatory responses. Functional SNPs may compromise an appropriate immune response and contribute to disease. This study demonstrate that the non-Indigenous population maintained a significantly different genetic profile when compared to the First Nations populations.

Foreword: Indigenous Research

First Nations research is guided under the principle of OCAP™ and the Tri-Council Policy Statement 2. These guiding documents ensure that both researchers and study participants are equals in the research process. As such, one of the defining features of Indigenous research is taking the researcher out of traditional academia and presenting themselves as a person and not as an unbiased facilitator of science. To do so, I will be taking part in the practice of "locating myself" as a person so that I, the researcher, have context as to why this research is important to me and why I choose to work with Indigenous peoples.

Locating myself in this research

I was born and raised in the Northwestern Ontario community of Ignace, a rural community of approximately 1000 people with a significant Aboriginal population. I am the son of Dale Barrett, a single mother of two from Newfoundland. I must admit that locating myself by birth is a foreign concept for me, as I do not know who my fathers' biological parents were and my mother is estranged from her own family. What I know of my heritage is that my mothers' great-grandmother was from Quebec and her great-grandfather was from Newfoundland, making me at least a 4th generation Canadian. From what I know, I am not an Indigenous person.

Having grown up with Aboriginal friends and neighbours I was able to witness some of the health inequities Aboriginal Canadians experience. Some of these inequities included medical health issues, such as diabetes, and others included racism leading to an inability to find employment. Being young and naïve I did not attribute this to racism but to classism, as I found myself and my mother also struggling to find any meaningful employment and this was largely

due to our social standings. It was not until I moved to Winnipeg for University as a 17-year-old were I truly started to understand the underlying racial culture Canada has founded itself on.

While in high school, I visited and stayed at Northern Ontario reserves while visiting friends, for work, and sporting activities. Since graduating high school I have spent my summers working construction and some of the projects I participated in were building septic systems for Mishkeegogamang Ojibway First Nations reserve and road construction and area maintenance for the Ojibway Nation of Saugeen First Nation. I believe these experiences have afforded me a culture perspective of some of Canada's First Nations people, particularly Ojibway people, which many fellow Canadians and University students will never be privy too.

My mother had never finished high school, as she was forced to quit by her family. She is a passionate advocator for equal rights for all people, but especially for women and education. Because of this my mother's house was a boarding residence for female high school students from Mishkeegogamang Ojibway First Nations reserve. Through this experience I became more accustomed to some of the cultural differences these young ladies had as compared to my own.

Having worked in lower income employment since I was 12 years old, I was able to earn enough financially to overcome what government student loans would not provide and left Ignace to attend University in Winnipeg at the age of 17. I worked as a Resident Assistant at St. Andrew's College amid my first degree, a Bachelor of Arts in psychology, and gained incredible insight into some of the different cultures which were not present in my rural Northern Ontario town. I enrolled in psychology as I wanted to have a profession which focused on aiding others. However, through self-discovery, I realized by the end of the degree that psychology would not be how I achieved this goal.

During my psychology degree I obtained my class A/1 driver's license and have since used it to finance my decision to return to academics and focus on helping others via the health sciences. Government assistance would not provide financing for a second bachelor's degree and I had to be diligent and dedicated to my cause in returning to academics. Working summers as a construction worker, garbage truck driver, logger, oil patch worker; and as a bouncer, librarian assistant, and student research assistant during the academic year, I have been fortunate enough to overcome economic disadvantage and earn a Bachelor of Science in Microbiology and now a Masters in Medical Microbiology.

While in university I have volunteered as a coordinator for the Footsteps First Nations breakfast program for underprivileged students at Hastings School in Winnipeg. Unfortunately, the program no longer has funding and I have had to shift my focus from volunteering to employment again. As such I am now a part-time worker for the Canadian Longitudinal Study on Aging.

I wish to take my mother's morals and assist others, particularly Aboriginal people and the economically disadvantaged, with medical care. All of these life events have guided me to my current research project. I would like to see this project eventually help Aboriginal people, and ideally all people, with effective and affordable therapeutics against *Mycobacterium tuberculosis* and any other pathogen which is effected by the Th17 pathway.

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Terms

APC - Antigen presenting cells

AIDS - Acquired Immune Deficiency Syndrome

CCR5 - C-C chemokine receptor 5

CD - Cluster of Differentiation

DC - Dendritic cells

DNA - deoxyribonucleic acid

ETB - extra-pulmonary tuberculosis

FNIGC – First Nations Information Governance Committee

FNIHB – First Nations and Inuit Health Branch

FNIHB – First Nations Information Governance Centre

HGP - Human Genome Project

HIV - Human Immunodeficiency Virus

HWE - Hardy-Weinberg Equilibrium

IL – Interleukin

MDR-TB - multi-drug resistant tuberculosis

MHC - major histocompatibility complexes

MTB - Mycobacterium tuberculosis

OCAP™ - Ownership, Control, Access and Possession

PHAC - Public Health Agency of Canada

PTB - pulmonary tuberculosis

RFLP - Restriction Fragment Length Polymorphisms

SDH - social determinants of health

SE - Standard Error

SNPs - Single Nucleotide Polymorphisms

TB - Tuberculosis

Th - T Helper cells

Th1 - T-helper cells 1

Th2 - T-helper cells 2

Th17 - T-helper cells 17

Treg - T regulatory cells

WBC - White Blood Cells

WHO - World Health Organization

XDR-TB - extensively drug-resistant tuberculosis

1.0 Chapter 1: Introduction to Research

1.1 Tuberculosis

Tuberculosis (TB), caused by MTB, is a disease that has effected humans for tens of thousands of years (Comas 2013, Daniel 2006). It is currently the second largest pathogenic killer in the world and rates of drug resistant versions of the mycobacteria are increasing in frequency (World Health Organization [WHO] 2015). TB is described as a "social disease" as infection is directly related to economic and social factors (Merriam-Webster 2004). As such, TB is influenced by many different factors such as genetic differences in the immune system, comorbidity with other diseases such as Human Immunodeficiency Virus (HIV), and the social determinants of health; poor nutrition, age, occupation, and crowded housing (Lönnroth 2009 and Harling 2014). One-third of the world's population is thought to currently have active or latent TB. Areas of high population densities and poverty display the highest levels of TB burden (WHO 2015, Rasanathan 2011). In contrast, Canada has some of the lowest TB rates in the world, however certain populations within the country experience a much higher burden of this disease (FNIHB 2012, Centre 2014). The likelihood of a Canadian-born Indigenous person[1] acquiring TB has risen to a frequency of 29.6 times that of a Canadian-born non-Indigenous person (Whitlock 2012).

As a global disease, TB is estimated to currently infect 2-3 billion people worldwide, however, only a relatively small proportion of these people (5-15%) will go on to develop the active disease of TB during their lifetime (WHO 2015). The WHO estimated that in 2014 alone there were 9.6 million new case of TB and 1.5 million TB related deaths worldwide (WHO 2015). This mortality rates brings TB up to the same global death rates of HIV and the

[1] Indigenous people born in what is present day Canada. These people are comprised of three groups: 1) First Nations, 2) Inuit, and 3) Metis.

comorbidity rates of the two are up to 12% of infected people (WHO 2015). There are, however, greater areas at risk to TB infection than others. Southern African countries, India, and many Asian counties have the highest incidents rates per 100,000 people when describing geographical landmasses (WHO 2015). Of note, different subgroups of people also experience high rates of TB when compared to national averages; examples include indigenous peoples of both Canada and Australia (Barry 2012, Health Canada 2012a).

An individual's or community's economic and social conditions help shape their health profiles, such attributes to health profiles are known as the social determinants of health (SDH) (Raphael 2009). An example of a SDH with an association to TB is that of undernutrition. TB is known to make the issues of undernutrition worse and undernutrition is known to weaken the immune system, this cascade effect thereby increases the likelihood of latent TB developing into active disease (WHO 2013). Numerous studies have demonstrated the impact of the SDH (food security, housing, medical care access, education and income) and the ability to resist and recover from diseases (Raphael 2009, Marmot 2006). These disparities in the SDH help explain the differences which influence the exposure and vulnerability to risk, and ability to recover from diseases such as TB (Rasanathan 2011). For First Nations on-reserves in Manitoba, these SDH are key contributors to disease susceptibility (Larcombe 2011, Boutilier 2013). SDH are tangibly observed in the crowded living conditions and poor ventilation in many on-reserve homes in Canada, additionally more than half of the houses have been investigated and labelled as in need of major repair (Melnyhuk 2016, Whitlock 2012).

Many other SDH which present social inequities have been correlated with greater risk in developing and spreading active TB and are disproportionately witnessed in Aboriginal demographics (Whitlock 2012, Centre 2014). These inequities are known to weaken the immune

system and include such things as increased prevalence of smoking (First Nations rates almost 3x higher than Canadian average [CDN ave]) and food-insecurity rates (54% First Nations vs 9% CND ave). Pervasiveness of heavy drinking in First Nations populations at ages 59 and below had heavy drink rates ranging from 59%-69% while over 60 was 38%, the Canadian average for pervasive heavy drinking in all age groups was 19%. Another factor included the estimated HIV prevalence rates in First Nations being almost twice as high as the Canadian average (FNIGC 2012, Gionet 2013, Peoples 2014).

Crowded housing and malnutrition have been linked to increased TB rates. Overcrowding raises the probability of being exposed to this infectious respiratory pathogen, which could increase the transmission rates of the disease especially to those more susceptible (Clark 2002). Malnutrition has been witnessed to be at a higher rate in those with active tuberculosis as compared to healthy controls, and malnutrition in a host may cause an enhanced susceptibility to disease due to host immunodeficiency (Gupta 2009).

An immune system unbalanced by these social determinant factors will have an increased probability of inactive/latent TB activating and an increased risk of transmission of disease. TB is an opportunistic pathogen and is adept at bidding its time hidden in the lungs until the environment is more befitting for its development.

Mycobacterium tuberculosis (MTB) is a highly infectious obligate aerobic rod-shaped intracellular bacterium from the Mycobaceriaceae family (Ryan 2004). It is primarily an infection of the lungs however it is capable of infecting other parts of the body. It infects host via bacilli in air-borne water droplets. It is slow dividing once in a host, taking more than 3 weeks to form colonies in a new host (Kaufmann 2005). MTB is a bacterium with relatively low rate of genetic mutation as compared to other bacteria (Cohen 2012). It is defined by two separate

clades, each of which representing several different strains of MTB, some of which are drug resistant. Clade I encompasses the Euro-American Haarlem strains and the Beijing strains, which have been found to be more virulent in humans than the clade II mycobacteria. Clade II is represented by the East African Indian strains which have demonstrated less virulence and replication potential in human hosts (Reiling 2013).

TB has a long-standing history, not only with humans but possibly with our early ancestors as well (Gutierrez 2005). The MTB complex possibly originated 70,000 years ago and the modern strains may have a common ancestor dating back to 20,000-15,000 years ago, with the present strains originating 1000-250 years ago (Comas 2013, Daniel 2006). TB was documented in Egypt more than 5000 years ago, India 3300 years ago, China 2300 years ago, and has possible references in the bible under the Hebrew term "schachepheth", which historically meant a wasting disease and is currently used in Hebrew for TB infection (Daniel 1999, Daniel 2006). The disease even predates European arrival in the Americas and could have been transmitted to humans via infected seals in Peru nearly 1000 years ago (Coghlan 2014). TB also has a long standing history of considerable impact on mortality as witnessed during the early 19th century when London, Stockholm, and Hamburg had mortality rates linked to TB as high as 800-1000/100,000/year (Daniel 2006).

Treatment of TB is highly effective with a success rate upwards of 85% for first time non multi-drug resistant TB strains (MDR-TB) without co-infection of HIV. Without chemotherapy it has been demonstrated that approximately 70% of those with a positive smear sputum test for pulmonary TB (PTB) would die within 10 years (WHO 2015). The 85% efficacy rate against non MDR-TB is based on a six-month course of four first-line drugs. MDR-TB has inhibited the ability of the two most powerful first-line drugs, rifampicin and isoniazid, in their ability to

effectively clear TB infections. MDR-TB requires a more expensive and toxic chemotherapeutic intervention that lasts up to 20 months and displays a much lower efficacy rate. The WHO estimated that there were 480,000 cases of MDR-TB and that 190,000 people died of MDR-TB worldwide in 2014 (WHO 2015). Because of this, TB is a disease that needs more than just medical intervention to successfully control. It requires social, environmental, and economic mediation if it is to be successfully treated as a pandemic (WHO 2015).

The estimated yearly global financial impact for TB diagnosis and treatment is in excess of $12 billion US (Kim 2016). This burden is extravagantly high for a disease that presents an 85% clearance rate. Research into new therapeutics and vaccines are currently underway with many new chemotherapeutics in phase I and phase II testing (WHO 2015). Financially, the research and development of these novel and repurposed therapeutics is above and beyond the $12 billion mentioned prior as that is solely for diagnosis and treatment.

1.2 Tuberculosis Treatment

In 2014, TB was estimated to have infected 2-3 billion people worldwide and causing 1.5 million deaths (WHO 2015). Treatment options for infection, are highly effective with a success rate upwards of 85% using a six-month course of four first-line drugs. However, without treatment approximately 70% of smear sputum positive individuals with PTB will die within 10 years of contraction (WHO 2015).

MDR-TB and extensively drug-resistant tuberculosis (XDR-TB) have increased in recent years with an estimated 190,000 people dying from the two combined in 2014 (Kumar 2015, WHO 2015). With MDR-TB, the two most powerful first-line drugs, rifampicin and isoniazid, are inhibited in their ability to treat the disease. The regimen for MDR-TB is much more intense

than regular MTB strains and has a much lower efficacy rate, with under 50% clearance in 2012 (WHO 2015). The regimen includes chemotherapeutics of a higher toxicity and cost, and could take as long as 20 months or more of implementation. XDR-TB is resistant to the two previously mentioned first-line drugs as well as any fluoroquinolone and at least one of the injectable second-line drugs; amikacin, kanamycin, or capreomycin (LoBue 2009). A successful treatment outcome for patients with XDR-TB was only 26% of those monitored in a 2012 (WHO 2015).

There are many reasons improvement in TB medications need to be pursued. The current 6-month regimen of 4 drugs may lead to compliance issues for continual use of the chemotherapeutics for the full suggested time frame. TB is often witnessed in those struggling with many of the SDH, such as income and housing, which can exasperate the issue of affordability of a 6 month, or longer, medical treatment. With MDR-TB and XDR-TB rates increasing, the need for a different approach to medication is easily discernable.

The effectiveness of the Bacillus Calmette–Guérin vaccine has been underwhelming and it is reasonable to believe that we must start trying to target other areas of the immune system if a fully successful vaccination or therapeutic is to become a realistic possibility. Treatment for TB is not entirely driven by biological factors or gene interactions, as it will require addressing the issues of social, environmental, and economic influences on health. However, therapeutic treatments, whether it be chemotherapeutics or vaccination based, do need to improve and will be the governing focus of this thesis.

In addition to all of the health concerns the global population faces in the fight against TB, the financial constraint is also staggering. In 2014 diagnosis and treatment of TB worldwide was estimated to be in excess of $12 billion US (WHO 2015). Research and development of new chemotherapeutics and vaccines was also in excess of the $12 billion US spent in 2014 (WHO

2015). A number of new or repurposed drugs are in advanced stages of clinical testing, including a few treatment regimens in Phase II and Phase III of trials, and there are 15 vaccine candidates on trial (WHO 2015).

However, until a therapeutic is developed which can address length of treatment issues, vaccination performance, or the discrepancies of the social determinants of health inequities; the global health burden of TB will continue to be an issue.

1.3 Tuberculosis in Canada

Canada has experienced a steady decrease in number of TB cases in the last 90 years, to the point where Canada now has some of the lowest TB rates in the world (FNIHB 2012, Centre 2014). In the 1920's, the death rates due to TB in Canada were at a percentage slightly higher than deaths due to cancer (Centre 2014). Once methods were put in place to help prevent or treat TB, such as improved living conditions after World War II and the introduction of first-line chemotherapeutics (1950's), the mortality and incidence rates began to decrease rapidly.

The all-time low for TB rates in Canada for the Canadian population as a whole was in 2010 at a rate of 4.6 people per 100,000 population, which is a decrease of almost half from the Canadian rates in 1990 when the numbers were 7.0 people per 100,000 population (PHAC 2015). The rates of TB cases among Canadian-born non-Aboriginal peoples is the lowest of any demographic in Canada, at an incidence rate of 0.7 people per 100,000 population (Centre 2014).

1.4 Tuberculosis in First Nations in Canada

Certain populations in Canada experience a much higher burden of certain disease than Canadian-born non-aboriginals; examples of these disease included chronic diseases, infectious

diseases, and cancer (PHAC 2015, Manitoba Health 2014, Larcombe 2005, Lodge 2006, Adelson 2005, Barnabe 2008). An example of this is the disproportionately higher rates of MTB infection in Canadian First Nations as compared to the non-Aboriginal Canadian-born population (Lodge 2006, Health Canada 2012a, Peoples 2014). Whereas the rates of TB in Canadian-born non-Aboriginal people have decreased significantly since 1970, Canadian-born Aboriginal people have seen an increase in TB cases over the last 45 years (Centre 2014). The disparity has seen the likelihood of TB in Canadian-born Aboriginal populations escalates to 29.6 times the rate as compared to Canadian-born non-Aboriginals (FNIHB 2012). This increase has furthered the gap in health parity of these populations in Canada.

In 2013 the Canadian-born non-Aboriginal population had an incidence rate of tuberculosis of 0.7 per 100,000 people and Canadian Metis had an incidence rate of 3.3 per 100,000 population (Centre 2014). In the same year Canadian First Nations living on reserves experienced a rate of 28.8 per 100,000 and Inuit people experienced 154.2 cases per 100,000 population (Centre 2014, Peoples 2014). TB infection for Aboriginal people in Canada have shown a large variance by geographical region as well, with the Aboriginal peoples in Manitoba, Nunavut, and Saskatchewan having the highest TB case rates in Canada in 2010 (Centre 2014, Whitlock 2012).

The high rates of TB in Canada's First Nations populations are not a new phenomenon. In the 1870s, TB in Plains First Nations groups were relatively unseen, however, by the late 1880s TB was acknowledged as the primary cause of morbidity and mortality in the same groups (Daschuk 2006). In the early 20th century, Canadian health officials believed that aboriginal rates of TB were so debilitating high that they proclaimed treatment of neighbouring "white"

communities meaningless unless the TB crisis was also addressed in First Nations communities (McCuaig 1999).

1.5 Tuberculosis in First Nations in Manitoba

Incident rates of tuberculosis among First Nations people are often provided as a representative whole and not by distinct geographical areas or distinct First Nations groups. The case numbers provided previously represent the Canadian First Nations as a whole, however differences can be seen between provinces. As an example in 2013 the First Nations people in the province of Ontario experienced TB rates of 4.4 per 100,000 whereas the First Nations people of Manitoba experienced TB rates of 70.9 per 100,000 (Peoples 2014). Differences are also found within a province between geographically similar, but historically different, First Nations groups (Olson 1999). An example of this was noted between the years of 1994-2004 where two individual northern Manitoba communities, one Dene and one Cree, had different average annual incidence rates of TB infection. During this time the Dene community experienced an average annual incidence of 636 per 100,000 people and the Cree community experienced 196 per 100,000 people (Lodge 2006, Kettner 2011).

First Nations in the province of Manitoba face a number of adverse socioeconomic and environmental conditions, which potentially influence the varying levels of infection. These conditions include crowded housing and poor ventilation, food insecurity, poverty, and barriers to access for appropriate medical care (Christopherson 2012, Standing Committee on Aboriginal Peoples, issuing body, & Canadian Electronic Library 2015, Slater 2013, Boutilier 2013, Larcombe 2011, 2012a).

1.6 Biological Risk Factors for Tuberculosis

There are a number of risk factors that may increase the host susceptibility to TB, including biological factors such as a unfavourable balance of the immune system, genetic factors and social factors including inadequate diet or substance abuse. Recently, there has been research investigating how biological and genetic differences may contribute to health outcomes and disease susceptibility in First Nations groups (Larcombe 2005, Lodge 2006, Larcombe 2011, Madduer 2012). Studies analyzing comorbidities and genetic factors contributing to different diseases have shed light on the role that genomics may play in susceptibility and/or resistance to infectious diseases and on health disparities (Larcombe 2005, 2008, 2011, 2012b, Boutilier 2013, Guo 2013, Ferguson 2012).

The transmission route of TB is via the lateral spread of airborne droplets and only a person with the active form of TB is capable of transmitting the virus. These droplets are spread from person to person through the coughing and/or spitting by an infected individual, which are then inhaled into the airways by the new host and rest in their alveoli (Fennelly 2012). The lungs are the predominantly affected area in humans leading to what is known as PTB, however other sites of the body can be affected, these other sites give rise to the condition which is recognized as extra-pulmonary tuberculosis (ETB).

Symptoms of infection include coughing, sometimes with sputum or blood present, weight loss, fever, bodily weakness, night sweats, and chest pains. TB infection can be defined by the early host response of phagocytic cells quickly infiltrating the alveoli, this incursion of cells includes alveolar macrophages and neutrophils (Schlesinger 1996). The subsequent response by the host immune system dictates either clearance, infection, or containment of the pathogen. Containment of TB can result in a long-term and persistent latent infection or, possible

development of active disease, and is likely heavily influenced by macrophages and neutrophils and their contents (Frieden 2003). At this time, it is ambiguous if the development of latent versus active TB is dose dependent or if there is a genetic component associated with defense against active TB (Garton 2008). However, there are suggestions that TB may bind to a variety of host cell receptors and the determination of latent versus active infection could very well be dependent on which receptor is chosen by the incoming mycobacteria (Collins 2001).

1.6.1 SNPs and disease association

Genetic factors have become a prime focus in disease research; in terms of disease diagnosis, determining predisposition to diseases, therapeutic development, and drug responsiveness. Single nucleotide polymorphisms (SNPs) have been identified as the most abundant form of genetic variations within the human genome and have been associated with altered gene functionality (Ziegler 2010). These alterations have the potential to affect disease susceptibility in the individuals with them (Bellamy 2003). Cascade effects which can either impair or benefit the overall immune response may be a consequence from any of these small genetic modifications.

SNPs have been associated with increased susceptibility to a variety of diseases, from sickle cell anemia to cystic fibrosis (Ingram 1956, Hamosh 1992). An example of SNP research directing potential therapeutic intervention was demonstrated within the Canadian First Nations group the Dene (Larcombe 2012b). Prior to this research it had been suggested that vitamin D deficiency played a possible role in the abnormally high rates of TB seen within this population. However, upon completion of SNP research on the vitamin D receptor (VDR), and the subsequent cytokinetic studies, it was determined that due to this VDR SNP that vitamin D

supplementation would have very little effect on enhancing immunity against intracellular pathogens, such as MTB, in populations such as the Dene (Larcombe 2012b).

SNPs in immunity related genes may effect immune response and this can lead to a suboptimal generation of immunity and increased sensitivity to disease (Lan 2006). With the ever growing number of discovered SNPs, and the already researched SNPs of a small number of genes in the Th1 and Th2 pathways in First Nations people of Canada, this research has been chosen to focus on Th17 related genes; IL-17A, IL-17 Receptor A, IL-23 Receptor, IFN-γR and the $P2RX_7$ ligand-gated cation channel for their appreciable roles in defending human hosts against TB.

1.6.2 SNPs and Tuberculosis

Most SNPs in the human genome are not of functional relevance, however there are a number of variants that may alter gene product expression or function (Kim 2008). These functional variants may contribute to disease pathogenesis, modify response to treatments or organ transplants, change observed phenotype, or vary disease risk or response (Bianco 2005, Lech-Maranda 2004, Milano 2016). SNPs may be found in the coding region of a gene, causing issues such as nonsense polymorphisms which prematurely terminate the mRNA, or they may be found in the promoter region of a gene, causing issues such as decreasing the binding affinity of necessary transcription factors (Kim 2008).

The C-C chemokine receptor 5 (CCR5)-Delta-32 polymorphism confers resistance to HIV-1 and is an example of a polymorphism with a tangible impact on disease prevalence. This polymorphism is chiefly found in northern European and western Asian populations, which

suggests that ethnic differences can confer protection against different specific diseases (Novembre 2005).

Specific SNPs have been associated with tuberculosis susceptibility, in controlled TB vs non-TB SNP association studies, and have been observed within different ethnic groups including Chinese, West African, and Mexican (Shi 2015, Cooke 2006, Niño-Moreno 2007). SNP plasticity is also seen between different cultures and ethnicities as different ethnicities demonstrate varying levels of SNP representation (Choudhury 2014).

While SNPs in one arm of the immune response, such as the Th17 pathway, may cause an increased susceptibility to specific diseases, it is key to remember that the immune system is an interactive system and that SNPs in other pathways, such as the Th1 or Th2 pathway, can also have functional consequences on the other immune response pathways.

Cytokines are a broad category of proteins which are essential for the activation, differentiation, and control of the immune system (Bhushan 2012, Hiscott 2011). Many cytokine SNPs have been examined and determined to have statistically significant associations with different diseases. These studies do not examine causality but the implications are as such that the SNPs may directly or indirectly cause the disease phenotype (Bhushan 2012). Cytokines which assist the host immune system in defense against TB, are well established stemming from murine knockout studies conducted in the early 1990s (Mayer-Barber 2015). TB associated cytokines have had an increasing number of described SNPs in promoter and coding regions which are associated with TB susceptibility (Yim 2010). SNPs in promoter regions have the potential to alter transcription factor recognition sites, which may possibly affect the transcriptional activation and alter the levels of cytokine production and cell recognition (Yim

2010). Coding region SNPs may alter protein shape and function, which could lead to non-functional proteins or proteins which function less than optimally (Ramensky 2002).

Promoter and coding region SNPs in cytokines and cytokine receptors have been associated with increased susceptibility to tuberculosis in different ethnic populations (Selvaraj 2004). Underlying genetic susceptibility could be in part the reason for such differences in rates of tuberculosis in contemporary populations, such as Canadian First Nations. Prior research of SNPs in the promoter and regulatory regions of specific genes involved in the immune response against TB within distinctive Manitoba First Nations groups have been observed (Larcombe 2005, Larcombe 2008, Decter 2013, Larcombe 2015). These identified differences may be associated with the diminished ability of these specific groups ability to combat MTB.

For this research it was hypothesized that a different frequency of SNPs in key Th17 related genes (interleukin (IL)-17A (IL-17A), IL-17 receptor A (IL-17RA), IL-23 receptor (IL-23R), interferon gamma receptor ligand binding chain I (IFN-γR)), and the $P2RX_7$ receptor, all of which may affect the immune system's ability to combat MTB, would be observed in three distinct Manitoba First Nations populations as compared to a non-Indigenous Canadian population. Including these five genes into the already established immune profile of these distinct groups has provided increased acumen on the balance of Th1, Th2, and Th17 immune pathways, as well as insight into the immunogenetics of these Manitoba First Nations groups.

The ultimate goal of identifying and understanding the genetic variations that may be responsible for a differential immune response is to advance the possibility for a successful treatment therapy. Vaccinomics, the investigation into vaccinations which are created specifically for certain populations to target diseases, is cognizant of the individual combinations of host and pathogen genomes which are being targeted (Moller 2010). The opportunity to

exploit the immune system, either artificially or chemically, in an attempt to obtain optimal levels of cell mediated response against specific pathogens will only be increased by the expanded understanding of individual populations genomes and their specific SNP frequencies. Though genetic variation may arise through random recombination or mutation events, it is often likely to occur due to environmental and evolutionary stressors which influence their usefulness. Identifying these variances in individual populations can lead to an enhanced method of treatment for at risk groups.

This research has two objectives. First, the detection, quantification, and comparison of functional SNPs in the Th17 arm of the human immune response (i.e. IL-17A, IL-17RA, IL-23R, and IFN-γR) as well as in the $P2RX_7$ gene, in a First Nations study cohort (Dene, Cree, Saulteaux) and will be compared to a non-indigenous Canadian control group. Secondly, designing and applying knowledge translation strategies and techniques sharing the research results is necessary for engaging communities with knowledge translation processes contributes to the effectiveness of the research. Sharing gives the research relevancy and encourages sustainability of ongoing and further research opportunities with Canadian Indigenous communities. The open sharing of knowledge and results facilitates community support, builds capacity within the community, and increases the community's knowledge base (Estey 2009).

There are different methods for engaging communities in the knowledge translation process including community-based participatory research, community partnered participatory research, and decolonizing methodologies (Cargo 2008). The different methods engage community members in different ways, some more applicable depending on circumstance of the community or foundations of the research. Development of knowledge translation techniques requires interaction with the community members and culturally respectful education through

Indigenous research forums and academic training. Understanding research from a First Nation's perspective will enhance the ability to work in this field.

2.0 Chapter 2: TB Immunology and Genetic Variability

2.1 Background Literature

MTB is the causative organism responsible for the global disease tuberculosis, which infects an estimated 30% on the world's population (WHO 2015). It is known as a social disease, and is a slow dividing and highly aerobic mycobacteria which, with the proper medications, has a high clearance rate of 85% (WHO 2015). However, even with this high clearance rate, there were still an estimated 1.5 million MTB related deaths in 2014 (WHO 2015). This high mortality rate was second only to HIV in 2014, and of growing concern is the increased prevalence of MDR-TB (WHO 2015). Chemotherapy for MTB involves multiple drugs and is a regimented process requiring months of administration. This arduous and involved process may cause difficulty in remote areas, such as remote Africa, India, and Canadian reserves, where TB is most epidemic.

2.2 The Host Immune Response to *Mycobacterium tuberculosis*

The human immune system is a compilation of cells, tissues, and molecules which mediate defense against pathogens and resistance to disease, in a process referred to as the "immune response" (Abbas 2012). The most crucial physiological function of the immune system is identifying invading microorganisms which could harm the host, and eliminate or contain them with as little repercussion to the host as possible (Abbas 2012).

According to WHO, in 2014 1.2 million of the people infected with TB were co-infected with HIV (WHO 2015). Of these co-infected people 340,000 passed away in 2014 (WHO 2015). Impairment of the immune system is a clear way of observing just how critical the system is in host longevity. The Acquired Immune Deficiency Syndrome [AIDS] is the final stages of the

HIV, it is a disease which greatly inhibits the human immune system (Abbas 2012). When a person has AIDS, the immune system is damaged and the person becomes much more susceptible to opportunistic pathogens such as influenza and MTB. Without the assistance of the immune system, individuals often rapidly succumb to the co-infections of these pathogens. There are two arms of the immune system, the "innate immune response" and the "acquired immune response" and both are important for the immune defense against *Mycobacterium tuberculosis*.

2.2.1 The Innate Immune Response

The innate immune response is accountable for mediating the initial protection of the host against infections. Innate immunity is characterized by the immune defenses which first evolved with the organism and is the immediate line of defense against incoming disease causing assailants. Innate immunity provides immediate protection via natural epithelial barriers, specialized cells, and natural antibodies whose purpose is to prevent the pathogen's initial entry into the host body or to eliminate microorganisms (Alberts 2015). The innate immune response is not specific to particular pathogens and is instead focused on targeting conserved pathogenic markers witnessed on numerous different microorganisms. The innate response is the quicker of the immune responses, taking only hours to deploy. In humans, this would include epithelial barriers and secretions which cleanse mucosal surfaces, non-specific immune cells such as macrophages and dendritic cells (DCs), and humoral factors like the compliment system or mannose-binding lectin (Peakman 2009). Where the adaptive immune response is estimated to have arose in vertebrates, and only vertebrates, some 500 million years ago, the innate immune response is seen in both vertebrates and invertebrates and is likely much, much older (Alberts 2015).

Epithelial barriers produce chemicals such as lysozyme and phospholipase A, which have antimicrobial properties, and many epithelial barriers produce mucosal layers which prevent microbial, mechanical, and chemical incursion (Murphy 2008). Epithelial barriers include systems such as the gastrointestinal tract, where the gut is constantly producing natural acids, and the intestines have their own intestinal microfloral environment making it difficult for invading pathogens to establish a niche in the environment (Purchiaroni 2013). The respiratory tract is one of the human physiological systems which features a mucosal barrier. This barrier helps prevent pathogens from adhering to host tissue and is also used to effectively sweep invading microorganisms from the respiratory tract through the mechanism of beating cilia which sweep the mucous, and any trapped particles or microbes, from the tract. These mucous layers also produce its own peptides, known as defensins, which play an effective role in inhibiting microorganism culturing.

If a pathogen is successful in crossing the epithelial barriers than many different kinds of phagocytic cells await them, including monocytes, DCs, neutrophils, and natural killer cells (Abbas 2012). All of these cells function under the same directive of looking for common non-specific pathogen motifs displayed on many pathogens.

Inflammation is the initial reaction to successfully invading pathogens. Inflammation is characterized by the migration of the monocytes mentioned previously, to the site of infection, modification to vascular tissue permeability including blood clotting to create a physical barrier, and secretion of soluble mediators (Abbas 2010). All of these factors are key in eliminating invading pathogens. In response to inflammation stimulation the monocytes are recruited, travelling through capillary walls, to the inflammatory site. At this site, the monocytes mature in size and complexity into the macrophages required to phagocytize the invading pathogen (Fisher

1976). Macrophages and neutrophils are the first phagocytic cells to respond at the site of infection, attacking the foreign particle by locating the conserved pathogen motifs mentioned earlier. Once the receptors are adhered to the phagocytic cells attempt to ingest the foreign pathogen. If successful, the foreign pathogen is held internally in the cell in a compartment known as the phagosome. Phagosomes are eliminated from the intracellular space once they adhere to another intracellular body known as the lysosome. The lysosome is a membrane bound organelle containing acid hydrolases which degrade the endosomal material (Luzio 2007). When the phagosome and lysosome receptors identify each other the cells fuse together creating a phagolysosome. The phagolysosome has the contents of the phagosome degraded by the lysosomes acid hydrolases, lysosomal proteases, and reactive oxygen species (Hemilä 1992). After degradation the contents of the phagolysosome are either dispelled to the extracellular fluid where they can be eliminated from the body, or they are bonded to the major histocompatibility complex II for the purposes of antigen presentation by the adaptive immune response (Mittal 2015). All the different possible antigens which become attached to the cell surface can elicit a response from specific immune amplification genes.

Cytokines are produced by many of the innate immune response cells. Cytokines are small soluble proteins released by cells which interact and communicate with other cells causing specific events (Zhang 2007). These specific events are responsible for the meditation of the inflammatory and immune reactions between leukocytes and between leukocytes and other cells (Abbas 2012). Communication is key for a successful immune response, if these cytokines falter or emit a poor signaling response than the immune response may ultimately be down-regulated and leave the host susceptible to invading pathogens.

DCs are professional antigen presenting cells (APC) found on external environments; such as skin, and the linings of the nose, lungs, stomach, and intestines and are specialized for antigen uptake and processing (Giacomini 2001). As APCs on external environments, they are one of the most important innate immune response cells and a key communicator to the adaptive immune response. After interacting with pathogens, DCs mature and migrate to lymph nodes where they are responsible for the generation of effector and memory T cells, as well as the induction of peripheral tolerance (Sallusto 2002). They are capable of activation through their surface expression of major histocompatibility complexes (MHC), with bound antigen, and by production of costimulatory molecules and secretion of immune regulatory cytokines, such as IL-12 (Giacomini 2001).

The innate immune response is a nonspecific response to pathogens, it effectively casts a wide net to eliminate as many non-self microorganisms as possible. However, many pathogens have successfully developed ways of evading innate immunity and as such the innate immune response is not always successful in host defense. If innate immunity fails, then the adaptive immune response takes over the immune response. The adaptive immune response is stimulated by cells of the innate immune response, specifically the APCs such as macrophages and DCs, as well as antigens produced by the invading microbes themselves. These systems are not sequential orders of defense but are, in fact, complementary systems which provide that which the other lacks. These systems regulate each other via cytokine expression and direct cellular contact (Trinchieri 2003). This complimentary system is such that if a mechanism of innate immunity changes in level of effectiveness or function, then the other system is affected as well. This change can lead to compensation by one system for the other or, more concerning, the down

regulation of functionality of the other system. If the system responds with the latter, then the host is more susceptible to incoming infections due to a weaker immune system response.

2.2.2 The Adaptive Immune Response

The adaptive immune response is the more specific arm of the immune system, capable of a considerable amount of diversity. As many pathogens have evolved to overcome innate immunity, adaptive immunity focuses on tending to these infections and microbes which have either escaped or over burden the innate immune response. As such, adaptive immunity is an additional level of protection afforded to vertebrates as this system is only found in this sub-phylum of organisms.

As the name implies, adaptive immunity allows the immune system to adapt to invading microorganisms. It does so upon repeat and/or prolonged exposures to the invading pathogen. Medicine has taken advantage of this phenomena of repeat exposure eliciting the adaptive immune response through the use of vaccinations (Janeway 2005). Adaptive immunity is only activated if the pathogen or its antigens are capable of bypassing the epithelial layers of the innate immune response. From here a response is triggered by the phagocytic and APCs of the innate immune response, which delivers these signals to the lymph nodes, and activates the adaptive immune response cells known as lymphocytes. These lymphocytes are recognized as B and T cells, and they respond to microbes in a tailor-made fashion (Abbas 2012). As an adaptive process, adaptive immunity requires more exposure time with the pathogen to optimize its response, as such it can require days to weeks to develop the immunomodulation effects to full capacity.

This optimization by lymphocytes is the reason that acquired immunity is a specialized combat schema adapted for targeting exact pathogens and mechanisms of those pathogens. The immune system has evolved to adjust the adaptive immune system to develop memory cells, which are naïve lymphocytes that survive latently for years after the pathogen or antigen has been eradicated (Abbas 2012). These cells mediate an expeditious adaptive immune response with an intensified response upon subsequent manifestations of the antigen.

The adaptive immune response has two distinct avenues of protection; humoral/B cell mediated and cell/T cell mediated. These different avenues of protection have evolved to combat specific types of infection and are guided by different molecules and cells. Humoral mediation provides defense against extracellular pathogens such as viruses and parasites. It does so by developing immunoglobulins which recognize an antigen by its cells surface markers, which is the same as the variable domain marker of the B cell itself, and then binds to the antigen. This binding can have a number of effects including opsonization, compliment activation, inhibitory or blockage effects, agglutination, or mast cell degranulation (Abbas 2012).

Cell mediated immunity, or T-cell immunity, attacks pathogens through the production of cytokines or via the attacking of infected cells. In T-cell immunity, naïve T lymphocytes respond to cell-associated microbial antigens in a string of sequential steps which ultimately lead to an increased total of antigen-specific T cells, as either memory T cells or effector T cells. This process happens initially in the peripheral lymphoid organs when naïve T lymphocytes are exposed to their specific antigen. If and when this happens, the naïve T lymphocytes are stimulated to differentiate and proliferate into the previously mentioned memory and effector T cells (Janeway 2005). The effector T cells are the cells responsible for producing the immune

response required in attempting to eliminate the pathogen. They are activated by the exact same antigens when re-exposed to them in the peripheral tissues or lymphoid organs.

T cells originate in the bone marrow and develop in the thymus. Before maturing to naïve T cells, the new T cells in the thymus, known as thymocytes, undergo positive selection to develop a capacity to bind either the MHC I or MHC II receptors and develop into Cluster of Differentiation 8+ (CD8+) or 4+ (CD4+) naïve T cells respectively. CD8+ cells are cytotoxic T lymphocytes (CTLs) whose designated responsibility is to kill host cells containing any infectious contaminant in its cytosol or nucleus, which eliminates the cellular reservoir of the infection. CD4+ T cells are known as T Helper cells (Th), their function is to secret cytokines which recruit and activate other leukocytes to assist in phagocytosis of the foreign material.

Naïve T cells need to encounter an APC which fits its MHC receptor before it can initiate its primary immune response. To encounter this complex, the naïve T cells must travel the body to secondary lymph nodes or the spleen, which are sites for APCs. Once the APC-MHC complex is formed a signal is transmitted within the T cell which changes the T cell response from pathogen recognition to pathogen elimination. The T cell differentiates and undergoes clonal expansion, a proliferation event which dramatically increases the number of T cells. Clonal expansion generates effector T cells, which are capable of a particular defense mechanism based on the type of T cell response. The effector cells are then required to travel back to the site of infection so that they may perform their pathogen killing function (Abbas 2012).

The type of invading pathogen determines which T cell mediate immunity is activated. CD8+ T cells, which are activated via the MHC I, are targeted towards intracellular pathogens, viruses, cytosolic pathogens, tumour cells, and transplanted tissue. CD4+ T cells, which are

activated by MHC II, targets extracellular pathogens, bacterial infections, protein antigens, and extracellular parasites (Sommer 2005).

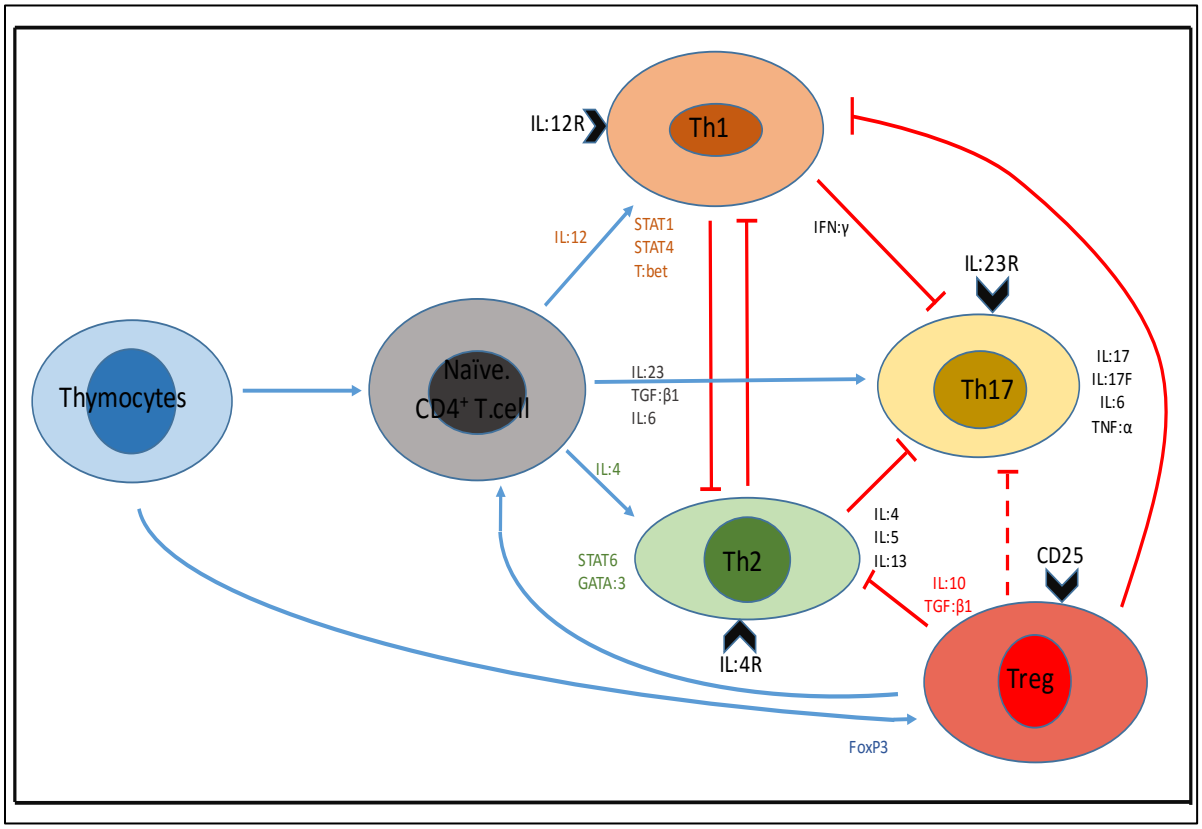
The MHC gene loci contain two sets of tremendously polymorphic genes, these polymorphic genes are the genes involved in antigen presentation of the MHC, and are the most polymorphic loci known in vertebrates (Sommer 2005). In humans, the complexes that make up the MHCs are known as Human Leukocyte Antigens (HLAs). The incredible diversity witnessed in the T-lymphocyte receptors is correlated with the high degree of variability of MHC-molecule. This diversity assists in determining the long term survivability of an organism and its population against diseases, parasites, and other infections.

Once the T cells are activated by the APC they begin to secret cytokines. These cytokines affect many functions, one of their main duties is to aid in the stimulation of proliferation of antigen specific T cells. This stimulation results in the expeditious expansion in the number of antigen-specific lymphocytes. As CD8+ cells proliferate they differentiate to recognize and attack pathogen infected cells, while CD4+ cells activate phagocytes to destroy pathogens sequestered into phagocytic vesicles (Janeway 2002). The CD4+ cells other main function is assisting B lymphocytes in producing antibodies (Abbas 2012).

CD4+ have the potential of differentiating into distinct subsets of cells which have different and specific immunological functions (Murphy 2008). Of the distinct subsets, the most highly researched are the T-helper cells 1 (Th1), T-helper cells 2 (Th2), T-helper cells 17 (Th17), and T regulatory cells (Treg) immune responses. Each cell subset is activated by a different pathway from the precursor CD4+ cells and the Treg subset. The T cells are also regulated by opposing Th subsets, which may produce inhibitory cytokinetic activity, thus limiting the extent of immune activation by each individual subset of cells (Murphy 2008).

Important cytokines are released during effector T cell proliferation which help direct the immune response to specific pathogens (Figure 1). Interleukins have the ability to communicate from leukocyte to leukocyte or from leukocyte to other types of cells to induce a response to pathogens (Abbas 2012). Cytokines are produced by CD4+ cells and are the main triggering mechanism for the stimulation of the T-helper cell generation.

Figure 1. T cell regulation by cytokines.



Possible developmental pathways of thymocytes. The blue arrows indicate upregulation. The red arrows indicate regulator inhibition. Each arrow is accompanied by the cytokines the individual cell produces with the coloured arrow indicating the cytokines effect on the other cells. The black triangles represent receptors.

2.2.3 Immune Responses in Cooperation

Both systems are reliant on the ability to determine "self" from 'non-self" cells (Medzhitov 2002). The systems are not isolated events and often work best as a coalition and overlapping as adaptive immunity is often responsible for enforcing innate immunity via APCs, compliment pathways, and cascade effects. A cascade event is an immunological event in which a strong response by one gene will in turn amplify the downstream response or responses of the successive gene. This cascade effect may be limited, or may even have negative repercussions, if one of the upstream genes has been altered to give a subpar response.

A successful immune response focuses the immune system in completing four main tasks; immunological recognitions, immune effector functions, immune regulation, and immunological memory (Murphy 2008). Any variation from optimal immune response functioning, in any of these four tasks, can lead to a compromised immune system and/or prolonged exposure to the pathogen in question. The effects could include delayed or non-existent containment or elimination, which ultimately lead to poor allocation of immunological resources and energy, and impoverished health outcomes of the individual.

As an example of these four immune system tasks in practice, the immune response to TB will be modelled. The immunological recognition to MTB is enacted once the MTB establishes an infection in the lung parenchymal cells. The MTB bacilli are assailed by White Blood Cells (WBCs) including neutrophils and alveolar macrophages, which are of the innate immune response and are considered the first line of defense (Schlesinger 1996). However, the ability of these first line of defense cells is doubtlessly influenced by the acquired immune response extrinsic factors which play a critical role in determining the outcome of TB infection (O'Garra 2013). Once immunological recognition has been determined, factors may be activated

which influence the immune responses decision in determining if the TB infection may be cleared or if the infection must instead be contained. These factors are the immune effectors and they perform their tasks via the compliment system of blood proteins, antibodies, and WBC activation (Decter 2013). Once the immune response has been activated, the key for immune regulation is ensuring that the response does as little damage as possible to the host itself. TB infection involves the activation of a number of inflammatory regulation cytokines, including IL-1 and IL-17, which, if left unchecked, could damage host cell tissue. Immunological memory is a foundational function of acquired immunity in which the B and T cells designate specific memory cells to produce effector cells in case of recurrent infection by the same pathogen. These memory cells produce a more rapid and more thorough immune response upon subsequent exposures from the same pathogen (Abbas 2012).

2.3 Host Immune Response to Mycobacterium tuberculosis

MTB is a unique pathogen as it may persist for a lifetime within the host as a non-infectious "latent" disease causing no symptoms of illness. However, MTB may also cause significant symptoms of malady in the virulent "active" form. The active form is the disease form and is often witnessed after a host's immune response fails or weakens due to other factors such as co-infections, fatigue, and age (Young 2008, Frieden 2003). Once MTB has come into contact with a human host the human hosts immune response has multiple strategies for eradication and/or containment of the mycobacteria. Unfortunately, the human immune response to TB infection is incompletely characterized and complex, as such, researchers are not completely confident in which eradication strategy is the most important or efficient of the possible strategies (O'Garra 2013).

2.3.1 Macrophages

Alveolar macrophages cause inflammation upon interaction with invading bacilli in the lung. Inflammation is one of the activation signals that triggers the recruitment of neutrophils, monocytes, B and T cells to the lungs. Commissioning all of these cells to this area leads to the formation of the pulmonary granuloma (Flynn 2011). Initially the host inflammatory response is strong, but if the disease is capable of evading the host immune system long enough eventually suppressing mediators are secreted to balance the inflammation (Sasindran 2011).

Macrophages and neutrophils are the first responders to the incoming mycobacteria, and the context of their activation can have a profound effect on the ensuing immune response. The three physiological outcomes that these innate immune response cells can affect are; containing the mycobacteria and developing a persistent latent infection, clearance of the pathogen by the immune response, or failure to clear or contain the mycobacteria and developing active disease (Frieden 2003, O'Garra 2003).

As discussed earlier the macrophage clears pathogens by ingesting foreign material in a phagosome and then combining with a lysosome for phagolysosomal degradation of the material. Macrophages are also capable of enveloping intracellular bacteria that have found their way into the cell through pathogenic means and introducing similar lysosomal degradation through a processes known as autophagosome development. The autophagosome envelopes the pathogen in the cytoplasm, much like the way the phagosome is generated by the macrophage against cytosolic pathogens, and then this autophagosome is delivered to the lysosome by activation with IFN-γ and the combined result can lead to MTB killing via acidification (Colombo 2006, Gutierrez 2004).

2.3.2 Lung Granuloma

One immune response that is synonymous with TB infection is the formation of the lung granuloma. The TB granuloma has been under investigation for numerous years and its exact defined role is still debated. The primary purpose of the granuloma is suggested to be that of a natural barrier, one in which the immune response is walling off the mycobacteria from interacting with the rest of the host. The granuloma is composed of a plethora of cells. In the center of the mass is a number of MTB infected macrophages, multinucleated giant cells, lipid rich epithelioid and foamy macrophages, and neutrophils (Russell 2009). Surrounding this central mass are lymphocytes, including CD8+ and B cells, but largely composed of Th cells and fibroblasts which encapsulate the granuloma (Peters 2003). Assorted cytokines and chemokines act as pro-inflammatory and inhibitory molecules in the formation of the granuloma (Peters 2003). There is evidence suggesting that MTB has adapted to this segregation from normal lung tissue and that the bacilli can survive within the center and even the periphery of these lung granulomas (Ulrichs 2004, Hernandez-Pando 2000).

2.3.3 Apoptosis

Apoptosis of infected macrophages is another method of mycobacterial elimination. Apoptosis is the process of programmed cell death, in which a cell undergoes biochemical and morphological changes which lead to the death of the cell and the destruction of the infectious material inside of the cell (Green 2011). Apoptosis could be described as cell suicide for the greater good of the host organism. Macrophages, specifically alveolar macrophages, are capable of undergoing apoptosis to eliminate MTB in a controlled manner. Again, MTB has found a way

of hindering the usefulness of this defense strategy. Apoptosis has been witnessed to be highly effective against some strains of MTB but much less competent against more virulent stains of MTB (Keane 2000). Virulence strains of MTB are strains which have developed strategies to avoid or modify the immune response in the mycobacteria's favour, whereas less virulent strains lay dormant until the optimal conditions present themselves naturally (Forrellad 2013). Virulent strains of MTB are capable of not only inhibiting the apoptotic ability of macrophages, but also are able to induce necrosis of infected cells which leads to the spread of the mycobacteria (O'Garra 2013). The inhibition of apoptosis also prevents cross presentation of the mycobacteria to DCs which in turn disrupts the T cell response (O'Garra 2013). Though apoptosis has only been proven to be effective against less virulent strains of MTB it is still an important defense strategy as these less virulent strains can still cause active TB infection.

2.3.4 T cells

If the immune system is to effectively eradicate or control a TB infection than a fully function CD4+ T cell response is required. Murine studies involving CD4+ T cell-deficient mice and human studies involving CD4+ deficient humans both had similar results in that both populations were highly susceptible to TB and, in the case of the murine studies, more likely to be overcome by disease burden (O'Garra 2013).

At the present time it is unclear how the CD8+ T cells contribute to defense against TB. IFN-γ activates macrophages and this may be the most important contribution to the eradication of TB by the CD8+ T cells (North 2004). As MTB is an intracellular pathogen, CD8+ cells have little to no direct contact with the bacilli, but CTL's ability to secrete the IFN-γ may be a useful feature in TB defense.

2.3.5 Interleukin mediated clearance

IL-23 induces the production of IL-17 from Th17 cells (Sasindran 2011). Th17 cells are a subset of CD4+ helper T cells which are characterized by their ability to abundantly produce IL-17 (Khader 2008). IL-17 is affluently produced during the early stages of MTB infection and plays a part in the formation of the lung granuloma and in Th1 cell recruitment, which are the main characteristics associated with defense against TB (Sasindran 2011, O'Garra 2013). However, the exact defined role of Th17 is still under review as studies have shown that antigen-dependent Th17 cells are only capable of partially inhibiting mycobacterial growth (O'Garra 2013). Other studies have demonstrated that IL-17 deficient mice are not reliant on IL-17 for control of TB infection, but IL-17 was shown to be important for granuloma formation (Sasindran 2011). IL-17 has been implicated in triggering other CD4+ cell responses, such as Th1, which plays a more crucial role in control of MTB infection such as the generation of IFN-γ (Sasindran 2011).

2.4 Modification of cellular environment by MTB

After gaining entry to the host macrophage, TB initiates the process of establishing a favourable living environment for long-term survival. MTB has evolved to inhibit the fusions of the phagosome and autophagosomes with lysosomes (Sturgill-Koszycki 1994). This inhibition prevents the acidification of the phagosomal compartment and allows the mycobacteria to grow in a more acclamatory habitat, creating their own microbiological niche (Sturgill-Koszycki 1994). TB restricts the phagosome maturation state to that of an early endosome. This early endosome state keeps the endosome in the recycling endosome pathway and granting TB access

to the endosomes iron supply which TB steals and uses for growth and metabolism (Collins 2001, Ratledge 2004). Another example of this host cell modification is the upregulation of the glyoxylate shunt to bring the TB infected macrophage carbon from fatty acids (Collins 2001). All of these environmental reorganization processes are of importance as it demonstrates the requirements of optimal MTB growth which, if fully understood, could be used for defensive strategies and medicine against the mycobacteria.

2.5 Genomic variability

This study is exploring the genomic variability of each of the study populations and the possible health influences that variability plays. To fully appreciate this research, a few key concepts require an explanation. All living organism are composed of basic genetic information, inherited from the parental organism(s), that provides the instructions for the building, maintenance and reproduction of the organism. The instructions for sustaining life are coded in a language consisting of four nucleotide letters; A, C, T, and G, which make up DNA. Each human is made up of one paternal and one maternal copy of the DNA code. This combined parental code is the offspring's genotype. A genotype is the genetic constitution of an organism, and it is the information inside all living organisms that is responsible for the organism's inheritable traits (Hartl 2012).

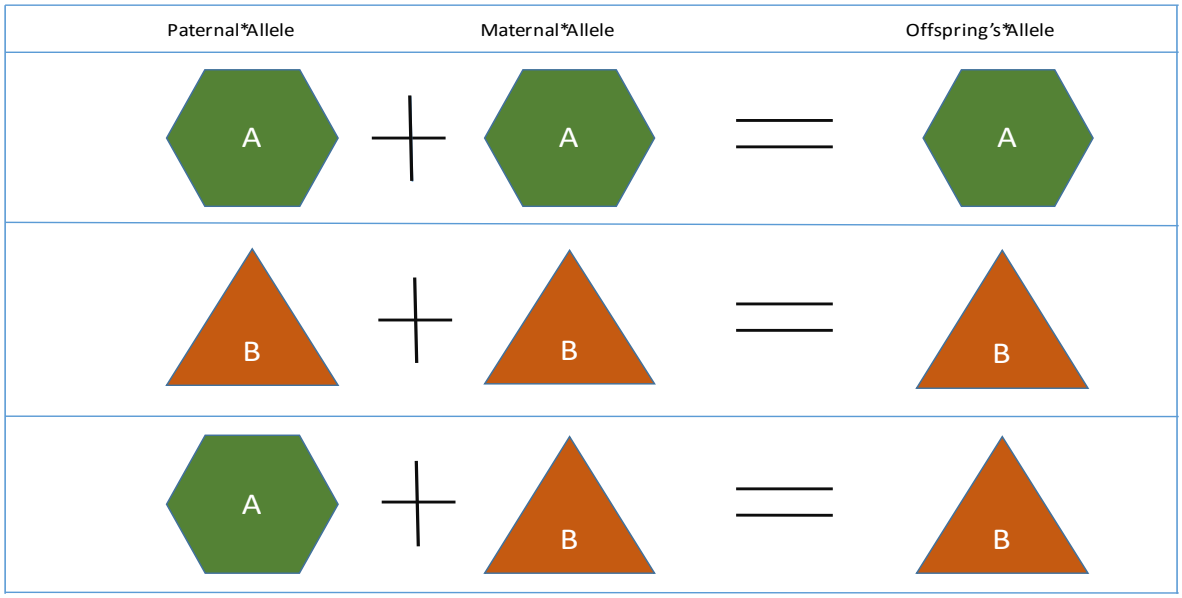
The offspring is not exactly the same as either parent, nor are they the same as any sibling (except for identical twins). A gene is comprised of two alleles, one from the maternal, the other from the paternal side (Hartl 2012). The offspring's genes will therefore have characteristics that are a combination of its parents and perhaps ones that are unique to the offspring. The inheritance of genomic material from two parents provides the offspring with genetic variability.

A living organism can be described by both the phenotype, which is the physiological representation of the genome, and by their genotype, which is the biochemical component of the genome. A genotype is the hereditary information of an organism, whereas the phenotype is the observed property (i.e. behaviour, development, or morphology) (Fletcher 2013). The distinction between genotypes and phenotypes may be best represented by considering that a genotype is (i.e. DNA) whereas a phenotype is what is on the outside (i.e. observable trait). As there are two paternal genes influencing an offspring, one of the genes will have a more dominant effect. If the paternal genotype is A, and the maternal is also A, then the offspring will exhibit A as well. However, if the paternal genotype is A and the maternal is B, and the B genotype is dominant, then the offspring will display the B phenotype (Figure 2).

If an individual has two copies of a gene which are identical, the gene from both maternal and paternal sides are the same, then that individual is said to be homozygous for that genotype (Fletcher 2013). However, if the gene under study has any differences between the maternal and paternal donor, then the gene is said to be heterozygous (Fletcher 2013). A simplified homozygous example would be if both maternal and paternal donors had brown eyes, the resulting phenotype and genotype would be brown. A simplified heterozygous example would be if maternal donor had brown eyes and paternal donor had blue eyes, the resulting phenotype would be brown but the genotype would have information for both brown and blue.

Genomic variance is a term used to describe the variation in phenotype caused by differences in genotype among individuals in a population (Hartl 2012).

Figure 2. Example of Allelic Inheritance.



In this example the B allele is dominant. When two identical alleles combine than the offspring's allele is the same and the offspring is said to be homozygous, which is seen in the first two examples. If the paternal and maternal alleles are different than the resulting offspring's genotype is heterozygous and the phenotype is that of the dominant allele, which is seen in the third example.

2.5.1 Single Nucleotide Polymorphism

A genetic polymorphism is the coexistence of multiple alleles at a locus in a DNA sequence that arise in an appreciable number in a population, i.e. more than 1 percent (Krebs 2014). A SNP is a polymorphism of a single nucleotide within a gene locus (Krebs 2014). These SNPs occur on average about once every 1330 base pairs in the human genome (Krebs 2014). The human genome is about 99.9% similar in all people, however the 0.1% is composed of genetic variation that make us individually unique. These SNPs are the most common type of genetic variation among people. This is witnessed overtly through physical characteristics, such as hair colour, or less distinguishable manners, such as disease susceptibility and resistance. These changes can be silent with no discernible outcome, the changes can be harmless; such as a phenotypic change in a gene not required for survival, or the changes may be harmful resulting in modifications to the way cells respond to pathogens with outcomes of ranging from prolonged cellular disorder to death.

An example of how SNPs may possibly protect individuals from diseases has been well documented in the battle against HIV. The CCR5 is a protein on the surface of white blood cells and is one of the cell motifs which macrophage targeting M-tropic HIV-1 strains initially recognizes and bind to infect host cells (Samson 1996). CCR5 may obtain a polymorphism known as CCR5-delta-32, a deletion polymorphism which completely prevents individuals with this polymorphism from producing functional CCR5 on their macrophages (Naif 2013). Individuals who are homozygous for this mutation are highly resistant to infection by the HIV-1 non-syncitia-inducing R5 strain, an M-tropic strain (Naif 2013). Not only that, but individuals who are heterozygous for this polymorphism are slower in developing AIDS and are more apt to stave of death due to AIDS related complications (Naif 2013). This is an excellent example of

how a polymorphism may positively affect health outcomes, however the reverse is also possible, as numerous SNPs may adversely affect health outcomes against certain diseases.

SNPs are a small part of the overview created by the Human Genome Project (HGP). The HGP is the completion of the human genetic map and since its completion in April of 2003 it has been used to identify over 1800 genes associated with disease. The SNPs identified when comparing to the HGP standardized genome helps establish variability at the possible SNP sites, as SNPs are the most common form of genetic variation. Since SNP association studies have begun in the late 1990s, there have been over 8 million identified SNPs with an estimate that the human genome has upwards of 10 million SNPs (Ziegler 2010). Though the vast majority of SNPs have not been associated with determinable physiological consequences, a small portion have demonstrated significant effect in disease prevention and susceptibility, and gene phenotypic variation (Yang 2010).

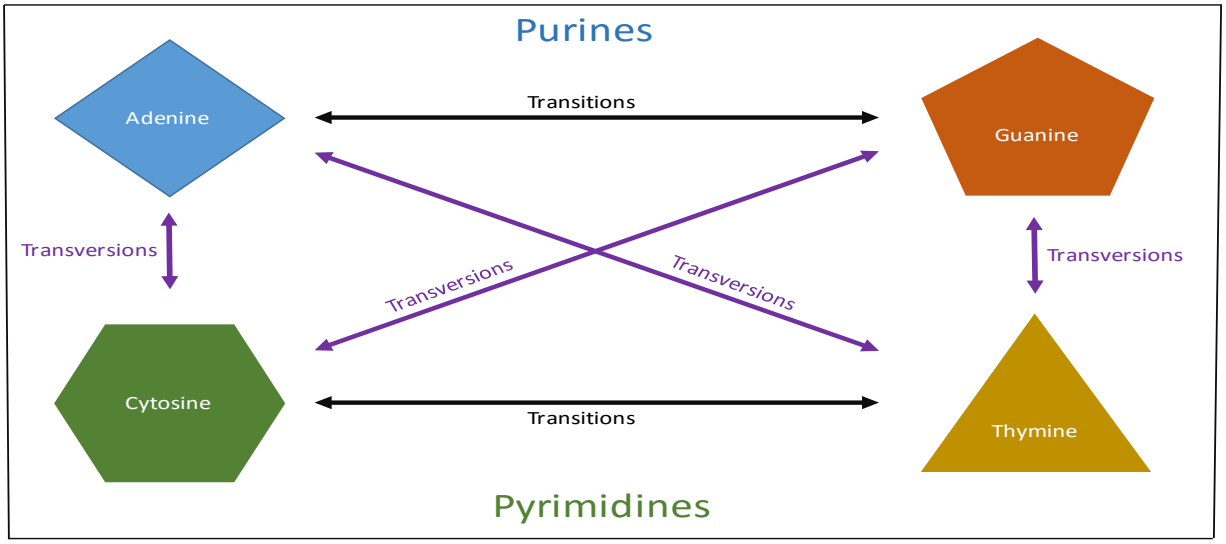
SNPs are found throughout a genes region, including untranslated regions, exons, and introns (Wang 2007). These changes in genetic code arise due to mutations that have either gone unchallenged by the environment for functional consequence or by being selected for via Darwinian evolution. That is to say, if a functional consequence has been observed than the environment may dictate keeping the genetic change as it could provide a selective advantage for those with it.

There are two wide categories of SNPs; transitions and transversions, both of which are point mutations. Transitions represent the simplest form of polymorphism and involve the change of a purine for a purine (A→G) or a pyrimidine for a pyrimidine (C→T) (Murray 2009). Transitions occur more often, at a rate of two-thirds of the SNPs found in nature (Ziegler 2010).

The other form of SNP is transversion in which a purine is exchanged for a pyrimidine or vice versa (Figure 3) (Murray 2009, Winter 1998).

SNPs can have different effects depending on is they are in coding or non-coding regions. If the SNP is in a non-coding region than it may have no effect, or it could affect a number of things such as gene splicing, gene promotion, and transcription factor binding. If the SNP is in the area of a coding sequence, then the changes can lead to silent changes; in which the amino acid sequence of the protein is not effected, or non-synonymous changes which can be further classified as missense and nonsense coding. Missense coding is when the amino acid sequence of the protein is changed and affects the viability of the protein for better or worse. Nonsense coding occurs when the transcription of the gene is prematurely ended by a misplaced stop codon (Murray 2009). A nonsense SNP likely results in a nonfunctional protein and is most often selected against via Darwinian evolution (Winter 1998).

Figure 3. DNA point mutations: transitions and transversions.



2.5.2 SNP identification, Th17 immune pathway and $P2RX_7$ genes and SNPs

The National Center for Biotechnical Information (NCBI) maintains a database of each SNP identified by researchers. Each SNP is given a reference number, or "rs" number, which is used to distinguish where the SNP is located in a gene and links it to research associated with that individual SNP (NCBI 2005). These reference SNP clusters can be integrated with other NCBI resources as they are a non-redundant set of markers designed for the purposes of annotating a specific reference genome sequence. The NCBI periodically updates the SNP database to include the new information on existing SNPs as well as adding newly identified SNPs to the library. Under NCBI surveillance, novel submissions at new positions will be given new rs identifiers and new submissions which match older existing data will be merged with the coordinant rs ID currently in use.

2.5.3 Interleukin 17A (rs2275913)

Th17 cells are widely appreciated for their role in coordinating autoimmune and inflammatory responses, as well as their role in defense against TB. Th17 cells are a subset of CD4+ T cells that are distinguished by their production of IL-17A, IL-17F, IL-21, and IL-22 and for the expression of surface IL-23 Receptor (IL-23R) (Torrado 2010). Of these cytokines the Th17 cells manufactures, it is IL-17 which is most abundantly produced. There are 6 different IL17s produced; they are IL-17A through IL-17F. Of the IL-17s developed, IL-17A has been specifically reported to assist in the host defense against different pathogens including mycobacteria. As a molecule IL-17A is composed of two monomers, as either a homodimer of two identical IL-17A's or as a heterodimer composed of one half IL-17A and one half IL-17F.

These monomers are connected by intramolecular disulphide bonds on cysteine residue (Miossec 2012). It is this IL-17A monomer which this research will be focusing on.

IL-17A is a pro-inflammatory cytokine that plays a role in host defense against certain pathogens (rheumatoid arthritis, inflammatory bowel disease) at epithelial and mucosal barriers (Jin 2013). It is involved in the production and upregulation of an environment of different cytokines, chemokines, and prostaglandins (Torrado 2010). An important outcome of these effects is the stimulation and attraction of neutrophils to the site of inflammation, which are rapidly recruited to the site of mycobacterial infection. IL-17A is a primary contributor to the formation and stabilization of the lung granuloma. The development of lung granuloma, a physical barrier sealing off the mycobacterium from the pulmonary tissue, is the primary human defense against pulmonary tuberculosis. IL-17A acts to regulate granulocyte homeostasis, chemokine expression, early cell recruitment (such as neutrophils), and to orchestrate germinal center formation (Torrado 2010). A down-regulation of IL-17 pro-inflammatory responses could lead to a diminished mature granuloma formation and thus a reduced containment of infecting MTB. In IL-17A knockout mice, the formation of the mature granuloma was followed by an impaired response to virulent MTB, thus emphasizing the importance of this Th17 cytokine (Yoshida 2010).

The IL-17A SNP rs2275913, also known as IL-17A G-197A on chromosome 6 at position 52186235, is located within the Nuclear Factor Activated T cells (NFAT)-binding motif, -197 base pairs upstream of the start codon (Espinoza 2011). Functional studies involving this SNP have led to inconsistent results, one suggesting an effect of IL-17A production and the other claiming no significant change (Rodriguez 2015). The functional study suggests that the mutant A allele is associated with significantly increased IL-17A mRNA production, when stimulated

with phytohaemagglutinin, and had a higher affinity for NFAT; a critical transcription factor involved in IL-17A regulation (Espinoza 2011).

rs2275913 is a transition mutation in which the dominant nucleotide, guanine, is mutated to an adenosine. There are studies suggesting that the rs2275913 wild type G allele and homozygous wild type genotype, GG, are associated with first time acquisition of PTB in a northern Spanish cohort (Ocejo-Vinyals 2013). This information was supported by a study involving PTB patients in southern Brazil (Milano 2016). However, Shi 2014 published conflicting results suggest a Chinese population with the mutant allele A was more at risk of acquiring TB than a control population (Shi 2014).

The mutant allele, A, is associated with increased susceptibility to rheumatoid arthritis in a Norwegian population with an N=1883 and the heterozygous genotype is associated with risk of poorly differentiated, TNM classification I/II subtypes of gastric cancer within the age groups of 40-65, but not with total gastric cancer risk, in a Chinese Han population with an N=2510 (Nordang 2009, Wu 2010). In the Chinese Han population there was no correlation to gastric cancer survival rates and observed polymorphism differences.

In 2014, a meta-analysis of 12 different studies of rs2275913 was undertaken in an attempt to determine if this SNP has an impact on Asian populations and cancer rates. The research was inconclusive but indicated that this SNPs wild type allele may be implicated in increased cancer risk for all types of cancer (Dai 2014)

The wild type A allele was seen to be associated with ulcerative colitis and AA genotype was found to be significantly associated with the severe form of ulcerative colitis, known as pancolitis, in a Japanese cohort with N=359 (Arisawa 2006).

2.5.4 Interleukin 17 Receptor A (rs4819554)

The cells capable of responding to the proinflammatory cytokine of IL-17A include DCs, macrophages, lymphocytes, and neutrophils; these cells interact with IL-17A through their Interleukin-17 Receptor A (IL-17RA). If optimal in response, many of these cells can aid in establishing an anti-microbial granuloma that can lead to the control of the mycobacterium by inducing latency or even the eradication of the pathogen. The same cells under less than optimal conditions may also be responsible for the dissemination of viable bacterium and may contribute to pathogenicity (Guirado 2013, Lowe 2012). The immune cells that express IL-17RA are ubiquitously expressed in various organs including the lungs, spleen, and liver (Torrado 2010). The IL-17 receptor family is composed of five members, listed as IL-17 Receptor A (IL-17RA) through IL-17 Receptor E. IL-17RA was named as such as it was the first of the family discovered, the others are named based largely on the sequence similarity to IL-17RA (Jin 2013). Most of the functional IL-17 receptors are found as heterodimers on the associated cells surface. IL-17RA is a heterodimer composed of IL-17 Receptor A and C, with the A monomer being the largest member or the receptor family (Gaffen 2009). IL-17RA is capable of recognizing both IL-17A and IL-17F, but not the other IL-17 cytokines (Jin 2013). IL-17RA is distinguished within its own family of receptors by having the largest cytoplasmic tail of the family. This cytoplasmic tail provides a docking station for key signaling intermediates.

Prior research has identified that defects in either, or both, of IL-17A and IL-17RA can lead to increased bacterial dissemination. This bacterial dissemination is correlated with poorer neutrophil recruitment and reduction in inflammatory mediators (Jin 2013). In an attempt to establish that IL-17A is not experiencing a significant difference in binding affinity, which could

destabilize the inflammatory balancing of IL-17A, the IL-17 Receptor A will be under investigation with the populations under study.

The IL-17RA polymorphism rs4819554, also known as A-947G on chromosome 22 at 17084145, is a promoter polymorphism with limited functional studies available. However, one study reports that leukocytes with higher levels of IL-17RA mRNA are those expressing the rs4819554 wild type AA genotype as opposed to the mutant GG genotype (Coto 2015). The same research group hypothesizes that the wild type A allele in this promoter region could create a more attractive site for Transcription Factor Activating Enhancer Binding Protein 4 (TFAP-4) to aptly bind too (Coto 2015).

rs4819554 undergoes a transition mutation of adenosine to guanine. The mutant G allele was found to be significantly associated with the Cw6 major histocompatibility marker which is considered the main biomarker risk factor for psoriasis. These findings were found in a Caucasian population from northern Spain with an N=1147 (Batalla 2015). The wild type A allele is associated with Aspirin Exacerbated Respiratory Disease in a Korean population of N=1379 (Park 2013).

2.5.5 Interleukin 23 Receptor (rs10889677)

Naïve $CD4^+$ T cells may be activated to differentiate into distinct pathways by numerous different cytokines and chemokines. Once activated into these distinct pathways the T cells will release and interact with specific cytokines associated with their individual responses. The ability of macrophages to inhibit the growth of MTB can be directed by these acquired immune response factors, as intense macrophage activation is dependent on the type of effector T cell promoted and the state of the overall immune response. The distinctive pathways the naïve T

cells can differentiate into are Th1, Th2, Th17 or Treg cells. If the immune system is working optimally than markers of invading pathogen determines which of the T cells pathways is activated as specific cytokines are expressed to help navigate this complex process (Abbas 2012). IL-12, IL-18 and IFN-γ promote the expression of the Th1 subset while IL-4, IL-5, and IL-13 induce the Th2 pathway (Dheda 2010). The Th17 subset is promoted through the expression of IL-6, IL-21, and TGF-β (Qin 2009). Th1 and Th17 cytokines prompt the maturation and activation of macrophages and granulocytes, the most notable of the cytokines are IFN-γ, TNF-α, IL-12, and Granulocyte–macrophage colony stimulating factor (GM-CSF) (Dheda 2010, Piper 2014). The activation of macrophages and granulocytes to restrict the growth of MTB stem from Th1 and Th17 amplification and is one of the key elements to the successful immunologic response to this pathogen (Dorhoi 2011. Flynn 2011).

Though the Th17 response is still not completely defined it has been demonstrated that Th17 cytokines do play an important function in the generation of the granuloma formation and are implicated in triggering the Th1 response, which is crucial for controlling MTB infections (O'Garra 2013, Sasindran 2011). The ability to differentiate into and maintain Th17 cells from CD4+ cells is one of the roles of the cytokine IL-23. IL-23 binds to the IL-23R (which is dimer composed of IL-23 Receptor and IL-12 Receptor Beta 1) on CD4+ T cells aiding in the cells differentiation to Th17 cells and to the initiation and sustainment of signaling pathways for the production of IL-17 (Khader 2008). The signaling of IL-23R may also promote the immunosuppression of Tregs which may lead to the downstream promotion of IL-17 (Zheng 2012).

A reduction in the IL-23R expression can lead to a decrease in IL-17 expression and therefore a decline in natural host defense of MTB due to decreased ability in granuloma

formation. The inclusion of IL-23 is thought to be required for the optimal response of Th17 cells against mycobacterial infection (Khader 2008)

The IL-23R SNP rs10889677, also known as IL-23R +2199A/C on chromosome 1 at 67259437, has undergone biochemical analysis in an attempt to identify how it effects the gene. The minor allele, A, disrupts the binding site for the micro-RNA, miR-let-7f, which increases the transcription of the IL-23R gene both *in vitro* and *in vivo* using the immortalized human T lymphocyte cell line Jurkat (Zheng 2012). The Zheng study also measure serum samples from healthy controls using an enzyme linked immunosorbent assay and found that mutant genotype AA individuals produced higher levels of IL-17 (Zheng 2012).

Jiang et al. investigated a Chinese Uygurs population, N=250, with active PTB in an attempt to establish a correlation between PTB and rs10889677. The wild type genotype CC was represented more frequently in PTB cases in the population and has been suggested to possibly be associated to susceptibility of the disease (Jiang 2015).

In a study population from Eastern and Southern China, N=10,600, the rs10889677 SNP was investigating for association with risk of all cancer types. The study identified that the mutant A allele was associated with multiple types of cancer; including breast and lung cancer, and nasopharyngeal carcinoma. As well, the homozygous wild type genotype, CC, was reported to have lower proportion of T regulatory cells and a higher proportion of T cell proliferation when stimulated with concanavalin A, a mitogenic plant abstract, than the homozygous mutant AA individuals (Zheng 2012, Gunther 1973). In a Chinese Han population, N=392, both the C allele and CC genotype were associated with idiopathic dilated cardiomyopathy (Chen 2009).

2.5.6 Interferon-gamma receptor ligand binding chain I (rs2234711)

IFN-γ is a pleiotropic cytokine that plays a key role in the modulation of many phases of the immune and inflammatory responses (Farrar 1993). IFN-γ belongs to a family of three distinct cytokines, which have been established via specific criteria, these three family members include IFN I, II, and III. IFN-γ, which falls into the type II family, is referred to as immune IFN, and is release from Th1 cells. IFN-γ is responsible for the activation and deactivation of T cells and natural killer cell.

Whereas IL-23 functions in the induction and differentiation of Th17 cells, this Th1 cytokine, IFN-γ, targets naïve T cells and inhibits the production of T helper 2 cells (Th2) and Th17 cells (Mangan 2006). IFN-γ acts to assist in regulating the inflammatory response of Th17 and induces the production of the Th1 response, which is one of the key elements to the successful immunologic response to MTB (Dorhoi 2011). Prior studies have demonstrated that hosts with IFN-γR deficient cells have an even greater ability to develop Th17 cells, regardless of IL-23 stimulation (Weaver 2006). As mentioned earlier, IL-17, the major cytokine produced by Th17 cells, is a key inducer or inflammation, which has the potential to incur cellular damage as a by-product. Due to the danger of possible cellular damage, a functional balancing system must be in place.

Prior studies have indicated that there are functional SNPs variances of IFN-γ within three of the populations which will be studied in this research; Dene, Cree, and Caucasian (Larcombe 2008). These function SNP variances may play a role in the differential rates of tuberculosis between the populations. This prior study has inspired further inquiry into the possibility of the INF-γR having a functional SNP difference between the populations under

review in this study, as prior studies indicate that cells deficient of IFN-γR may develop even greater Th17 cell responses (Weaver 2006).

IFN-γR SNP rs2234711, also known as IFN-γR T-56C on chromosome 6 at 137219383, is located in the gene promoter area and the mutant allele C has been observed to express higher levels of Th1 than the wild-type allele T (Xiang 2014). It is suggested, though not confirmed, that the mutant C allele increases the binding affinity of the TFAP4 binding site (Jüliger 2003, He 2010). TFAP4 assists in the regulation of cell proliferation, and can act as an activator on the target gene (D'Annibale 2014, Lin 2010). This increased binding affinity has been associated with susceptibility to some infectious diseases, including tuberculosis (He 2010).

rs2234711 has been shown to display inconsistent evidence for suggesting association to TB susceptibility. In a study involving the three West African countries of Gambia, the Republic of Conakry, and Guinea Bissau, the wild type T allele of rs2234711 was found to be associated with smear-positive or TB culture positive participants, N=1301 (Cooke 2006). This finding was mimicked in a Chinese Han population, N=1434 (Lü 2014). However, in a separate Gambian study with N=640, there was no tangible association seen with either allele (Awomoyi 2004).

In another study on Hepatitis B Virus (HBV), the rs2234711 mutant C allele was found to be associated with viral clearance in a Chinese population with an N=983 (Zhou 2009).

2.5.7 $P2RX_7$ (rs3751143)

The $P2RX_7$ gene encodes a transmembrane polypeptide that is 595 amino acids long and acts as a receptor for a ligand-gated cation channel. This receptor is highly expressed on cells of hemopoietic origin such as macrophages, DCs, lymphoid cells of all subtypes, and mast cells, and these are activated by adenosine triphosphate (ATP) (Shemon 2006). When $P2RX_7$ protein is

stimulated by ATP it opens a cation channel which allows for the transaction of potassium efflux and calcium influx. This efflux/influx phenomenon is involved in the induction of apoptosis. This same apoptotic process has been described as a possible method for the killing of mycobacteria infected macrophages (Niño‐Moreno 2007). As previously mentioned, apoptosis is a controlled method of killing infected macrophages which inhibits the mycobacteria's ability to spread to adjacent cells.

As a highly polymorphic gene within humans, the $P2RX_7$ gene has many possible SNP sites to consider. However, the rs3751143 SNP, also known as $P2RX_7$ A1513C on chromosome 12 at position 121184501, has been studied extensively and its biochemically effects are now understood. This SNP induces a transversion mutation from the dominant allele of adenosine to the recessive allele of cytosine. This point mutation causes the amino acid sequence to change as well, as a missense mutation is introduced at this position with a glutamic acid being substituted with an alanine. If the genotype becomes a homozygous mutant, CC, then the observed phenotypic trait is that of an almost complete loss of function of $P2RX_7$ leading to lack of ATP-induced mycobacterial killing of infected macrophages (Niño‐Moreno 2007). The heterozygous genotype for this particular SNP is also highly compromised as reductions of up to 75% in mycobacterial killing apoptosis function has been observed (Fernando 2005).

The biochemical characterization of rs3751143 is significant as it provides addition information as to why SNP research of the particular polymorphism has seen a correlation of the mutant C allele with higher prevalence of TB. Numerous examples of this higher prevalence has been observed as the mutant C allele has identified as associated with TB susceptibility in numerous studies (Singla 2012, Sehajpal 2010, Mokrousov 2008, Niño-Moreno 2007, Ben-Selma 2011, Fernando 2007, Tekin 2010).

Meta-analysis research of nine separate rs3751143 TB studies was undertaken and amalgamated information on 2195 cases of TB and 2036 controls. This amalgamation of information identified that the C allele and CC genotype was significantly associated with increased susceptibility to TB in all populations, but was specifically an even more immense contributor to susceptibility of TB in Asian populations. In Asian populations the C allele was also associated with increased susceptibility to ETB as well, though this association was not witnessed in African and Latino populations (Wu 2014).

2.6 Gaps in knowledge and rationale for study

Previous research has shown that SNPs in key immune regulatory genes have played a critical role in host immune response to MTB and other respiratory infections (Adelson 2005, Barnabe 2008, Ferguson 2010). First Nations in Manitoba have been shown to maintain a high frequency of SNPs in immune regulatory genes that may impact their immune response to some infectious pathogens (Larcombe 2010). SNPs that have functional consequences for Th1 and Th2 immune response have been identified in Dene, Cree, and Saulteaux First Nations, but the Th17 pathway and the $P2RX_7$ gene have not been explored in these groups, despite their important role in immune and inflammatory responses (Larcombe 2008, Larcombe 2012b, Khader 2008). There has also been documentation of differences not only between First Nations groups and Caucasians, but within the First Nations groups (Dene, Cree, Saulteaux) as well. The Dene, who have some of the highest tuberculosis rates of the aforementioned groups, have the most skewed SNP profile (favouring Th2 cell generation and activation); while the Cree, who have intermediate tuberculosis rates between the Dene and Caucasians, have a less skewed Th2 profile of SNPs (Larcombe 2008). Immunogenic differences between Aboriginal groups likely reflects

differences in social and cultural history, current and historical geographic location, historically significant interactions with original European settlers of Canada, as well as contact and experience with infectious pathogens (Adelson 2005).

SNPs affecting the IL-17 pathway may contribute to TB susceptibility although previous research has had conflicting results. Ocejo-Vinyals 2013 and Milano 2016 found the wild type allele to be associated with first time acquisition of PTB whereas Shi 2014 found the more at risk groups to have an increase mutant A allele profile (Ocejo-Vinyals 2013, Milano 2016, Shi 2014). In the IL-17 studies on rheumatoid arthritis in a Norwegian population the results were inconsistent. The same group of researchers were not able to replicate their findings of a correlation between the A allele and rheumatoid arthritis while investigating a separate New Zealand population (Nordang 2009). The study inquiring about gastric cancer in a Chinese Han population only found a correlation between the AG genotype and the age group of 40-65 year olds (Wu 2010). The Chinese Han research is particularly peculiar as there was no correlation found in any other age group and no correlation with observable gastric cancer survival rates (Wu 2010).

A significant gap in knowledge is the lack of IL-17RA SNP rs4819554 research pertaining to tuberculosis. The information gathered for the purposes of this study was obtained from studies focusing on psoriasis and asthma. At this point in time there are no rs4819554 association studies involving TB.

There is one study with data suggesting the IL-23R SNP rs10889677 wild type genotype has a tangible association with susceptibility to MTB in a Chinese Uygur population; however, there are numerous findings of IL-23R SNPs having a correlation with the prevalence for other

diseases (Crohn's Disease, Inflammatory Bowel Disease, idiopathic dilated cardiomyopathy, and numerous cancers) within other populations (Jiang 2015, Zheng 2012, Chen 2009).

The IFN-γR SNP rs2234711 has been shown to display inconsistent evidence for suggesting association to TB susceptibility (Cooke 2006, Lü 2014). It is reasonable to suggest that further meta-analysis studies are required to determine the overall effect the SNP does, or does not, have on TB susceptibility.

$P2RX_7$ is a highly polymorphic gene and though rs3751143 has been biochemically defined, there is reason to believe that other SNP sites within the gene may have functional consequences on gene activity as well.

Numerous studies have demonstrated the impact of the SDH (food security, housing, medical care access, education and income) and the ability to resist and recover from diseases (Raphael 2009, Marmot 2006). These disparities in the SDH may help explain the differences that influence the exposure and vulnerability to risk, and ability to recover from diseases such as TB (Rasanathan 2011). For First Nations on-reserves in Manitoba these SDH are key contributors to disease susceptibility (Larcombe 2011, Boutilier 2013). Recently, there has been research that investigates how biological and genetic differences may be contributing to health outcomes and disease susceptibility in First Nations groups (Larcombe 2012b, Maddur 2012, Adelson 2005, Barnabe 2008). Studies analyzing comorbidities and genetic factors contributing to different diseases have shed new light on the role that genomics may play in susceptibility and/or resistance to infectious diseases and on health disparities (Larcombe 2008 and 2012, Chen 2009, Cooke 2006, Ferguson 2010. Shemon 2006). Past research with First Nations population identified potential genes variants in the Th1 and Th2 immune pathways that may increase or decrease their disease risk (Barnabe 2008, Larcombe 2008 and 2012b and 2015). However, gene

variants affecting the Th17 pathway genes of IL-17A, IL-17RA, IL-23R, IFN-γR and the $P2RX_7$ gene have not been investigated within this population (Torrado 2010, Gaffen 2009, Khader 2008, Mangan 2006, Niño‐Moreno 2007).

Much of the research in the past has been done "on" Aboriginal people rather than "with" Aboriginal people. Due to this there has been a legacy of harm, distrust, and resentment built around Aboriginal research with Western research practices (Masching 2006). Knowledge translation and dissemination has been increasing within the academic world, however it is still not a prominent focus within medical and genetic research (Cargo 2008). Even recent research with First Nations communities and people in Canada have not made knowledge translation a primary objective within its research as seen with Greenwood, Oen, or Murdoch (Greenwood 2000, Oen 1998, Murdoch 2012). Academic publications are currently the gold standard for research but if research is to be relevant and engaging to First Nations and other non-academic communities than it is the researcher's responsibility to involve the partners at every stage of research.

2.6.1 Hypothesis and Objectives

In addition to social and environmental factors that influence health, it is hypothesized that distinct First Nations groups (Dene, Cree and Saulteaux) will have a different frequencies of SNPs in the key Th17 immunity related genes of IL-17A, IL-17RA, IL-23R, IFN-γR, and the $P2RX_7$ gene, as compared to a non-Indigenous Canadian group.

The primary objectives of this study were as follows:

1) Detect and document the frequency of SNPs in the promoter and coding region of five distinct genes; IL-17A (rs2275913) a cytokine involved in the regulation of

granuloma formation; IL-17RA rs4819554 the receptor for IL-17A expressed on innate immune cells of mucosal layers in the body; IL-23R rs10889677 the receptor for IL-23 expressed on naïve T cells and a guiding factor in the expression of the Th17 pathway; IFN-γR rs2234711 the receptor for IFN-γ on naive T cells and a guiding factor in the repression of the Th17 pathway; $P2RX_7$ rs3751143 is a cation channel which induces mycobacterial killing apoptosis in infected macrophages. This will be done in three Manitoban First Nations (Dene, Cree, and Saulteaux) populations and a non-Indigenous Canadian population.

2) Perform knowledge translation activities/communications on the information gathered and communicate this information in a culturally respectful and appropriate manner.

3.0 Chapter 3 Scientific and Knowledge Translation Methods

This section will describe the participating populations in this study, the methods for ethics approvals, data and sample collection, and knowledge translation.

3.1 Study participants

In Manitoba there is a population of approximately 150,000 Indigenous peoples who identify as First Nations, Inuit or Metis (Government of Manitoba 2016). Representing over 50% of the Indigenous peoples in Manitoba, the First Nations comprise the majority of this group. The First Nations people in Manitoba identify as belonging to the Athapaskan (Dene) or Algonkian (Cree, Saulteaux, Oji-Cree) language groups. The four First Nations populations in this study are from three distinct geographic regions (northern taiga, central boreal forest, southern prairie/parkland) in Manitoba. To further represent the distinct geographical areas of each of the communities the Canadian Band Remoteness Classification was included in their descriptors.

3.1.1 Dene First Nations

Dene people inhabit the boreal forest and the taiga (barrenlands) of the northern prairies and subarctic regions of the territories of Canada and Alaska (Figure 4). The Dene language belongs to a northern Athabaskan language family, which is distinct from the Algonquin language family, Canadian Inuit and American Eskimo peoples. “Dene” is a common Athabaskan word, which means “people”. Pre-contact Dene lived in the subarctic region in small

groups of hunter-gatherers, which moved across the landscape with the migrating caribou herds (Ward 1995). Following the migrations of caribou south brought the Dene people into direct conflict with northern Cree peoples. In northern Manitoba there are currently two Dene communities, both of which are considered to be a remoteness area 4 according to the Canadian Band Classification Manual, which means they are not accessible by road year round (Chan 2000). Both of the Dene communities have distinct histories. One of the groups moved across northern Alberta and Saskatchewan before arriving in northwestern Manitoba, the other's traditional lands were in northern central Manitoba, Nunavut and Northwest Territories. Due to these distinct histories and current classification of remoteness this study has separated the Dene communities, Dene 1 and Dene 2, for the purposes of data collection and analysis. Samples were collected from individuals in the two communities that had populations of 303 and 604 in 2006 (Statistics Canada 2016a, Statistics Canada 2016b).

3.1.2 Cree First Nations

Cree people in Canada encompass the largest geographical area of any of the First Nations people found in the country, spanning from the shores of Labrador all the way across the country into the area of the Rocky Mountains. Within Manitoba, Cree territory is expansive, overlapping with the southern territory of the Saulteaux and extending northward into the lands of the Dene (Figure 4). Pre-contact Cree people lived as hunter-gatherers often residing in small units of 8-12 people called a "lodge". In the prairie provinces the Cree people were a highly nomadic population, covering great distances for hunting, trade, and warfare (Abel 2005).

As discussed above, the Cree are members of the Algonquin language group. The Cree study participants were from a Cree community in the northern central part of Manitoba that had

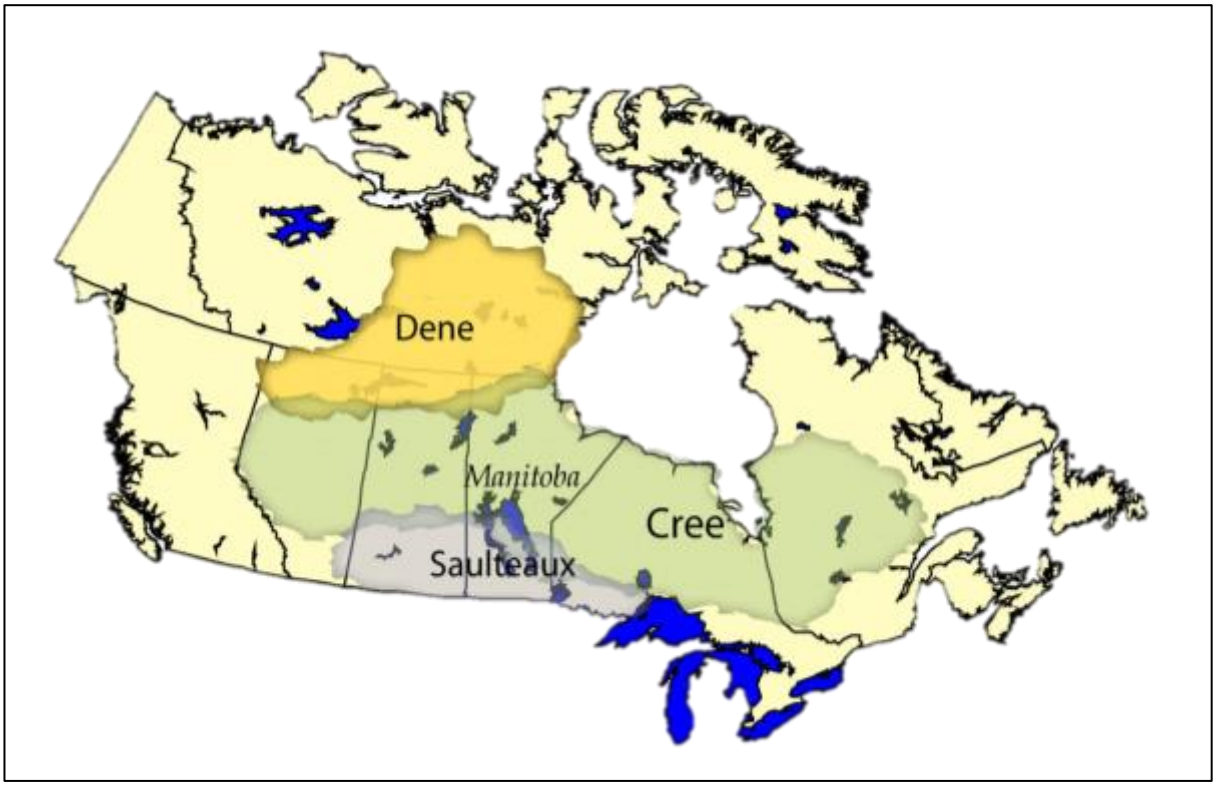
a population of 2096 in 2006 (Statistics Canada 2016c). This community is considered a remoteness area 2 on the Canadian Band Classification Manual and as such has road access year round (Chan 2000).

3.1.3 Saulteaux First Nations

Saulteaux people are a branch of the Ojibwa nation, and the "Saulteaux" name has its origins from French settlers who became involved with the Ojibwa near the French settlement of Sault Saint Marie, Ontario, a primary location of trade during the 17th century (Ward 1995). The term Saulteaux is derived from the French word "saulteurs" which means "people of the rapids" (Ward 1995). The Saulteaux became distinguishable from the Ojibwa as they moved westward with the expanding trade route, settling along the western edges of Lake Superior and into the Manitoba Interlake area; as well as the Red, Assiniboine, and North Saskatchewan rivers (Peers 1994). Before European contact in North America, the Saulteaux were a hunter-gather peoples who followed seasonal routes for collecting their provisions. In present day, the majority of Manitoba Saulteaux individuals reside in southern part of the province and may also be referred to as "Plains Saulteaux" (Figure 4).

Ojibwa/Saulteaux belong to the Algonquin language family and as such are closely related to Cree. This Algonquin language family is further divided into the Anishinaabe speaking peoples of which both the Saulteaux and Cree are members. Samples from the Saulteaux participants were collected from one community in the southern part of Manitoba. This community is not remote and is accessible year round by road (Chan 2000). In 2006 the population was 427 (Statistics Canada 2016d).

Figure 4. Geographical Distribution of Dene, Cree, and Saulteaux.



Used with permission from L. Larcombe (2016)

3.1.4 Non-Aboriginal Control Population

The control sample included individual volunteers residing in Winnipeg who were healthy, over 40 years of age, and were not of First Nations ancestry. These individuals were typically, but not exclusively, employees of the University of Manitoba or the laboratories at the adjacent Health Sciences Centre.

3.2 Ethics

This research has been conducted with the First Nations in Manitoba following the foundations of the First Nations Principles of OCAP™ (Ownership, Control, Access and Possession) (FNIGC 2007). OCAP™ is an ethical document written and sanctioned in 2007 by the First Nations Information Governance Committee. It is a document championing self-determination, equality, and the protection of First Nations people and any information generated from research involving First Nations people and/or their communities. OCAP™ has had an extensive impact on other research bodies in Canada, including the Tri-Council Agencies. Past examples of genetic research infringed on human rights, as such power balance was of utmost importance for both the participants involved as well as the researchers. Both OCAP™ and the Tri-Council Policy Statement 2 clearly identify that research needs to be beneficial and relevant for both the researcher and the participating community (FNIGC 2007, CIHR 2015). Both sanctions advocate for all possible outcomes to be outlined before any research takes place. It is important for First Nations peoples' ability to self-determine all research concerning them, protecting themselves and any data or information generated from investigation done with them, and determine where the information generated will be physically stored. As such, the outline of the research was discussed and shaped with the assistance of the Chief and Councils of the First

Nations groups involved; Northlands Denesuline First Nations at Lac Brochet, Sayisi Dene First Nations at Tadoule Lake, Tootinaowaziibeeng Treaty Reserve First Nation at Valley River, and Nisichawayasihk Cree Nation at Nelson House (Appendix A1-A6).

The DNA samples and all test results are physically stored at the Basic Medical Sciences Building, which is in connection to the Health Sciences Center, in Winnipeg, Manitoba. However, as part of the "possession" directive of OCAP™, this possession is only as place-keepers of biological material and data as the people and groups donating are the owners of the material and data. The owners of the material and data can request to withdraw at any time and associated data collected and biological materials stored will be appropriately disposed of. There is also a timeline for possession of the biological samples, once that timeline is reached the communities involved will be contacted and discussion on what they wish to do with the biological materials will take place. The researchers have permission to keep the physical data generated for finishing research projects as defined by the project descriptors.

Prior to the initiation of the research the objectives, protocols and methods of the study were reviewed and approved by of the Health Research Ethics Board's (HREB) guidelines as defined by the University of Manitoba (Appendix A7-A13).

3.3 Sample collection and processing

Individuals were invited to enroll into the study by local radio announcements, at public events in the community and through community meetings. Individual informed consent was obtained before blood or buccal sample draw. The consent form was read and explained to the participant in English and was translated into the local language (Dene, Cree or Saulteaux) by a research assistant when requested. This included a verbal and written overview of the research,

what was required of the participant, and confidentiality provisions (Appendix A14-18). The genetic portions of the research involved only genes related to TB immunity and with the understanding that upon completion of the research that the genetic material will be disposed of and not used for any other study without further consent.

The collection of samples for the study was done by convenience sampling and included adults and children (with their own and their guardians informed consent) in four separate First Nation communities (2 Dene, 1 Cree, 1 Saulteaux) and a "control" sample of non-Aboriginal individuals. Age, sex, TB history (active, latent, or no prior contact with TB) and self-identified ethnicity was collected for each participant enrolled into the study. Individuals were excluded from the study if they were a first degree relative of another participant. In 2005 Dr. Larcombe, with the assistance of Dr. Andrew Lodge who was at that time a summer student of the University of Manitoba Faculty of Medicine, gathered whole blood samples from participants residing in a Dene community in Northern Manitoba (Dene 1). The blood tubes were labeled with the participants de-nominated study number and the date of collection. All blood samples were collected in BD Vacutainer® K2 EDTA (K2E) Plus Blood Collection Tubes. The samples were kept at 4° Celsius and transported from the community by direct air to Winnipeg, Manitoba. Once the samples reached Winnipeg they were immediately taken to the University of Manitoba Basic Medical Sciences Building for DNA separation and DNA extraction. Blood samples were collected from the non-Aboriginal control population from 2005 to 2008.

In the summer of 2006 the same procedure was repeated in a Cree community in central Manitoba (Cree). In the summer of 2008, buccal swabs were collected at the Saulteaux community (Saulteaux) and in 2010 buccal swabs were collected at the second Dene community (Dene 2) after the request from the Chief and Council to be involved in the study. Two buccal

swabs were collected using Cap-Shure sterile cotton tipped applicators with aerated tip protector by Puritan®. Buccal cells were collected rather than blood because these samples are more stable for the purpose of transportation. After collecting the buccal cells on the Puritan® swabs they are allowed to dry and were stored individually in paper envelopes for transport to the University of Manitoba Basic Medical Sciences Building. This method of collection is a less invasive method for the study participants, as not everyone is comfortable with needles, as well as the whole blood extraction process being costlier than that of buccal swab collection. The disadvantage of DNA collection via this method is that the amount of DNA which can be recovered from buccal swabs is significantly lower than from separated whole blood.

3.3.1 Extraction of DNA from whole blood samples

Whole blood samples were spun at 3500 rpm for 10 minutes to separate the three blood components: the clear plasma layer (top), the white blood cell layer (middle), and the dark red blood cell layer (bottom). The top blood plasma layer is composed of the extracellular matrix that surrounds blood cells. It is used as a suspension material that provides structural and biochemical support to the surrounding cells. Blood plasma is mostly composed of water, cofactors and proteins but does not carry intact DNA (Michel 2010). The dark red bottom cell layer is composed of red blood cells, known as erythrocytes, which are responsible for transporting oxygen throughout the body. These red blood cells lack a nucleus and thus have no DNA (Kabanova 2009). The middle white blood cell layer is referred to as the "buffy coat" layer and contains the white blood cells, which have nuclei containing DNA, and platelets. The DNA extraction is accomplished through the separation of the buffy coat. Buffy coat extraction from blood samples yields a high concentration of DNA, much higher than the average buccal swab

concentration. To extract the DNA from the buffy coat of cells a QIAGEN© QIAamp DNA Mini Kit was used. The procedures were performed as outlined by the QIAGEN© QIAamp DNA Mini and Blood Mini Handbook (QIAGEN 2012). The process involves cell wall degradation by a cell lysis buffer, a proteinase step to breakdown peptides, an ethanol wash to precipitate the DNA from the rest of the material, multiple wash buffers to rid the sample of extraneous molecules, and an elution buffer as a final step to separate the DNA out by itself for stable and appropriate storage. The extracted DNA was stored at -70 °Celsius.

Optimal concentration for DNA via these extraction processes is 100 ug/ml. A concentration of less would result in inadequate amplification of the product, and would need further amplification techniques, and a higher concentration could result in decrease amplicon specificity (New England BioLabs 2016). Spectrophotometry was used to quantify the purity of DNA from the blood and buccal swabs after extraction and after Repli-g amplification of the DNA. Spectrophotometers measure the amount of light a substance absorbs to determine how much of a given substance is present and how much of the measured body is composed of contaminants. Nucleic acids, the building blocks of DNA, absorb ultraviolet light at a specific wave pattern of 260 nm. The more light absorbed at this frequency, the higher the nucleic acid concentration is in the sample.

To control for other contaminants in the samples, such as proteins and organic compounds, the spectrophotometer was set to measure a ratio of light absorbed at 260 and 280 nm (A260/280) which indicates purity of the nucleic acid/DNA sample. An approximate ratio of 1.8 is expected for pure precipitations of DNA (Bio-Rad 2016). Protocols were followed for nucleic acid spectrophotometry as outlined by the Bio-Rad™ SmartSpec Plus Spectrophotometer Instruction manual.

3.3.2 Extraction of DNA from Buccal Swabs

DNA collection by buccal swabs are a more cost efficient and less invasive method of obtaining genetic DNA samples. This method also provides increased DNA stability for the purposes of transport of the samples collected off-site where there is no ability to store or process blood samples. The buccal swaps undergo the same extraction process but with one additional initial step of washing the cotton swabs with the ionic water-based solution, phosphate-buffered saline (PBS), which assists in disengaging the cheek cells from the swab head.

3.3.2.1 Repli-g Amplification of DNA from Buccal Swabs

The DNA yield from buccal swabs is often significantly less than that obtained by blood extraction. Buccal swab DNA concentrations often are found to be between 1-6 ug/ml which is less than optimal for subsequent analysis of single nucleotide polymorphisms. QIAGEN© REPLI-g Mini Kits, which are whole genome replication kits, were used to increase the yield of DNA. The further amplification techniques incur additional monetary costs and time expenditures for buccal samples. These kits provide a means to amply DNA to a concentration up to 200 ug/ml regardless of the starting template concentration. Samples were diluted to a concentration of 100 ug/ml for subsequent analysis. This was ideal with the low yield buccal swabs. The Repli-g kits were also used to amply DNA extracted from blood if the original stock was running low.

Qiagen© REPLI-g Mini Kits uses the technique of Multiple Displacement Amplification (MDA), which involves denaturing whole DNA products into single stranded DNA and binding random hexamers to these strands. Once the strands are bound DNA synthesis is initiated by the

enzyme Phi-29 polymerase, an enzyme from the bacteriophage phi-29, at a constant temperature. The Phi-29 polymerase does not disassociate from the genomic template that allows for multiple fragments to be generated. These fragments are up to 100kb and display no sequence bias. Phi-29 also has a 3'→l' proofreading exonuclease which generates a 1000-fold higher fidelity ability as compared to PCR's Taq polymerase. As compared to whole genome amplification via PCR, Repli-g provides lower locus bias, higher uniformity of amplification across the entire genome, and lower mutation rates during amplification (QIAGEN 2016).

3.4 DNA Amplification using PCR

Polymerase Chain Reaction (PCR) was used to amply specific areas of the DNA. The use of small customized strands of DNA between 15-30 nucleotides long, known as primers, were used to establish the areas of amplification (Dieffenbach 1993). Two primers were incorporated per area of amplification, a "forward" and "reverse" primer, which were complimentary to the 3' ends of the sense and anti-sense strands, respectively. DNA polymerase is the enzyme that adds nucleotides to single stranded DNA sequences during DNA replication. This research uses Life Technologies Inc. Platinum Taq High Fidelity DNA Polymerase for all PCR amplification. Isolated from *Thermus aquaticus*, a thermophilic bacterium, Taq DNA polymerase is a polymerase enzyme which is thermo stable even at temperatures exceeding 90°C (Saiki 1988). It is this ability to withstand elevated temperatures that make this isolate so well suited for PCR.

Primers are short DNA strands, usually between 15-30 nucleotides in length, which are designed to anneal to a specific sequence of template DNA. The forward and reverse primers are designed to be the starting and end points, respectively, of a specific DNA fragment that is to be amplified. Primer design and determination of annealing temperatures is a complex process that

is based on cytosine-guanine content. The higher the cytosine-guanine content of the primer, the higher the melting temperature will need to be. The primers for this research are outlined in Table 1.

Table 1: Forward and reverse primers for SNP analysis.

Gene	**Forward Primer 5'→3'**	**Reverse Primer 5'→3'**
IL-17A[1]	AACAAGTAAGAATGAAAAGAGGACATGGT	CCCCCAATGAGGTCATAGAAGAATC
IL-17RA[2]	CGCGTGCTAAGAAGGAGACT	GGGGACACTTGAGTTCCTGG
IL-23R[3]	CTGTGCTCCTACCATCACCA	TGCTGTTTTTGTGCCTGTATG
IFN-γR[2]	GCCCATCTCAGCCCTGCTCA	ATTGTGGCTCGGCTGTGGCC
P2RX$_7$[4]	AGACCTGCGATGGACTTCACAG	AGCGCCAGCAAGGGCTC

1: Wu 2010; 2: NCBI 2016a; 3: Chen 2009; 4: Niño-Moreno 2007

PCR reagent mixture was consistent throughout the entire project. Each PCR was performed using a Life Technologies Inc. Platinum Taq High Fidelity 500 Reactions PCR kit. These kits included Platinum Taq polymerase, HiFi PCR Buffer, and MgSO4. The HiFi Buffer provides a stringent primer-annealing condition over a wider range of annealing temperatures and Mg2+ concentrations that increases the specificity of primer annealing. The Taq enzyme requires a divalent cation for its activity. This is the reason MgSO4 has been supplied (Slack

2011). The reagent mixture was an admixture totaling 25µl; 21.5µl of master mix and 3.5µl of template DNA. The dNTPs kit utilized was the Life Technologies Inc. 100mM dNTP Set PCR Grade. A dNTP master mix was created by combing 25µl of each nucleotide into a VWR® 1.5 Disposable/Conical Micro-Centrifuge Tubes. The composition of the master mix is presented in Table 2.

Table 2. PCR Master mix reagents and concentrations.

Reagent	**Stock Concentration**	**µL/rxn**
HiFi PCR Buffer	10x	2.5 µl
$MgSo_4$	50 mM	1.0 µl
dNTP mix	100 mM mix	0.2 µl
Forward A	10µM	0.5 µl
Reverse B	10µM	0.5 µl
ddH_20		16.7 µl
Platinum Taq HiFi	1.0 unit	0.1 µl
DNA Template	100 µg/ml	3.5 µl
	Reaction Volume	**25 µl**

PCR is a process of 3 specific steps; denaturation, annealing, and elongation. During the denaturation step, the double stranded DNA sample (or "template strand") is heated until it separates into single stranded constituents. Consequently, the single stranded template strands are cooled to the ideal "annealing" temperature where the forward and reverse primers attach to the template strands giving the DNA polymerase a place to begin DNA synthesis. Starting at the

primers, the DNA polymerase begins adding complimentary nucleotides to the single stranded template strands in a process known as "elongation". The polymerase adds nucleotides until the temperature is raised or lowered again. The process of PCR repeats the three steps for a specific number of cycles, exponentially increasing the amount of DNA from the specific primer set, until the entire reaction is halted by a cooling step. Once halted, the amplified DNA is available for further experimentation or appropriate storage in a cold freezer.

As mentioned the amount of template strands after the first cycle will ideally double. During the second cycle, half of the templates will be from the newly synthesized strands of the first cycle and hence will only be fragments of the DNA genome. Therefore, half of the second cycle products will only be as long as these new products. As the cycles repeat, the majority of the products will only be as long as the newly synthesized strands, a fixed length product, as the elongation processes cannot exceed the area where the second primer annealed. This way exponential amplification takes place between the two primer sequences, which will yield high concentrations of double-stranded DNA of the same identical length. The parameters of temperature and duration are controlled by thermocyclers. The specific sequence primers; and stringent parameters of temperature, number of cycles, and length of each cycle, are absolutely essential for successful and optimal amplification. All thermocycler procedures were performed with a *Bio Rad T100™ Thermal Cycler*.

Thermocycle parameters are found on Table 3. Each parameter set is followed by a final elongation step and then placed on infinite hold at 4°C.

Table 3: Thermocycle parameters for each gene.

Gene	Initial temperature step (ºC) and time (seconds). Not repeated	Denature temperature (ºC) and time (seconds).	Annealing temperature (ºC) and time (seconds).	Elongation temperature (ºC) and time (seconds).	Number of cycle repeats (cycle consists of – Denature, Annealing, Elongation)	Final elongation temperature (ºC) and time (seconds) before 4ºC infinite hold
IL-17A	96, 90	96, 15	58, 30	72, 45	35	72, 180
IL-17RA	95, 120	95, 15	59.6, 30	72, 45	36	72, 300
IL-23R	94, 300	94, 30	63.6, 45	72, 55	36	72, 600
IFN-γR	95, 180	95, 20	68, 50	72, 50	31	72, 300
$P2RX_7$	94, 300	94, 30	54, 30	72, 45	30	72, 300

3.5 Restriction Fragment Length Polymorphisms using Restriction Enzymes

Restriction Fragment Length Polymorphisms (RFLP) analysis is a molecular method of genetic analysis allowing for the identification of unique patterns in genetic material in specific DNA regions. This technique takes advantage of polymorphisms in individual genetic codes to identify similarities and differences. Even though individuals of a species share essentially the same genetic material and make up, these slight differences account for individual variations in phenotype.

Cutting a DNA region with known variability is the bases of this molecular methodology. Restriction enzymes are used to "cut" the DNA. Restriction enzymes are isolated proteins that recognize specific sequences of DNA and break apart, or "cut", the DNA at these designated sites. However, if there is a polymorphism in the site inhibiting the cutting of the DNA than the end products will be the same length of the original fragment.

Once the procedure of cutting is complete, the DNA fragments are then separated by agarose gel electrophoresis to determine the relative number of fragments and fragment size per sample or participant. Each gene has its own length of DNA fragment and individual coding sequence; therefore, a different restriction enzyme is used for every gene within the study. Each gene with corresponding restriction enzyme and allele base pair length is presented in Table 4.

Table 4: Individual restriction enzyme used and cut sites for both alleles of each gene.

Gene	**Restriction Enzyme**	**Cut sequence for restriction enzymes (^ represents cut sites)**	**Alleles (base pair length)**
IL-17A	EcoNI	5'- CCTNN ^ NNNAGG-3' 3'-GGANNN ^ NNTCC-5'	G (34, 68) A (102)
IL-17RA	AciI	5'-C ^ CGC-3' 3'-GGC ^ G-5'	A (2, 52, 107) G (2, 44, 52, 63)
IL-23R	MnlI	5'-CCTC$(N)_7$ ^ -3' 3'-GGAG$(N)_6$ ^ -5'	C (82, 70) A (152)
IFN-γR	AfeI	5'-AGC ^ GCT-3' 3'-TGC ^ CGA-5'	T (419) C (115, 304)
$P2RX_7$	HaeII	5'-RGCGC ^ Y-3' 3'-Y ^ CGCGR-5' R= A or G Y= C or T	A (317) C (118, 199)

The cut sequences for the restriction enzymes were determined by NEB.com (New England BioLabs)

3.5.1 Gel electrophoresis

Gel electrophoresis is a quick, uncomplicated, and sensitive analytical tool for separating nucleic acids or proteins. Electrophoresis is the use of an electric field to transport charged molecules through a solvent. There are two types of separating matrixes (gels) that may be used; either agarose or polyacrylamide. Agarose is more economically feasible and used for larger fragments requiring less resolution whereas polyacrylamide gels are more sensitive to small

bands requiring higher resolution. With the fragment sizes that this research was investigating (larger than 50 base pairs), agarose was the gel composition chosen.

The intercalating agent, ethidium bromide, is used in the agarose gel electrophoresis as a staining agent. When exposed to ultraviolet, ethidium bromide will fluoresce with an orange colour at an almost 20-fold intensity increase.

The gel composition, voltage, and time for both PCR and RFLP procedures are listed in table 5.

Table 5: Gel composition, voltage, and length of run for PCR and RFLP procedures.

Gene	**PCR agarose gel composition (%) voltage (V), and length of procedure (minutes)**	**RFLP agarose gel composition (%) voltage (V), and length of procedure (minutes)**
IL-17A	2.5, 120, 30	3.0, 120, 27
IL-17RA	2.5, 120, 25	3.3 100, 60
IL-23R	2.5, 120, 25	3.0, 120, 27
IFN-γR	2.5, 120, 20	3.0, 120, 25
$P2RX_7$	2.5, 120, 30	3.0, 120, 30

3.6 SNP Identification

The combination of the two nucleotides or alleles in an individual gene is what constitutes the genotype. Genes can be presented as either a heterozygous pair, with both alleles represented, or a homozygous pair, where both alleles are the same. Homozygous pairs are also

broken down further into homozygous wild type or mutant pairs. The wild type allele is the allele coding for the wild type phenotypic trait, and the mutant allele codes for the mutant phenotypic trait. An example of this was seen in Chapter 1 section 1.7. Figure 2, with B representing the wild type allele and A representing the mutant allele. The genotypes for each of the individual genes are reported in Table 6.

Table 6. Wildtype and mutant alleles for each SNP.

Gene	**Wild Type Allele**	**Mutant Allele**
IL-17A rs2275913	G	A
IL-17RA rs4819554	A	G
IL-23R rs10889677	C	A
IFN-γR rs2234711	T	C
$P2RX_7$ rs3751143	A	C

Each of the observed gene polymorphisms were detected and distinguished via the allele counts, which represents the two nucleotides that could be detected at the specific SNP site. The total number of alleles observed, two per participant, is represented in the allele count under each cohort (Table 8). For IL-17A rs2275913 analysis, the frequency of G and A alleles were determined with the wild type allele represented as G (Ocejo-Vinyals 2013). IL-17RA SNP rs4819554 was also represented by A and G alleles with the A allele being wild type (Coto 2015). IL-23R SNP rs10889677 had C as its wild type allele and A as the mutant allele (Zheng 2012). The $P2RX_7$ SNP rs3751143 is represented by the wild type allele A and the mutant allele C (Niño- Moreno 2007). IFN-γR had a wild type allele T and mutant allele C in the SNP rs2234711 (Xiang 2014).

3.7 RFLP Analysis

RFLP analysis was performed to determine the genotype of each gene. An example of an RFLP for all SNPs are found in Figures 5-9. Once RFLP analysis and gel electrophoresis was completed the observed alleles were recorded according to the genotypes displayed. Allelic and genotypic frequencies were determined by direct counting of alleles for each of the study cohorts.

Figure 5. RFLP results on 3% Agarose gel for IL-17A. Ladder (100 base pair ladder), G/G genotype (samples 3, 5, 8-10), G/A (samples 4), and A/A genotype (samples 1, 2, 6, 7).

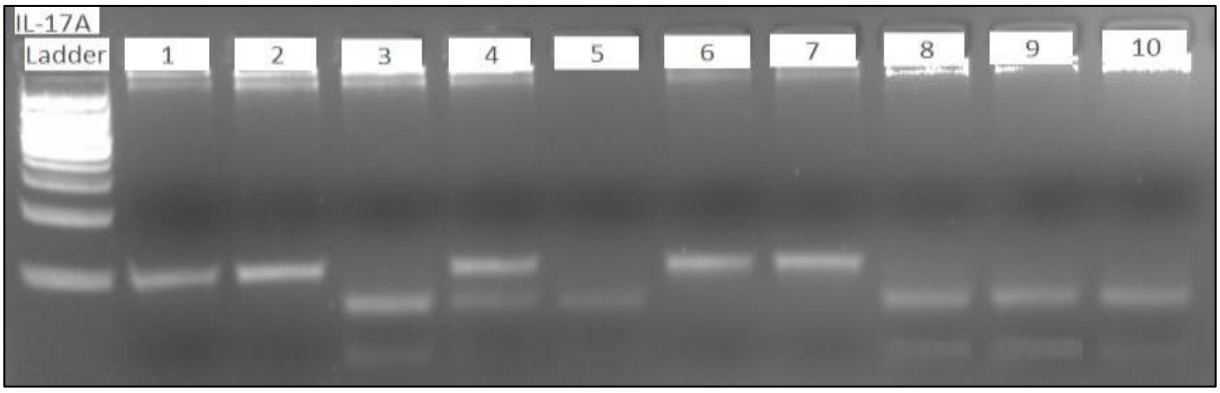


Figure 6. RFLP results on 3% Agarose gel for IL-17RA. Ladder (100 base pair ladder), A/A genotype (samples 4, 5, 8. 9), A/G (samples 2 and 10), and G/G genotype (samples 1, 3, 6, 7).

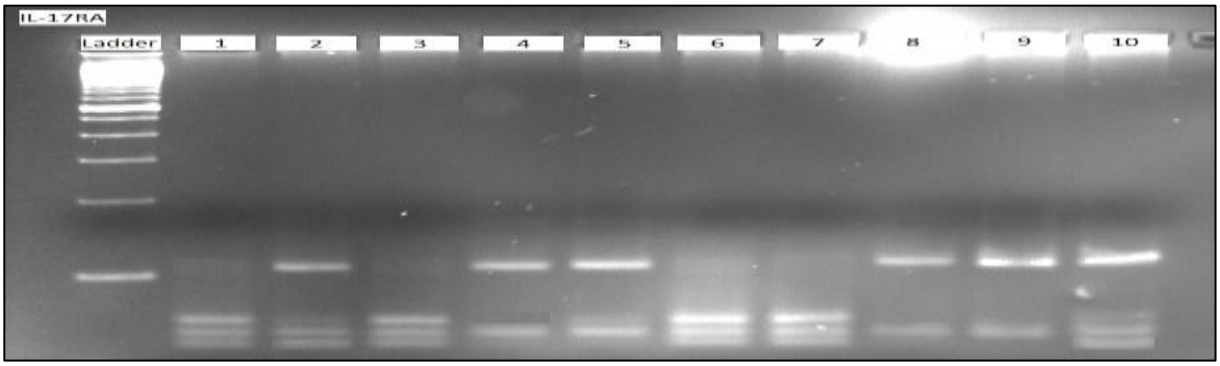


Figure 7. RFLP results on 3% Agarose gel for IL-23R. Ladder (100 base pair ladder), C/C genotype (samples 2, 4, 5, 8, 9), C/A (samples 1, 6, 7, 10), and A/A genotype (sample 3).

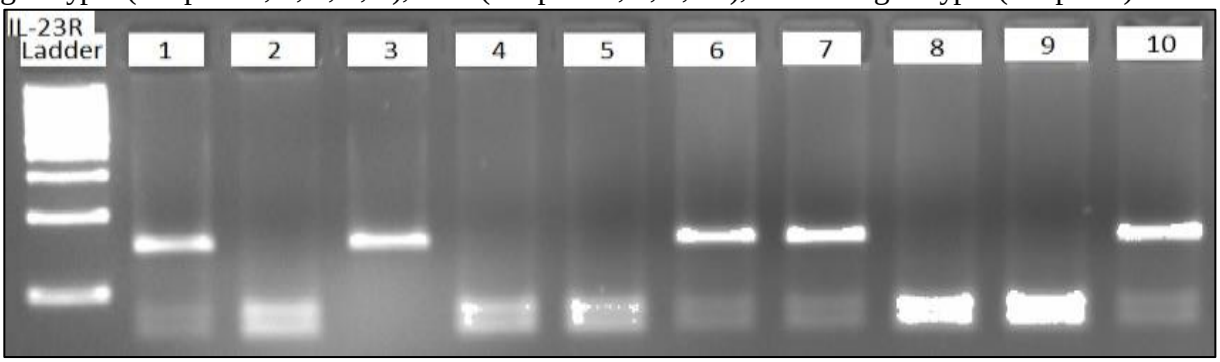


Figure 8. RFLP results on 3% Agarose gel for IFN-γR. Ladder is 100 base pair ladder, T/T genotype (samples 1-3, 6), T/C (samples 5, 7, 9-10), and C/C genotype (samples 4 and 8).

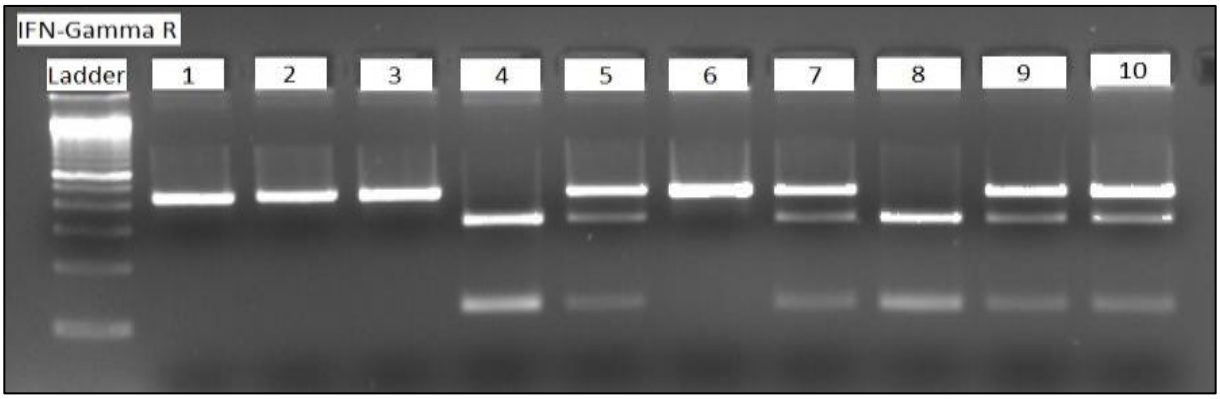
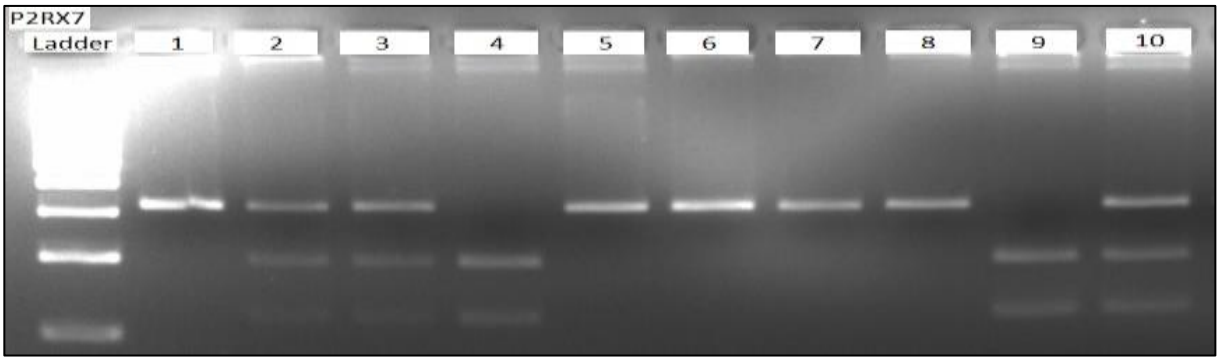


Figure 9. RFLP results on 3% Agarose gel for P2RX$_7$. Ladder (100 base pair ladder), A/A genotype (samples 1, 5-8), A/C (samples 2, 3, 10), and C/C genotype (samples 4 and 9).



3.8 Statistical Analysis

To determine if any of the observed SNP frequency variances were significantly associated with different populations statistical analysis was conducted. First the populations were assessed via a Pearson's chi-square test or a Fisher exact test to determine if the populations were in Hardy-Weinberg Equilibrium (HWE). HWE is a principle stating that genetic variation will remain constant for populations from generation to generation in the absence of selective pressures (Hendrick 2011). The principle theorizes that the frequencies of both genotype and alleles will remain constant in a large random mating population as long as no extrinsic influences disrupt the population. Disruptive influences can include things such as genetic drift, natural selection, genetic flow, mate choice, or mutations. Typically, one or more of the possible disruptive influences occurs within a real population. As such, HWE conditions are hypothetical conditions are unlikely to be observed in human populations. A Pearson chi squared test was used to test for deviation from the HWE For samples with N of 5 or greater in each of the observed genotypic frequencies to decide whether the observed frequencies were sufficiently close to the expected HWE proportions (Hendrick 2011). For samples with genotypes of N under 5 a Fisher exact test was used as this test if more adept when the sample size is of a smaller degree (Hendrick 2011). A p-value of ≤ 0.05 was required to reject the null hypothesis of HWE.

The allele and genotype frequencies for each population were compared using the Pearson chi squared tests to determine if they were significantly different from one another. A p-value of ≤ 0.0045 for population comparisons was required to reject null hypothesis after correcting for multiple populations using Bonferroni correction. Each will be stated with the appropriate tests in Chapter 3.

3.9 Knowledge Translation Methods

It is unlikely that the creation of new knowledge in itself will lead to the improvement of population health for those involved in the knowledge creation. The improvement of health of populations requires the development of actions to translate the knowledge into practical application (McDonald 2016). Knowledge translation in this research was accomplished by general explanation and a hands-on laboratory DNA extraction tutorial, the creation of a one-page infographic to be dispersed within the community at community health centers, and the development and presentation of academic posters for community members.

Typically, knowledge translation of research is facilitated through conferences, publications, and academic networks. An important feature of knowledge translation is the acknowledgment of the study participants and the researcher as equals. As such, the three knowledge translation practices used in this research will be represented in the first person perspective instead of the traditional third person perspective found in academia.

An important focus of my research project was to investigate and understand how health information related to genetics can be effectively communicated to the research partners. During the research project a group of ten young adults and two Elders visited Dr. Larcombe's research laboratory to learn about health research at the University. Five of the young adults and one Elder were from the northern remote Dene community of Lac Brochet, Manitoba and five other students and one Elder were from Tadoule Lake, Manitoba. During their visit they were given a guided tour of the laboratory and participated in a DNA extraction tutorial I facilitated. The tutorial (Figure 10), along with an explanation of the research done for this project in Dr. Larcombe's lab, was used to increase the understanding of genetics and to develop an interest in the fields of science, medicine, and research (Figures 21-24).

I designed a one-page infographic to distribute to the communities involved at their local health centres (Figure 25). The infographic has been developed with community capacity in mind, explaining the different influences on susceptibility to disease such as TB and how this research may make an impact for community members.

Throughout my research project, I have composed posters specifically to present my research and to make it accessible to non-academic audiences. One poster was presented at the 2015 *Canadian Student Health Research Forum* in Winnipeg, Manitoba and the Canadian Society for Immunology Annual Meeting in Winnipeg, Manitoba in 2015 (Figure 26). With this poster I explained the connection between gene polymorphisms and their potential impact on tuberculosis, this poster was a brief introduction to this research study and introduced the first three genes and early results. Another poster I presented was at the *3rd Annual Indigenous Health Symposium* by myself and at the *Northern Manitoba Research Network* held at the University College of the North in Thompson, Manitoba by a representative from the Assembly of Manitoba Chiefs (Figure 27). This poster was created with community capacity in mind, describing both the importance and the relevance of the research to the communities involved. A final poster I created was for the 2016 *Canadian Student Health Research Forum* in Winnipeg (Figure 28). This poster was designed to display the results and discuss the conclusions of this study.

Figure 10: DNA extraction tutorial for visiting Dene students.

DNA Extraction tutorial

Introduction: You have heard about DNA in popular movies like Jurassic World or Captain America. How it makes all living things and how crazy scientists can alter it to make dinosaurs and super soldiers. In real life, working with DNA is a bit more complex than the movies make it, but it's not so complex that you can't work with it yourself! Today we will be extracting DNA from split peas to prove it does really exist! (Unfortunately you will not be able to grow a dinosaur with it, I have tried…)

Background: Deoxyribonucleic Acid (DNA) is what makes you you! If DNA was a language, the words would be known as Genes. Most people's "gene words" are spelled very similarly, however, small changes in these words are what makes us unique. These changes are what decide things like hair and eye colour.

All living things have DNA and we will be examining split peas to prove it!

Tools:

1. 1/2 cup of split peas (100ml)
2. 1/8 teaspoon table salt (less than 1ml)
3. 1 cup cold water (200ml)
4. 2 tablespoons liquid detergent (30 ml)
5. Test tubes
6. Enzymes!...wait, what are enzymes??? (See title below "Enzymes!")
7. Denaturing buffer
8. One strainer
9. One bowl/measuring cup
10. A pencil

Procedure:

- Blend a 1/2 cup of split peas together with 1 cup of cold water and the salt until smooth.
- Pour your thin pea-cell soup through a strainer into another container (like a bowl or measuring cup).
- Add liquid detergent…why am I washing my pea soup? See title below "why am I washing my pea soup?"
- Let the mixture sit for 5-10 minutes.
- Pour the mixture into test tubes until each is about 1/3 full.
- Add a pinch of enzymes to each test tube and stir gently. Be careful! If you stir too hard, you'll break up the DNA, making it harder to see.
- Tilt your test tube and slowly pour denaturing buffer into the tube down the side so that it forms a layer on top of the pea mixture. Pour until you have about the same amount of alcohol in the tube as pea mixture.
- Denaturing buffer is less dense than water, so it floats on top. Look for clumps of white stringy stuff where the water and buffer layers meet.

Conclusion: DNA is a long, stringy molecule. The salt that you added in step one helps it stick together. So what you see are clumps of tangled DNA molecules!

DNA normally stays dissolved in water, but when salty DNA comes in contact with denaturing buffer it becomes undissolved. This is called precipitation. The physical force of the DNA clumping together as it precipitates pulls more strands along with it as it rises into the buffer.

You can use a pencil or wooden stir stick to collect the DNA. If you want to save your DNA, you can transfer it to a small container filled with alcohol. (But I am serious, you cannot grow a dinosaur with it!)

Help!??!:

Why am I washing my pea soup? - Blending separated the pea cells. But each cell is surrounded by a sack (the cell membrane). DNA is found inside a second sack (the nucleus) within each cell. To see the DNA, we have to break open these two sacks.

So how does detergent break the sac? The sacks are made of fat, they are fat sacks. The soap attaches to the fat sacks and washes them away leaving the clean DNA!

Enzymes! - Enzymes are the proteins in your body that help make things happen! They have a variety of different jobs, but in this case they act like DNA scissors! The DNA in the nucleus of the cell is molded, folded, and protected by proteins. These enzymes cut the DNA away from the protection proteins.

4.0 Allelic, Genotypic, and Knowledge Translation Results

4.1 Results

Genetic diversity is of the utmost importance within the immune system. This diversity is responsible for the high degree of variation of the T and B cell receptors of the adaptive immune response, ensuring that an individual's host immune reaction is incredibly adept at fending off a large spectrum of invading pathogens. However, even with this substantial plasticity the immune system is only as capable of protecting the host as the weakest arm of the system allows. Polymorphisms which affect gene functionality can cause increased or decreased disease susceptibility. The Th17 arm of the adaptive immune response is a key member of the immune system and SNPs in genes of this response may cause a decrease in the ability to protect the host. Cytokines are involved in regulation, development, and behavioural aspects of the immune response and are needed to amplify and direct particular immunological pathways. Receptors on the other hand, assist in downstream signaling effects and communicate to cells the appropriate response in regards to the environment around the cell. SNPs have been shown to be associated with differential susceptibility or resistance to diseases, as well as increased or decreased severity of disease outcomes. These deviations in disease outcomes in relation to SNPs are due to altered gene expression and functionality and their downstream immune consequences (Rodriguez 2015, Ocejo-Vinyals 2013, Arisawa 2006, Zheng 2012, Chen 2009, Cooke 2006, Niño- Moreno 2007, Wu 2014). This chapter will describe the frequency of SNPs detected in IL-17A, IL-17RA, IL-23R, IFN-γR, and the $P2RX_7$ gene as well as the relative phenotypic expression for each of these genes and the genotype frequencies expression in three distinct First Nations groups and a non-

Indigenous Canadian population. The statistical analysis related to any relationship between ethnicity and SNP frequency will also be completed.

4.2 Genotype frequencies for the study cohorts and assessing Hardy-Weinberg Equilibrium

Table 7 displays the genotype frequencies for the non-Indigenous, Dene 1, Dene 2, Cree and Saulteaux study cohorts for IL-17A, IL-17RA, IL-23R, IFN-γR and $P2RX_7$ results. To assess whether or not the alleles are in Hardy-Weinberg Equilibrium, the Chi-square or Fisher-Exact test were used for each study population and the results are included in Table 7. The study cohorts were assessed for their compliance to HWE. Pearson chi-squared was used for populations with absolute observed or expected genotypic values of 5 or more for each genotype. If the values were less than 5, than a Fisher exact test was conducted.

Distinguishing that the cohorts were in HWE was the basis of the null hypothesis. If the Pearson chi-squared or Fisher exact p-value was less than or equal to 0.05 than the null hypothesis was rejected.

Table 7. Hardy-Weinberg Equilibrium table as conducted by Pearson Chi Square Test or Fisher Exact Test and the accompanying P-Values for IL-17A, IL-17RA, IL-23R, IFN-γR, and $P2RX_7$. * = Fisher exact test. $P \leq 0.05$ is significant and indicated in bold type.

IL-17A (rs2275913)	Genotype			Hardy-Weinberg Equilibrium	
Population	GG N (%)	GA N (%)	AA N (%)	χ^2	p-Value
Non-Indigenous	23 (23)	49 (50)	27 (27)	0.0071	0.9327
Dene 1	23 (33)	42 (61)	4 (6)	3.7600	0.1526
Dene 2	31 (60)	15 (29)	6 (12)	3.2406	0.0718
Cree	26 (57)	15 (33)	5 (11)	1.4268	0.2323
Saulteaux	32 (57)	17 (30)	7 (13)	3.2724	0.0705
IL-17RA (rs4819554)	Genotype			Hardy-Weinberg Equilibrium	
Population	AA N (%)	AG N (%)	GG N (%)	χ^2	p-Value
Non-Indigenous	65 (66)	32 (32)	2 (2)	*	0.8854
Dene 1	20 (29)	42 (61)	7 (10)	4.5960	**0.0320**
Dene 2	21 (39)	30 (56)	3 (6)	*	0.4161
Cree	8 (17)	27 (59)	11 (24)	1.4727	0.2249
Saulteaux	26 (47)	25 (45)	4 (7)	*	0.8812
IL-23R (rs10889677)	Genotype			Hardy-Weinberg Equilibrium	
Population	CC N (%)	CA N (%)	AA N (%)	χ^2	p-Value
Non-Indigenous	40 (40)	47 (48)	12 (12)	0.1017	0.7498
Dene 1	48 (70)	18 (26)	3 (4)	*	0.8580
Dene 2	34 (63)	16 (30)	4 (7)	*	0.8192
Cree	26 (56)	16 (35)	4 (9)	*	0.9014
Saulteaux	38 (68)	18 (32)	0 (0)	*	0.6788
IFN-γR (rs2234711)	Genotype			Hardy-Weinberg Equilibrium	
Population	TT N (%)	TC N (%)	CC N (%)	χ^2	p-Value
Non-Indigenous	41 (42)	41 (42)	16 (16)	1.0809	0.2985
Dene 1	34 (49)	26 (38)	9 (13)	1.2857	0.2568
Dene 2	24 (44)	21 (39)	9 (17)	1.3343	0.2480
Cree	31 (67)	12 (26)	3 (7)	*	0.7739
Saulteaux	32 (59)	20 (37)	2 (4)	*	1.0000

$P2RX_7$ (rs3751143)	Genotype			Hardy-Weinberg Equilibrium	
Population	AA N (%)	AC N (%)	CC N (%)	χ^2	p-Value
Non-Indigenous	71 (72)	22 (22)	6 (6)	4.7392	**0.0295**
Dene 1	22 (32)	14 (20)	33 (48)	28.4970	**0.0000**
Dene 2	21 (41)	13 (25)	17 (33)	12.0976	**0.0005**
Cree	35 (76)	9 (20)	2 (4)	*	0.7798
Saulteaux	33 (60)	18 (33)	4 (7)	*	0.8663

Evaluating the P-value ≤ 0.05 for statistical significance, the appropriate test accessing for HWE suggest that all but four alleles were in HWE. The four of which fail to meet HWE parameters are the IL-17RA (rs4819554) Dene 1 cohorts with a $p = 0.0320$, and then three cohorts in the $P2RX_7$ (rs3751143) SNP; Dene 1, Dene 2, and non-Indigenous all failed to meet the P-value parameters ($p=0.0295$, $p\leq 0.05$, $p\leq 0.05$).

The Dene 1 and Dene 2 community samples demonstrated a significant difference in observed genotype frequency for the $P2RX_7$ (rs3751143) SNP as compared to the other populations and as such failed to meet a p-value greater than 0.05. IL-17RA (rs4819554) Dene 1 also failed to be in HWE and this could be due to a selective pressures, such as a lower population density. The failure for these populations to reject the null hypothesis suggests that these alleles may not be in HWE.

Comparisons between individual communities were done for both allelic and genotypic frequencies. Either the Pearsons chi-square or Fisher exact test were used to evaluate the null hypothesis (Appendix Table A1-A2). The null hypothesis for the allelic and genotypic comparisons was that the groups were dependent and homogenous. To determine the appropriate p-value appropriate for the null hypothesis Bonferroni correction for multiple comparisons was

used ($p \leq 0.0045$). It was hypothesized that the First Nations cohorts would exhibit a different frequency of SNPs as compared to each other and the non-Indigenous Canadian cohort.

Table 8. Total allele frequency counts and percentages for First Nations and Non-Indigenous study cohorts

Gene	Study Cohort	Wild Type Allele N (%)	Mutant Allele N (%)
IL-17A		**G**	**A**
	First Nations Group	313 (70)	133 (30)
	Non-Indigenous	95 (48)	103 (52)
	Dene 1	78 (62)	48 (38)
	Dene 2	77 (74)	27 (26)
	Cree	67 (73)	25 (27)
	Saulteaux	81 (72)	31 (28)
IL-17RA		**A**	**G**
	First Nations Group	274 (61)	174 (39)
	Non-Indigenous	162 (82)	36 (18)
	Dene 1	73 (58)	53 (42)
	Dene 2	72 (67)	36 (33)
	Cree	43 (47)	49 (53)
	Saulteaux	77 (70)	33 (30)
IL-23R		**C**	**A**
	First Nations Group	360 (80)	90 (20)
	Non-Indigenous	127 (64)	71 (36)
	Dene 1	105 (83)	21 (17)
	Dene 2	84 (78)	24 (22)
	Cree	68 (74)	24 (26)
	Saulteaux	94 (84)	18 (16)
IFN-γR		**T**	**C**
	First Nations Group	321 (72)	125 (28)
	Non-Indigenous	123 (63)	73 (37)
	Dene 1	84 (67)	42 (33)
	Dene 2	69 (64)	39 (36)
	Cree	74 (80)	18 (20)
	Saulteaux	84 (78)	24 (22)
P2RX7		**A**	**C**
	First Nations Group	276 (62)	166 (38)
	Non-Indigenous	164 (83)	34 (17)
	Dene 1	52 (41)	74 (59)
	Dene 2	55 (54)	47 (46)
	Cree	79 (86)	13 (14)
	Saulteaux	84 (76)	26 (24)

4.2.1 IL-17A rs2275913 Allele and Genotype Frequencies

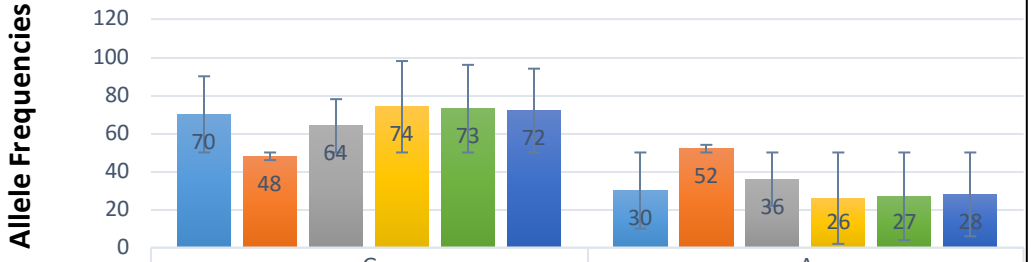
The frequencies of the A and G alleles of this IL-17A SNP show that the non-Indigenous cohort maintains an almost equal distribution of both alleles (48%, 52%). However, all of the individual First Nations groups, as well as the total of the First Nations groups (Indigenous), display a higher percentage of the G allele (Indigenous 70%, Dene 1 64%, Dene 2 74%, Cree 72%, Saulteaux 73%) (Figure 11). The Indigenous, Dene 2, Cree and Saulteaux groups have almost identical percentages of G allele that are also the highest of the groups under study. The Dene 1 frequencies are closer to the other First Nations groups than the non-Indigenous group.

For IL-17A, the only groups which reject the null hypothesis p-value of ≤ 0.0045 was the non-Indigenous group compared to all other groups. This defines the non-Indigenous group as independent and heterogeneous from the other groups. The allele frequencies of all of the First Nation cohorts were not significantly different. The genotype frequencies observed for IL-17A among the six groups displayed high expression frequencies of the wild type G/G genotype in the Indigenous (50%) and in three First Nations communities; Dene 2 (60%), Cree (57%), and Saulteaux (57%) and lower expression frequency of the same genotype in the non-Indigenous (23%) and Dene 1 (33%) cohort (Figure 12). Indigenous, Dene 2, Cree, and Saulteaux have similar trends in that they had the highest expression rates of the wild type genotype with a decreasing trend in the heterozygous (40%, 29%, 33%, 30%) and mutant (10%, 11%, 11%, 13%) expression. The non-Indigenous (50%) and Dene 1 (60%) had the highest frequency of the heterozygous allele, however they had different frequency trends. Dene 1 had the trend of heterozygous (61%), homozygous wild type (33%), homozygous mutant (6%) for their high to lower frequency rates. Non-Indigenous had the similar amounts of both homozygous genotypes (23% wild type, 27% mutant). Dene 1 had the lowest frequency of homozygous mutants and the

non-Indigenous had the lowest rates of homozygous wild type and highest rates of homozygous mutants.

IL-17A rs2275913 genotype frequencies had significant variance against the null hypothesis. Three of the First Nations cohorts were homogenous, as the Dene 2, Saulteaux, and Cree were all close to a p-value of 1.0 when compared to each other. The non-Indigenous and Dene 1 rejected the null hypothesis and are considered independent and heterogeneous from all of the other populations, except for Dene 1 when compared to Cree. The non-Indigenous cohort rejected the null hypothesis when compared to the Indigenous as well.

Figure 11. IL-17A Allelic frequencies as percentages and p-values for First Nations and non- Indigenous cohorts

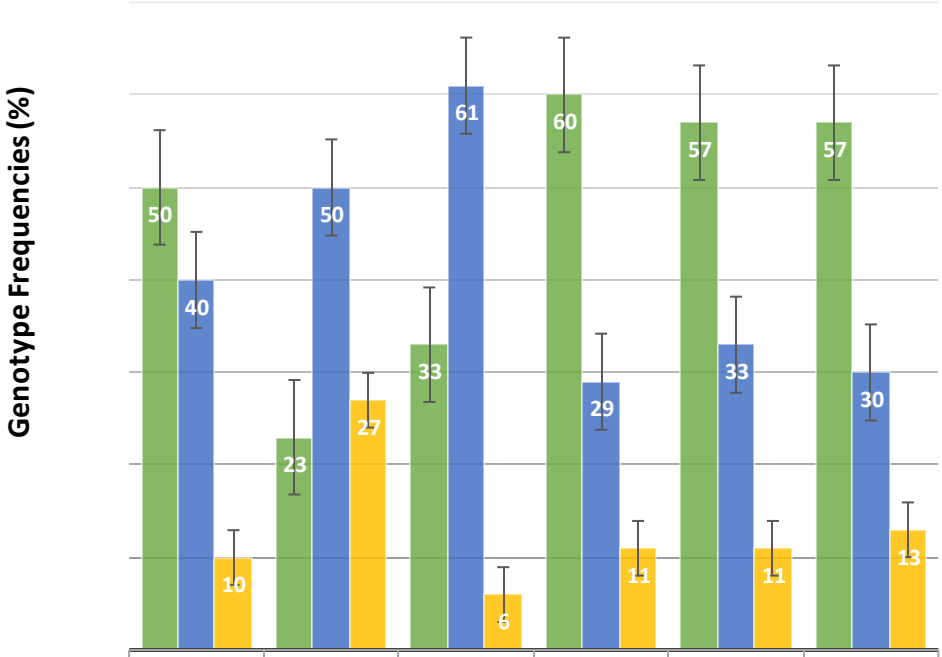


	G	A
Indigenous N=224	70	30
Non-Indigenous N=99	48	52
Dene 1 N=69	64	36
Dene 2 N=52	74	26
Cree N=46	73	27
Saulteaux N=56	72	28

IL-17A	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
Indigenous		**0.0000**				
Non-Indigenous			**0.0043**	**0.0000**	**0.0001**	**0.0000**
Dene 1				0.0895	0.1511	0.1507
Dene 2					0.8478	0.7760
Cree						0.9359
Saulteaux						

p-values calculated using Pearson chi square/Fisher exact tests (* = Fisher exact test) and corrected for multiple populations using Bonferroni correction. $p \leq 0.0045$ is significant and indicated in bold type (Standard Error (SE) bars included).

Figure 12. IL-17A Genotype frequencies as percentages and p-values for First Nations and non-Indigenous cohorts and non-Indigenous cohorts.



	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
G/G - Wild Type	50	23	33	60	57	57
G/A - Heterozygous	40	50	61	29	33	30
A/A - Mutant	10	27	6	11	11	13

IL-17A	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
Indigenous		**0.0000**				
Non-Indigenous			**0.0018**	**0.0000**	**0.0003**	**0.0001**
Dene 1				**0.0022**	0.0120	**0.0029**
Dene 2					0.9231	0.9656
Cree						0.9512
Saulteaux						

p-values calculated using Pearson chi square/Fisher exact tests (* = Fisher exact test) and corrected for multiple populations using Bonferroni correction. $p \leq 0.0045$ is significant and indicated in bold type (Standard Error (SE) bars included).

4.2.2 IL-17RA rs4819554 Allele and Genotype Frequencies

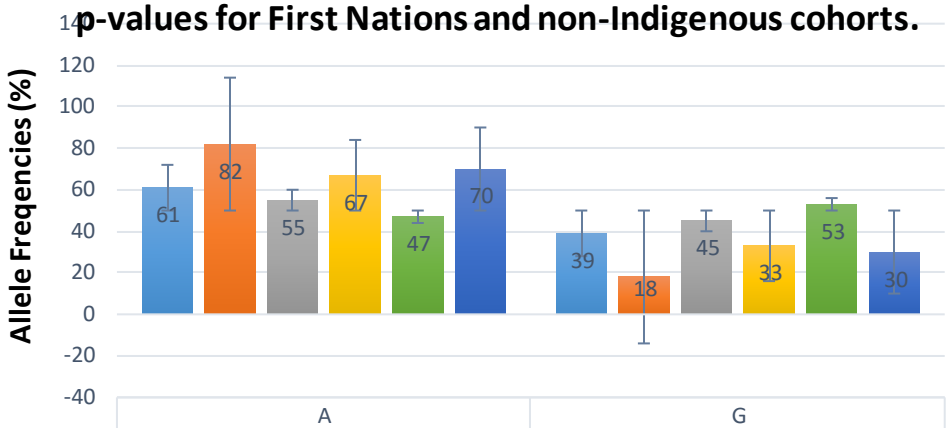
Previous research indicates that the A allele in the IL-17RA SNP produces more IL-17RA mRNA and the A allele may bind with higher affinity to TFAP-4 which would induce transcription of IL-17RA. The non-Indigenous (82%) group had the highest A allele percentage. The Dene 2 (67%) and Saulteaux (70%) had relatively close percentages to one another. The Cree (47%) population had a lower representation of A allele, relatively, to the other groups. The Indigenous group had an A allele frequency in the middle of the First Nations groups (61%) (Figure 13).

The non-Indigenous group is statistically independent and heterogeneous from the Indigenous group, both Dene communities, and the Cree community. The Cree are heterogeneous from not only the non-Indigenous group, but from the Dene 2 and Saulteaux communities as well.

Non-Indigenous had the highest frequency of wild type A/A genotype (66%) with a decreasing trend to heterozygous (32%) and then mutant (2%) genotype expression. Indigenous, Dene 1, and Dene 2 had a trend of highest to lowest genotype frequencies starting with heterozygous (55%, 61%, 56%), wild type (34%, 29%, 39%), and mutant genotype (11%, 10%, 5%). Cree displayed similar frequencies for both homozygous genotypes, wild type (17%) and mutant (24%), and a high rate in heterozygous genotypes (59%). Saulteaux had similar frequencies for both wild type (47%) and heterozygous (46%) genotypes, and low levels of mutant type (7%). The similarities between the groups was a display of low mutant genotypic rates in all groups except the Cree; as well as similar rates of heterozygous individuals in Indigenous, Dene 1, Dene 2, and Cree populations (Figure 14).

The non-Indigenous group is statistically different from every population, except Saulteaux, for IL-17RA rs4819554. The Cree cohort rejected the null hypothesis in reference to Saulteaux and are thus heterogeneous from one another. The Dene 2: Cree p-value was only slightly over the significance cut off (P = 0.0068).

Figure 13. IL-17RA Allelic frequencies as percentages and p-values for First Nations and non-Indigenous cohorts.



	A	G
Indigenous N=224	61	39
Non-Indigenous N=99	82	18
Dene 1 N=69	55	45
Dene 2 N=54	67	33
Cree N=46	47	53
Saulteaux N=56	70	30

IL-17RA	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
Indigenous		**0.0000**				
Non-Indigenous			**0.0000**	**0.0028**	**0.0000**	0.0171
Dene 1				0.2437	0.0586	0.0844
Dene 2					**0.0045**	0.5968
Cree						**0.0008**
Saulteaux						

p-values calculated using Pearson chi square/Fisher exact tests (* = Fisher exact test) and corrected for multiple populations using Bonferroni correction. $p \leq 0.0045$ is significant and indicated in bold type (Standard Error (SE) bars included).

Figure 14. IL-17RA Genotype frequencies as percentages and p-values for First Nations and non-Indigenous cohorts.

	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
A/A - Wild Type	34	66	29	39	17	47
A/G - Heterozygous	55	32	61	56	59	46
G/G - Mutant	11	2	10	5	24	7

IL-17AR	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
Indigenous		**0.0001**				
Non-Indigenous			**0.0008**	* **0.0031**	**0.00003**	* 0.0415
Dene 1				0.4025	0.0867	0.1114
Dene 2					0.0068	* 0.6482
Cree						**0.0023**
Saulteaux						

p-values calculated using Pearson chi square/Fisher exact tests (* = Fisher exact test) and corrected for multiple populations using Bonferroni correction. $p \leq 0.0045$ is significant and indicated in bold type (Standard Error (SE) bars included).

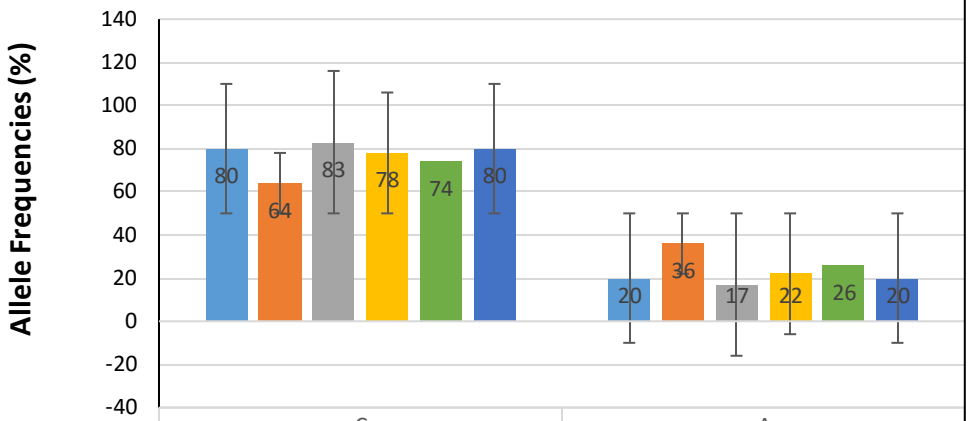
4.2.3 IL-23R rs10889677 Allele and Genotype Frequencies

SNPs for IL-23R displayed significant differences between the First Nations groups and the non-Indigenous group. All groups favoured the C allele, however the First Nations groups displayed a greater frequency (74-83%) of the C allele (Figure 15). The mutant A allele is the allele causing a functional change, as it disrupts the microRNA binding of miR-let-7f, this disruption promotes the transcription of the IL-23R gene (Zheng 2012). The A allele was more frequently identified in the non-Indigenous cohorts (36%) compared to the Indigenous groups (17-26%). IL-23R had allelic frequency differences between the non-Indigenous group as compared to the Indigenous, Dene 1, and Saulteaux and are considered homogenous with the Dene 2 and Cree.

All of the First Nations groups had similar genotypic frequency trends to one another for IL-23R (Figure 16). All First Nations groups (Indigenous, Dene 1, Dene 2, Cree, Saulteaux) displayed their highest frequency of genotype towards the wild type genotype C/C (65%, 70%, 63%, 56%, 68%), an intermediate expression of the heterozygotic genotype (30%, 26%, 30%, 35%, 32%), and a low expression of the mutant A/A genotype (5%, 4%, 7%, 9%, 0%). The non-Indigenous cohort had similar frequencies of wild type (40%) and heterozygous (48%) genotypes, while also displaying low frequencies of the mutant genotype (12%). All populations, including the non-Indigenous population, had low frequencies of the mutant genotype with the Saulteaux population not having a single mutant genotype individual in the study population.

IL-23R rs10889677 demonstrated that the non-Indigenous group was heterogeneous from Indigenous and Dene 1. All other groups are considered homogeneous.

Figure 15. IL-23R Allelic frequencies as percentages and p-values for First Nations and non-Indigenous cohorts.

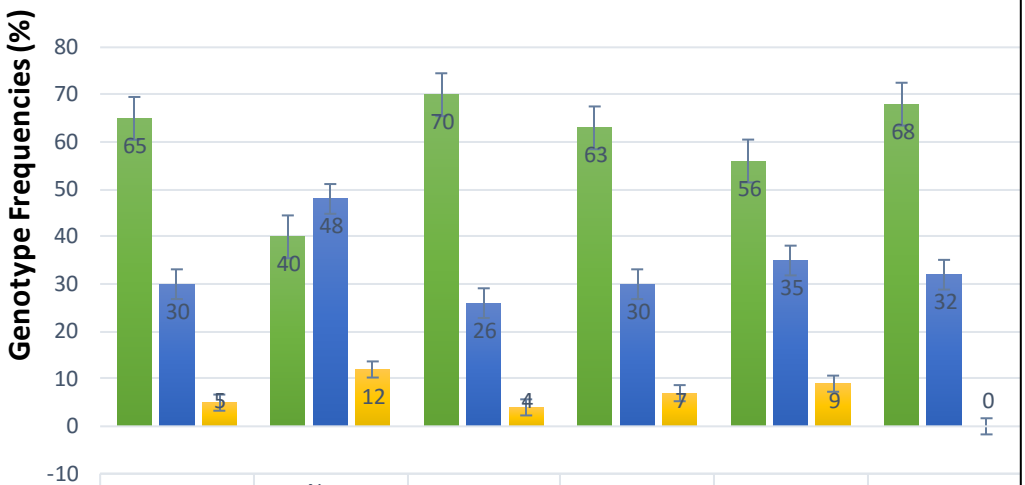


	C	A
Indigenous N=225	80	20
Non-Indigenous N=99	64	36
Dene 1 N=69	83	17
Dene 2 N=54	78	22
Cree N=46	74	26
Saulteaux N=56	80	20

IL-23R	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
Indigenous		**0.0000**				
Non-Indigenous			**0.0002**	0.0137	0.4025	**0.0002**
Dene 1				0.3427	0.1119	0.7815
Dene 2					0.5236	0.2459
Cree						0.0783
Saulteaux						

p-values calculated using Pearson chi square/Fisher exact tests (* = Fisher exact test) and corrected for multiple populations using Bonferroni correction.
$p \leq 0.0045$ is significant and indicated in bold type (Standard Error (SE) bars included).

Figure 16. IL-23R Genotype frequencies as percentages and p-values for First Nations and non-Indigenous cohorts.



	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
C/C - Wild Type	65	40	70	63	56	68
C/A - Heterozygous	30	48	26	30	35	32
A/A - Mutant	5	12	4	7	9	0

IL-23R	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
Indigenous		**0.0000**				
Non-Indigenous			**0.0018**	0.0284	0.1930	0.0448
Dene 1				* 0.6441	* 0.3012	* 0.3229
Dene 2					* 0.7798	* 0.1508
Cree						* 0.0703
Saulteaux						

p-values calculated using Pearson chi square/Fisher exact tests (* = Fisher exact test) and corrected for multiple populations using Bonferroni correction. $p \leq 0.0045$ is significant and indicated in bold type (Standard Error (SE) bars included).

4.2.4 IFN-γR rs2234711 Allele and Genotype Frequencies

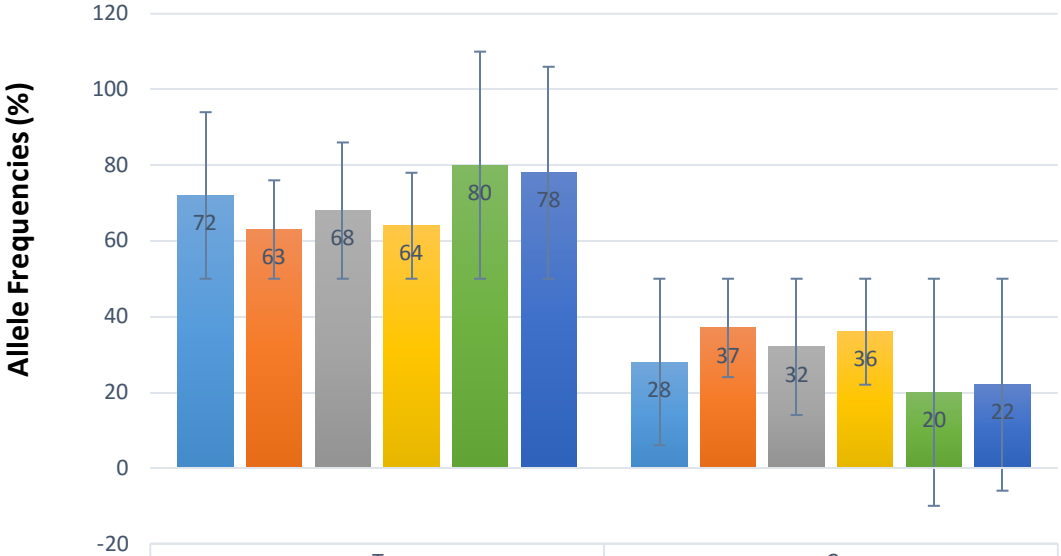
In functional studies of IFN-γR, the mutant allele C displays higher levels of Th1 cells than Th17 (Xiang 2014). It is suggested, though not confirmed, that the C allele lies within what may be the TFAP4 binding site (He 2010). The wild type T allele may have lower binding affinity that may be associated with susceptibility to some infectious diseases (He 2010). All populations favoured the T allele, however the Cree (80%) and Saulteaux (78%) have a greater representation of T alleles and the Non-Indigenous (63%) and Dene 2 (64%) had the lowest frequencies of T allele (Figure 17).

Once corrected for with Bonferroni, only the Cree cohort displayed noticeable frequency differences when compared to the non-Indigenous group for the IFN-γR SNP.

All of the First Nations groups (Indigenous, Dene 1, Dene 2, Cree, Saulteaux) had the same trend for the IFN-γR SNP genotypes (Figure 18). They all had the wild type genotype (54%, 49%, 44%, 67%, 59%) as the genotype with the highest frequency, which then trended downwards to heterozygous (36%, 38%, 39%, 26%, 37%) and finally mutant genotype (10%, 13%, 17%, 7%, 4%). Non-Indigenous also had the mutant genotype (18%) as their lowest frequency genotype. However, the highest frequency for the non-Indigenous group was the heterozygous genotype (43%), which was also the highest heterozygous frequency of all the populations. Though the two Dene groups had the wild type genotype as their dominant genotype they had similarly high levels of the heterozygous genotype. This is contrasting compared to the Indigenous, Cree, and Saulteaux, all of whom had a significant gap in frequency from the homozygous wild type to the heterozygous genotype.

None of the populations rejected the null hypothesis for IFN-γR rs2234711 and are considered homogenous and dependent.

Figure 17. IFN-γR Allelic frequencies as percentages and p-values for First Nations and non-Indigenous cohorts

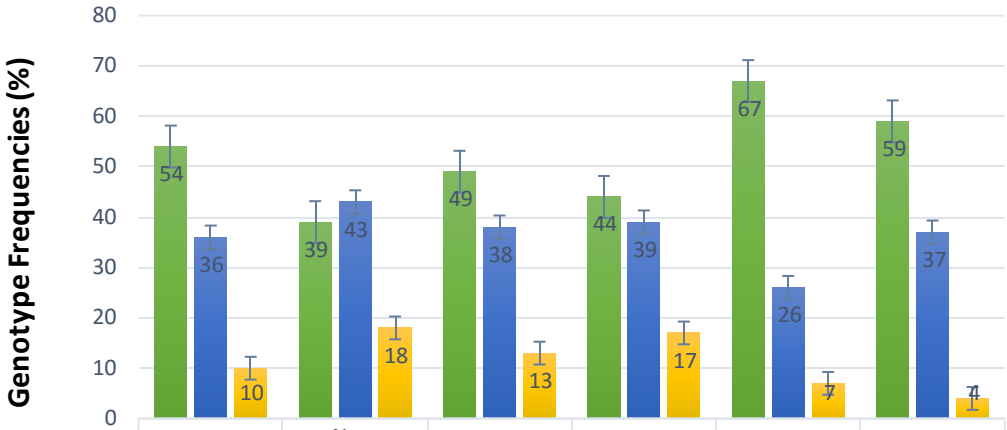


	T	C
Indigenous N=223	72	28
Non-Indigenous N=98	63	37
Dene 1 N=69	68	32
Dene 2 N=54	64	36
Cree N=46	80	20
Saulteaux N=54	78	22

IFN-γR	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
Indigenous		0.0199				
Non-Indigenous			0.3119	0.8453	**0.0026**	0.0072
Dene 1				0.4865	0.0391	0.0927
Dene 2					0.0098	0.0247
Cree						0.6547
Saulteaux						

p-values calculated using Pearson chi square/Fisher exact tests (* = Fisher exact test) and corrected for multiple populations using Bonferroni correction. $p \leq 0.0045$ is significant and indicated in bold type (Standard Error (SE) bars included).

Figure 18. IFN-γR Genotype frequencies as percentages and p-values for First Nations and non-Indigenous cohorts



	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
TT - Wild Type	54	39	49	44	67	59
TC - Heterozygous	36	43	38	39	26	37
CC - Mutant	10	18	13	17	7	4

IFN-γR	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
Indigenous		0.0880				
Non-Indigenous			0.6188	0.9371	0.0144	0.0290
Dene 1				0.8065	0.1459	0.1720
Dene 2					0.0567	0.0602
Cree						* 0.4541
Saulteaux						

p-values calculated using Pearson chi square/Fisher exact tests (* = Fisher exact test) and corrected for multiple populations using Bonferroni correction.
$p \leq 0.0045$ is significant and indicated in bold type (Standard Error (SE) bars included).

4.2.5 $P2RX_7$ rs3751143 Allele and Genotype Frequencies

Observed SNP profiles for $P2RX_7$ had two separate groups. The high A allele group included non-Indigenous (83%), Cree (86%), and Saulteaux (76%); and the medium level A frequencies; Indigenous (62%), Dene 1 (58%), and Dene 2 (54%) (Figure 19). The mutant allele C has functional studies demonstrating that it produces a missense sequence in the coding strand that abolishes the function of the $P2RX_7$ protein. The apoptotic function can be preserved via the heterozygous genotype, though at a reduced rate of functionality (Fernando 2005). A homozygous CC genotype displays the phenotypic trait of complete loss of function of the protein that leads to a lack of ATP-induced mycobacterial killing of infected macrophages (Fernando 2005, Niño- Moreno 2007).

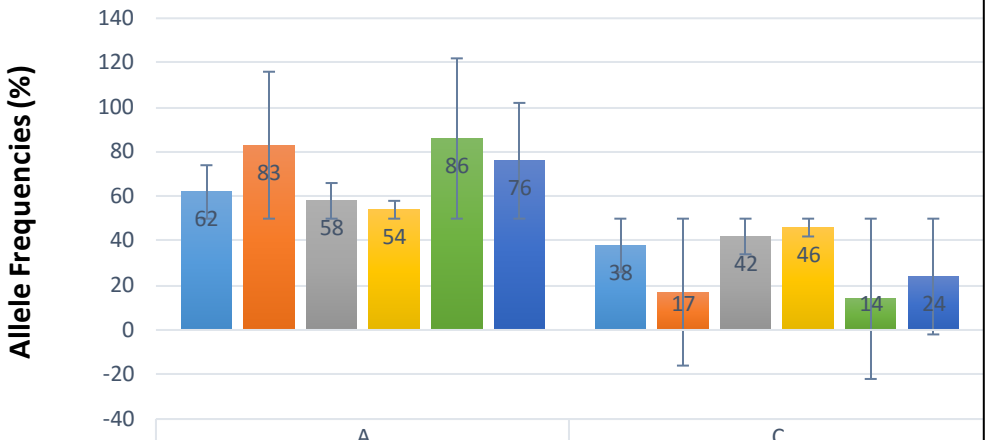
$P2RX_7$ demonstrated a radical allelic frequency variance between both of the Dene communities and all the other groups (non-Indigenous, Cree, and Saulteaux). The non-Indigenous group reject the null hypothesis towards the Indigenous as well, though this is likely heavily influenced by the profound differences of the two Dene groups.

For the $P2RX_7$ SNP, the cohorts of non-Indigenous, Cree, and Saulteaux all demonstrated the same general trend (Figure 20). Those three groups all had high frequencies of the wild type genotype (72%, 76%, 60%), intermediate frequencies of the heterozygous genotype (22%, 20%, 33%), and low levels of the mutant genotype (6%, 4%, 7%). When compared to these three groups, both Dene groups frequency trends differ considerably. Both Dene groups, Dene 1 and Dene 2, had similar values when compared to the others for the heterozygous genotype (20%, 26%). However, the Dene groups had much lower levels of homozygous wild type (32%, 41%) and much higher levels of homozygous mutants (48%, 33%) in comparison to the other groups. The Dene groups had reverse trends from one another for their homozygous genotypes, with

Dene 1 having higher frequencies of mutants followed by wild type, and Dene 2 having the opposite trend. The Indigenous had a high frequency of homozygous wild type (50%) and equal levels of of heterozygous (25%) and mutant (25%) genotypes.

$P2RX_7$ rs3751143 demonstrated that Dene 1 and Dene 2 are similar to one another but have a high degree of variance from all other groups. The overall First Nations group is different from the non-Indigenous group, though is likely heavily influenced by the breadth of difference between the Dene communities and the non-Indigenous group. The non-Indigenous group is not significantly different from the other communities and both Cree and Saulteaux are considered homogenous to one another.

Figure 19. P2RX$_7$ Allelic frequencies as percentages and p-values for First Nations and non-Indigenous cohorts

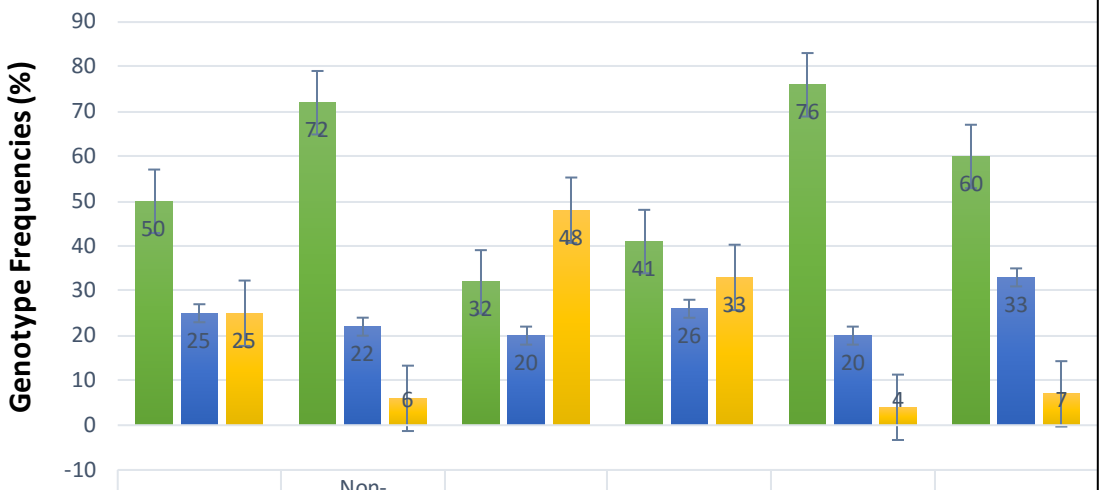


	A	C
Indigenous N=221	62	38
Non-Indigenous N=99	83	17
Dene 1 N=69	58	42
Dene 2 N=51	54	46
Cree N=46	86	14
Saulteaux N=55	76	24

P2RX7	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
Indigenous		**0.0000**				
Non-Indigenou			**0.0000**	**0.0000**	0.5131	0.1699
Dene 1				0.0681	**0.0000**	**0.0000**
Dene 2					**0.0000**	**0.0006**
Cree						0.0883
Saulteaux						

p-values calculated using Pearson chi square/Fisher exact tests (* = Fisher exact test) and corrected for multiple populations using Bonferroni correction.
$p \leq 0.0045$ is significant and indicated in bold type (Standard Error (SE) bars included).

Figure 20. $P2RX_7$ Genotype frequencies as percentages and p-values for First Nations and non-Indigenous cohorts



	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
A/A - Wild Type	50	72	32	41	76	60
A/C - Heterozygous	25	22	20	26	20	33
C/C - Mutant	25	6	48	33	4	7

P2X7	Indigenous	Non-Indigenous	Dene 1	Dene 2	Cree	Saulteaux
Indigenous		**0.0001**				
Non-Indigenous			**0.0000**	**0.0000**	0.8395	0.3166
Dene 1				0.2808	**0.0000**	**0.0000**
Dene 2					**0.0004**	**0.0034**
Cree						* 0.2520
Saulteaux						

p-values calculated using Pearson chi square/Fisher exact tests (* = Fisher exact test) and corrected for multiple populations using Bonferroni correction. $p \leq 0.0045$ is significant and indicated in bold type (Standard Error (SE) bars included).

4.3 Knowledge Translation Results

The three knowledge translation methods were only measured subjectively at the time of thesis preparation. As previously mentioned I will be discussion knowledge translation methods and results in the first person.

The DNA extraction tutorial was given in a small setting with 11 of the 12 visitors actively participating. Initially there was a little apprehension as to what was happening, but once DNA and genetics were explained with popular culture references and the guests started the hands-on portion of the procedure the apprehension disappeared and was replaced with smiles and curiosity (Figures 21-24). Three individual students took the time to inform me later during their trip that it was their favourite part and they felt like they had a better understanding of what DNA is. One student has chosen to follow a career in nursing, and while this is not research or a basic science based profession, it is a health related profession requiring continuing education. As this is subjective it is difficult to say if the visit to the Health Sciences Center, Dr. Larcombe's laboratory, or the DNA extraction tutorial had any direct effect on the individuals, none-the-less it provided 12 non-academic research partners with more insight into the overall world of health research.

The posters were presented at conferences comprising hundreds of other researchers, participants, and community members from Manitoba and the rest or Canada (Figures 26-28). The posters themselves did not win any awards but the one minute presentation of my poster during the Canadian Society of Immunology (CSI) Annual Meeting 2015 (Figure 26) was acknowledged in a presentation competition during the CSI 2015 event as one of the most interesting. No quantitative or qualitative data was collect and the award is subjective to the

judges and their personal biases. However, with the acknowledgment it did help disseminate the information of First Nations research in the Immunology community.

The community infographic will only be dispersed upon the completion of the research and whether it has an effect on the community or not will not be known until after the project is completed (Figure 25). This however does present a possibility for future directions for this research and for knowledge translation understanding and application.

Figure 21: DNA extraction tutorial with Dene students (Left to Right: Raven Yassie, Catlin Semple, Ty Gazayou, Patricia Dettanikeaze, Matthew Cutlip)

Figure 22: DNA extraction tutorial with Dene students and Elder (Left to Right: Dana Tssessaze, Catlin Semple, Ty Gazayou, Raven Yassie, Lizette Denechezhe, Patricia Dettanikeaze, Matthew Cutlip, Jessica Thorassie, Shirlena Cheekie)

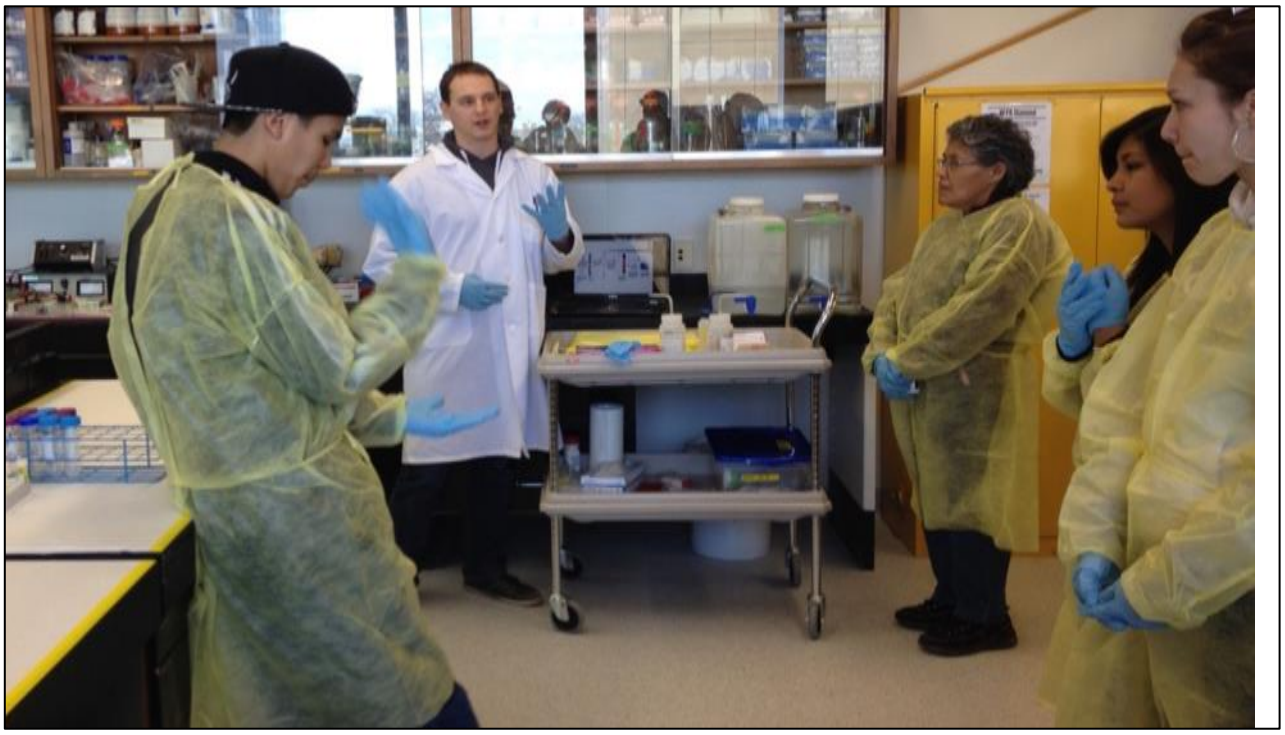


Figure 23: DNA extraction tutorial with Dene students and Elder (Left to Right: TJ Powderhorn, Lizette Denechezhe, Shirlena Cheekie, Jessica Thorassie)

Figure 24: DNA extraction tutorial with Dene Elder Lizette Denechezhe

Figure 25: Community health infographic

Genetics and the Immune Response to Tuberculosis (TB): Why is it important to First Nations? – C. Semple

TB Facts

- TB is the second leading pathogenic killer in the world after HIV.
- TB is treatable, but requires 4 different medications over a period of 6+ months.
- Without medications up to 85% of people do not survive the infection.
- Canadian-born Indigenous people have TB rates 30x higher than a Canadian-born non- Indigenous person.
- TB is influenced by many things, such as genetics and housing conditions.

Putting the health puzzle together

Genetics is one of the many puzzle pieces in human health. The goal is to identify the genetic pieces and try to make them fit with the rest of the health puzzle pieces such as the social determinants of health.

How this research may help First Nations health

- Unique populations respond differently to TB based on their genes.
- By identifying changes in different TB fighting genes, a new vaccine or medicine could hopefully be discovered to improve the current vaccine and treatment.
- Tailor-making medicines for a specific population.

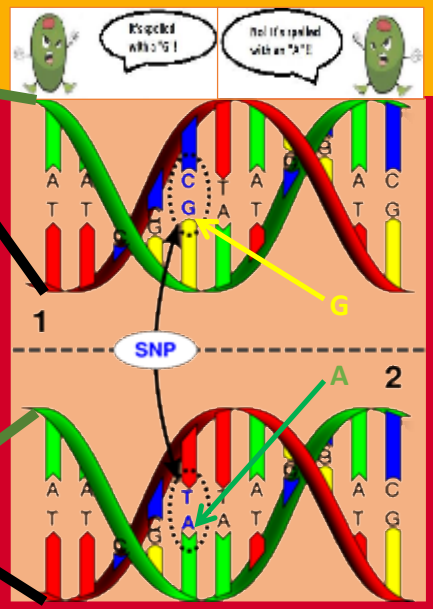
Social and Biological Determinants of Health

This is a First Nation woman's artistic representation of health culture, hobbies, and her DNA.

This research looks at SNPs in 5 different genes that help fight infections like TB

DNA and Health

- Small in the DNAchanges can have a big impact on how well the body does different things, like getting rid of diseases
- Spelling differences in the DNA code are known as Single Nucleotide Polymorphisms, or "SNPs".



This research discovered that SNPs in First Nations people have made their immune responses become very good at fighting off extracellular pathogens (i.e. parasites, strep throat) but not as good at fighting intracellular pathogens (i.e. TB, E. coli)

L. Larcombe Lab 2016

Figure 26: Poster for 28th Annual Canadian Student Health Research Forum 2015 held at the Brodie Centre Winnipeg, Manitoba and for the Canadian Society for Immunology 28th Annual Meeting held at the Fairmont Winnipeg, MB.

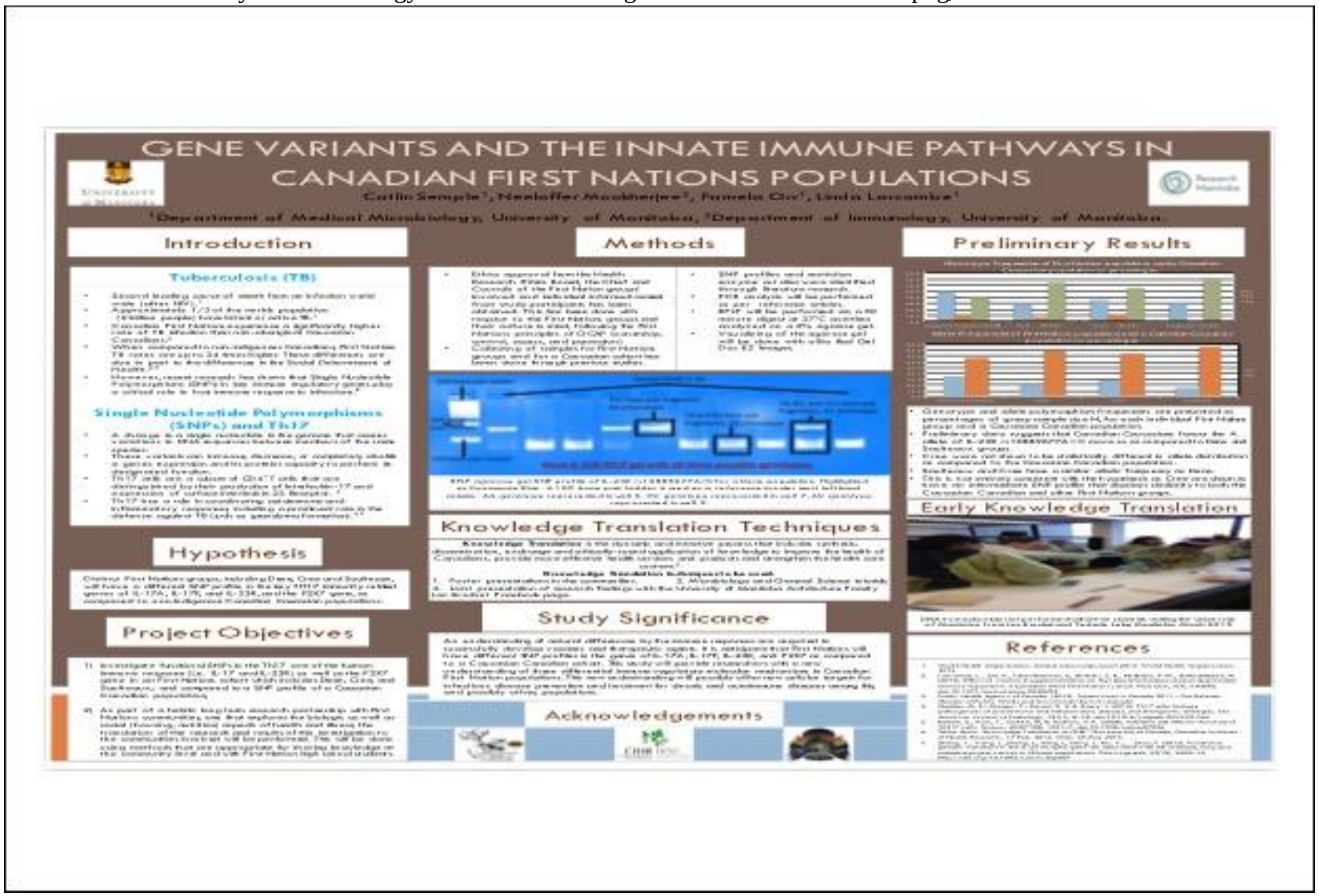


Figure 27: Poster for Centre for Aboriginal Health Research 3rd Annual Indigenous Health Symposium held at the Basic Medical Sciences Building Winnipeg, Manitoba and *Northern Manitoba Research Network* held at the University College of the North in Thompson, Manitoba.

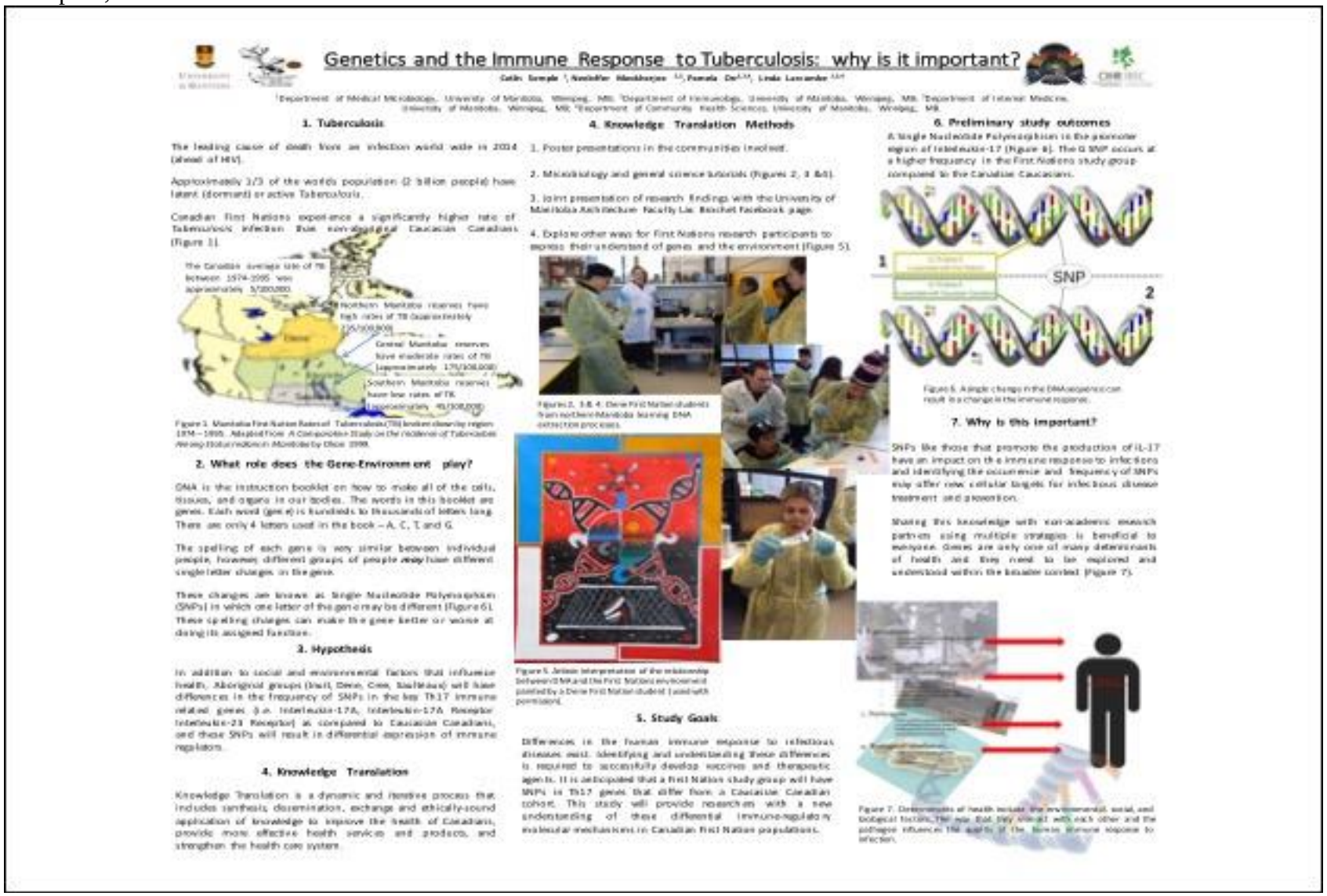


Figure 28: Poster for 29th Annual Canadian Student Health Research Forum 2016 held at the Brodie Centre Winnipeg, Manitoba.

4.4 Summary

Genetic diversity has aided the human immune system throughout its evolution. Without genetic diversity humankind would not have been able to defend itself against the large amount of pathogens which it has encountered. In this study the SNPs of key genes affecting the Th17 arm of the immune system and the $P2RX_7$ cation channel were investigated within four First Nations communities and a non-Indigenous Canadian cohort. The frequencies of both allele and genotype were measured in terms of frequency, if the populations were in HWE, and if the populations were homogenous or heterogeneous. The allelic frequencies demonstrated differences between the groups investigated. The most common observation was that the non-Indigenous cohort had a different frequency or only a slightly similar frequency as compared to the overall Indigenous population and that the two Dene communities were the only groups with similar frequencies to one another for all alleles. The $P2RX_7$ gene also suggested that both of the Dene communities are different in frequency from all other populations yet similar to one another. These findings were echoed by the genotypic frequencies, with a few exceptions. The IL-17A and IL-17RA genotypic profile demonstrated less difference in frequency throughout the individual groups, and the Saulteaux group was more similar in frequency when compared to the non-indigenous groups genotype. Cree also had a different allele profile from non-Indigenous for INF-γR but not for their genotype, and the Dene 1 and Dene 2 were considered different in genotype frequency for the IL-17A SNP but not for their allele frequency. These results demonstrate that the non-Indigenous population maintained a significantly different immunogenetic profile when compared to the First Nations populations and that there is less statistically significant frequency differences among the First Nations groups. These variations

are likely due to the different historical and selective pressures each group faced in their ancestral pasts.

The knowledge translations activities that were selected for this research took a number of approaches. By directly interacting with First Nation high school students I was able to demonstrate some of the research methods and explain how they contribute to health research. It is difficult to assess quantitatively if the knowledge translation techniques had the desired effects beyond the students themselves to the broader community but the subjective feedback from the students' was all positive. One student found that the experience spurred her interest in nursing, for another it helped with her level of comfort for attending the University of Manitoba during the next semester. One student found that the knowledge translation activities at the University provided encouragement for him to finish high school (Per. Comm. Larcombe).

The long term impacts of the knowledge translation activities is yet unknown, however, the community and Chief and Council continue to be research partners and are interested in finding ways to encourage their youth in research.

5.0 Chapter 5 Discussion, Conclusion, and Future Directions

5.1 Discussion

First Nations experience some of the highest incidences of TB, not only in Canada, but in the world (FNIHB 2012, Centre 2014, Whitlock 2012). There is the possibility that a different immunogenic profile contributes to the differences observed. Previous research that analyzed gene polymorphisms related to the Th1 and Th2 immune response in the Dene, Cree, and Saulteaux identified differences in the frequency of a number of key gene polymorphisms (ie. cytokine promoter regions, vitamin D receptors, vitamin D binding protein, killer-immunoglobulin receptors) in the Manitoba First Nations groups compared to non-Indigenous populations (Larcombe 2005, Larcombe 2008, Decter 2013, Larcombe 2015, Braun 2014).

This project hypothesized that SNP frequencies in the Th17 and $P2RX_7$ immune genes of distinct First Nations communities would be different when compared to a non-Indigenous Canadian cohort. These gene polymorphisms have defined functional consequences effecting the immune system defence against TB. IL-17A, IL-23R, IFN-γR, and $P2RX_7$ have prior association studies suggesting links with TB infection and IL-17RA has prior studies suggesting associations with other diseases. The hypothesis was found to be supported as the non-Indigenous group had a different SNP profile as compared to the First Nations groups in 3 of the 4 Th17 related genes and in the $P2RX_7$ gene.

Mutations are ultimately responsible for the occurrence of gene variants and SNPs, but their maintenance and perpetuation in a population is the result of selective pressures that "favour" the mutation. A mutation that provides a selective benefit for an individual (perhaps enabling a new response to a deadly pathogen) will survive and proliferate in a population to have a frequency that geneticists recognize as a SNP. CCR5-delta-32 mutation is an example of a

polymorphism with a tangible impact on disease prevalence that has increased in frequency in some populations due to natural selection pressures (Novembre 2005). Global populations will have different SNP patterns depending on their history of contact and isolation with other populations, and their exposure to pathogens in the microbial environment.

The biologic and cultural histories of populations have distinctive effects on the genetic variation seen in contemporary populations. Events such as founder effects and bottlenecks or other events such as gene flow can possibly explain differential SNP frequencies in individual populations. Founder effect is seen in populations which descend from a small founder population that has a lower degree of genetic variability than the parent population or by chance has a low or high frequency of particular alleles (Hedrick 2011). The lack of genetic diversity can have a negative effect on a population over the course of time (Hedrick 2011). Founder effects and bottlenecks can result in a small pool of genes having a greater representation of rare SNPs which are found in lower frequencies in other populations. Gene flow is the genetic exchange between groups due to movement between groups (Hedrick 2011). The genetic landscape of American Indigenous peoples changed after Columbus came to the America's in 1492 and with the establishment of European settlements in Canada beginning with Samuel de Champlain in the early 17th century (Kornwolf 2002). European explorer's, missionaries and traders contributed to the Indigenous gene pool but they also brought with them diseases that changed the demographics of North and South America. The rapid decline of the Indigenous population at the time of contact with Europeans had a profound effect on the cultural and genetic diversity of Indigenous Americans (Waldram 2006). The effects of these events on specific Indigenous groups was highly variable. Assessing the extent of their effects on allelic

diversity in the small populations analyzed in this study are beyond the scope of this research but might be investigated using additional genetic, cultural and historic information (Hackett 2002).

This chapter will compare the frequency of alleles of IL17A, IL-17RA, IL-23R, IFN-γR, and $P2RX_7$ in the study cohorts to other data sets to explore their potential associations with disease. By comparing the Manitoba First Nations SNP profiles to or with those found in other global populations, it may be possible to understand the adaptive processes that influenced the frequency of the SNP alleles in the current study populations. This study did not analyze ancestry markers from the study participants because, at the request of the Chiefs and Councils who supported this work, it was not a component of the study, and ethnicity was self-reported.

5.2 Allele and genotype frequencies and disease associations in global populations

This section will examine allelic and genotypic population profiles for IL17A, IL-17RA, IL-23R, IFN-γR, and $P2RX_7$ that are associated with disease in other studies. This was performed to investigate functional consequences of the individuals SNPs as they relate to different diseases. Many different disease and cancers have been associated with the SNPs in question, these have been included along with any available TB associated studies (Table 9). These population comparisons may give insight into what disease the First Nations allele and genotype profiles may be more susceptible or resistant to and possibly provide starting points in determining medications if the related disease do become significant within the communities.

Table 9: Global SNP frequencies associated with disease.

IL-17A Populations	Disease Associated	GG	GA	AA	G	A	Genotype or Allele associated with disease
Northern Spain[1]	PTB	51	40	9	70	30	GG
Chinese[2]	TB	38	43	19	60	40	AA, GA
Southern Brazil[3]	PTB	82	15	3	90	10	GG – G
Norwegian[4]	Rheumatoid Arthritis	42	46	12	65	35	G
Japan[5]	Ulcerative Colitis	31	55	14	58	42	AA – A
Chinese[6]	Cervical Cancer	30	24	46	53	47	AA – A
Chinese Han[7]	Lung Cancer	28	50	22	54	46	AA – A
IL-17RA Populations	**Disease Associated**	**AA**	**AG**	**GG**	**A**	**G**	**Genotype or Allele associated with disease**
Spanish[8]	Psoriasis	57	38	5	76	24	G
Northern Spanish[9]	Chronic Kidney Disease	63	33	4	80	20	A
Korean[10]	New onset diabetes after renal transplantation	48	48	4	72	28	A
IL-23R Populations	**Disease Associated**	**CC**	**CA**	**AA**	**C**	**A**	**Genotype or Allele associated with disease**
Chinese Uygur[11]	PTB	29	49	22	53	47	CC
Eastern Chinese[12]	Breast cancer	7	42	51	28	72	A
Southern Chinese[12]	Breast cancer	7	42	51	28	72	A
Eastern Chinese[12]	Lung Cancer	6	41	53	27	73	A

Southern Chinese[12]	Lung Cancer	6	40	54	26	74	A
Eastern Chinese[12]	Nasopharyngeal Cancer	6	41	53	27	73	A
Southern Chinese[12]	Nasopharyngeal Cancer	6	41	53	27	73	A
Chinese Han[13]	Idiopathic dilated cardiomyopathy	10	50	40	65	35	C
Hungarian[14]	ankylosing spondylitis	43	46	11	65	35	AA
North American Caucasian[15]	Graves' ophthalmopathy	62	33	8	79	21	CC – C
Spanish[16]	Crohn's Disease	35	54	11	62	38	A
Spanish[16]	Ulcerative Colitis	34	50	16	59	41	A
IFN-γR Populations	**Disease Associated**	**TT**	**TC**	**CC**	**T**	**C**	**Genotype or Allele associated with disease**
West Africa[16]	PTB	25	49	26	53	47	TT,TC
Chinese Han[17]	PTB	37	45	18	61	39	T
Gambian[18]	Malaria	35	36	29	53	47	TT, CC
$P2RX_7$ Populations	**Disease Associated**	**AA**	**AC**	**CC**	**A**	**C**	**Genotype or Allele associated with disease**
India[19]	TB	58	37	5	77	23	C
North Indian Punjabi[20]	TB	54	43	3	75	25	C
Russian[21]	TB	64	31	5	80	20	C
Mexico[22]	PTB	76	23	1	87	13	C
Tunisia[23]	ETB	67	25	8	80	20	CC, AC – C
Southeast Asians[24]	ETB	34	56	10	34	66	C
Turkey[25]	ETB	53	38	9	74	26	CC, AC – C

1: Ocejo-Vinyals 2013; 2: Shi 2014; 3: Milano 2016; 4: Nordang 2008; 5: Arisawa 2008; 6: Quan 2012; 7: He 2015; 8: Batalla 2015; 9: Coto 2015; 10: Kim 2012; 11: Jiang 2015; 12: Zheng

2012; 13: Chen 2009; 14: Sáfrány 2009; 15: Huber 2008; 15: Oliver 2007; 16: Cooke 2006; 17: Lü 2014; 18: Koch 2002; 19: Singla 2012; 20: Sehajpal 2010; 21: Mokrousov 2008; 22: Niño-Moreno 2007; 23: Ben-Selma 2011; 24: Fernando 2007; 25: Tekin 2010

5.2.1 IL-17A rs2275913 global comparison

IL-17 plays a role in host defense against certain pathogens, including TB, at epithelial and mucosal barriers (Jin 2013). It is a primary contributor to the formation and stabilization of the lung granuloma. Espinoza found that the IL-17A SNP rs2275913 mutant allele A is associated with significantly increased IL-17 mRNA production when stimulated with Phytohaemagglutinin (Espinoza 2011). All of the First Nation cohorts in this study had a higher frequency of the wild type G allele as compared to mutant A allele, which has been associated with TB in some populations but not others. Two studies, identified the GG genotype and G allele as associated to PTB susceptibility in Spanish and Brazilian populations and one study found the opposite allele – the A allele was associated to TB susceptibility in a Chinese population (Ocejo-Vinyals 2013, Shi 2014, Milano 2016). This inconsistency generates difficultly in associating the IL-17A SNP as influential for MTB containment. The G allele is also associated with autoimmune disorder rheumatoid arthritis in a Norwegian population.

5.2.2 IL-17RA rs4819554 global comparison

IL-17RA is a heterodimer composed of IL-17 Receptor A and C (Gaffen 2009). IL-17RA is capable of recognizing IL-17A, and according to Coto, leukocytes with higher levels of the rs4819554 wild type AA genotype will express higher levels of IL-17RA mRNA than those with the mutant GG genotype (Jin 2013, Coto 2015). First Nation communities Dene 1, Dene 2, and Cree had significantly higher frequency of G alleles when compared to the non-Indigenous population. The Dene communities are homogenous with the East Asian population as well. This

suggest that the First Nations groups of Dene and Cree may produce lower levels of IL-17RA mRNA than the non-Indigenous and Saulteaux groups.

The few studies regarding disease for rs4819554 demonstrated opposite allelic association from one another. Rs4819554 effects on chronic kidney disease due to chronic inflammation was associated with the A allele and the autoimmune disease psoriasis is associated with the G allele (Batalla 2015, Coto 2015).

As there are no prior MTB studies regarding rs4819554 it will require addition investigation to determine if the First Nations allelic and genotypic profiles are of consequence in the immune responses effort against MTB.

5.2.3 IL-23R rs10889677 global comparison

IL-23 binds to the IL-23R on CD4+ T cells aiding in the cells differentiation to Th17 cells and to the initiation and sustain signalling pathways for the production of IL-17 (Khader 2008). The IL-23R SNP rs10889677 mutant A allele disrupts the binding site for the micro-RNA, miR-let-7f, which increases the transcription of the IL-23R gene (Zheng 2012). Non-Indigenous had the highest frequency of A allele and were statistically different from the Dene 1 and Saulteaux communities and displayed very little homogenity with the Dene 2 community. The East Asian group is statistically different from all of the First Nations communities. This suggest that the non-Indigenous, Cree, and to a lesser extent the Dene 2 study cohorts, have the possibility of transcribing more IL-23R as compared to the Dene 1 and Saulteaux communities.

Zheng identified that an increased rs10889677 AA genotype produces higher serum IL-17 protein levels in healthy controls (Zheng 2012). Global studies identified the A allele in association with different cancers and bowel disorders (Zheng 2012, Cooke 2006). The Non-

Indigenous groups had the highest frequency of AA genotypes and are statistically different from Dene 1 cohort. Dene 2, Cree, and the Saulteaux groups had genotype frequencies which were homogenous with both non-Indigenous and Dene 1 cohorts, however the Dene 2, Cree, and Saulteaux demonstrated greater similarity to the Dene 1 population. This might suggest that the Dene 1, Dene 2, Cree, and Saulteaux communities may be producing lower levels of IL-17 than the non-Indigenous cohort.

First Nations communities have higher frequencies of the C allele and the CC genotype for this SNP. The lone IL-23R rs10889677 study involving TB was done in a Chinese Uygurs population (N=250) and attributed the association of rs10889677 wild type CC genotype with the susceptibility to PTB (Jiang 2015). The C allele is also associated with Idiopathic dilated cardiomyopathy in a Chinese Han population (Chen 2009).

5.2.4 IFN-γR rs2234711 global comparison

IFN-γ acts to assist in regulating the inflammatory response of Th17 and induce the production of the Th1 in response to intracellular infection (Dorhoi 2011). Prior studies have demonstrated that hosts with IFN-γR deficient cells have a greater ability to develop Th17 cells, regardless of IL-23 stimulation (Weaver 2006). The IFN-γR SNP rs2234711 mutant C allele has been observed to have higher levels of Th1 than the wild-type allele (Xiang 2014). It is suggested, though not confirmed, that the mutant C allele increases the binding affinity of the TFAP4 binding site (Jüliger 2003, He 2010). An association of the T allele to PTB in West African and Chinese Han populations has been reported, while Koch discovered an association of the heterozygous genotype with protection from malaria (Koch 2002, Cooke 2006, Lü 2014). The Dene and Saulteaux communities and the non-Indigenous cohort are statistically similar

with higher levels of C allele than the Cree, though the Saulteaux are very close to being statistically different from the non-Indigenous group (p=0.0072).

The higher levels of C allele in the non-Indigenous, and Dene communities may potentially have an upregulation of Th1 cells over Th17 cells as compared to Cree and Saulteaux.

5.2.5 P2RX7 rs3751143 global comparison

The $P2RX_7$ gene encodes a transmembrane polypeptide which acts as a receptor for a ligand-gated cation channel. Its activation leads to infected macrophage apoptosis and results in MTB death (Niño-Moreno 2007, Wu 2014**).** When rs3751143 genotype is homozygous recessive, CC, then the observed phenotypic trait is an almost complete loss of function of P2X leading to lack of ATP-induced mycobacterial killing of infected macrophages (Niño-Moreno 2007). If the heterozygous genotype for this particular SNP is encoded than a highly compromised reduction, of up to 75%, in mycobacterial killing apoptosis has been observed (Fernando 2005). Supporting this is that the mutant allele C and CC genotype is associated with increase susceptibility to infection by MTB in numerous studies (Singla 2012, Sehajpal 2010, Mokrousov 2008, Niño-Moreno 2007, Ben-Selma 2011, Fernando 2007, Tekin 2010). The non-Indigenous group shares a similar allelic and genotypic frequency with both the Cree and Saulteaux, and all three of these groups are similar in allelic frequency with the NCBI European group. Contrastingly, the Dene displayed a significant higher CC genotype and C allele frequency as compared to any other group, both in this study and globally. This suggests that the Dene may have an abrogated $P2RX_7$ and that macrophages infected with MTB in this population may be incapable of killing the mycobacteria via apoptosis when infected.

5.3 Th17 and $P2RX_7$ SNP profiles and health implications in First Nations

The Th17 immune response is beneficial in maintaining mucosal barriers against pathogens such as MTB (Dubin 2008). SNPs in the Th17 immune pathway have been shown to have functional effects for maintaining these barriers and have been associated with diseases including tuberculosis, cancers and autoimmune conditions. SNPs in three of the four Th17 related genes, IL-17A rs2275913, IL-17RA rs4819554, and IL-23R rs10889677; occur in high frequencies in First Nations cohorts in this study, favouring a decreased Th17 response. These SNPs may influence the immune system towards a lowered Th17 response and as past research has demonstrated the immune system balance may favour a Th2 response (Larcombe 2008). In First Nations IL-17A rs2275913 favoured the G allele as compared to the non-Indigenous population for all communities, which suggest that the communities do not produce as much IL-17A mRNA as the non-Indigenous population. If this is the case than the First Nations communities would have less pro-inflammatory response and possibly less effective granuloma development. Non-Indigenous also had the genetic profile suggesting an a more attractive NFAT binding site in the IL-17RA promoter compared to the two Dene communities and the Cree community, though not the Saulteaux community. If the SNP functions as such this again would indicate that the non-Indigenous population is more successful at producing higher levels of IL-17A mRNA as well (Coto 2015). For IL-23R rs10889677, all groups favoured the C allele, however the First Nations communities displayed a greater frequency of C allele than the non-Indigenous group. A functional IL-23R is required for an optimal IL-17 response as it has been demonstrated that mice deficient in the IL-23R do not preserve the Th17 response or IL-17A mRNA expression throughout an MTB infection (Torrado 2010). Non-Indigenous have a greater frequency of A alleles, which should disrupt the miR-let-7f microRNA binding site and thus

allowing for greater transcription of IL-23R (Zheng 2012). All three of these SNPs suggest that non-Indigenous Canadians have a more effective Th17 and IL-17A response as compared to these First Nations groups. As such, the Th17 immune response to certain diseases or conditions, such as MTB, might be downregulated in the First Nations cohorts. It is likely that one SNP might not affect the immune response, but the occurrence of numerous SNPs and/or the interaction of SNPs may differentially regulated the Th17 immune response. Though the Th17 immune response may be downregulated there is not enough empirical evidence suggesting these specific SNPs may be associated with the increased rates of TB. The immunogenic profile is also confounded as the IFN-γR rs2234711 SNP promotes the Th1 pathway in the non-Indigenous, Dene, and Saulteaux groups in comparison to the Cree community, though the Saulteaux only had a small degree of homogeny with the other groups.

The most intriguing discovery was the difference in the $P2RX_7$ rs3751143 frequencies of the Dene communities as compared to the other study population and the global allele and genotype frequencies. The homozygous CC genotype, which the Dene favoured, has a phenotypic trait of complete loss of function of the protein that leads to a lack of ATP-induced mycobacterial killing of infected macrophages and the heterozygous genotype also experienced high levels of loss of function (Fernando 2005, Niño- Moreno 2007). Though it is likely a variety of factors such as a panel of SNPs effecting TB susceptibility, this abrogated cation channel may constitute a single SNP with a profound effect on incidents of TB in the Dene populations. Of the First Nations communities involved, the Dene communities have had the highest rates of TB infection. With a number of studies identifying the mutant genotype having association with TB, PTB, and ETB infection, this novel finding within this population suggests further information should be gathered regarding possible therapeutic exploitation.

The Dene communities were separated to reflect their distinct histories and to identify any allelic or genotypic differences between the communities. The only gene that showed any statistical differences was the genotype for IL-17A, though the allele frequencies were statistically the same.

The occurrence of SNPs that might dysregulate the Th17 response is only one of many factors that potentially effect the rates of infectious diseases in First Nations communities. Poor housing conditions and other social determinants of health have an influence on disease transmission and outcomes and on many aspects of physical and mental health. Conditions on Canadian First Nations reserves have been well documented and are associated with health and wellbeing deficiencies for the residences (Larcombe 2011, Boutilier 2013, Kovesi 2007, Riva 2014). Tuberculosis has significant association with social determinants of health such as lower income levels, crowded housing conditions, poor ventilation, and isolation from resources such as medical care (Clark 2002). Substandard housing often has common disease associated factors within the structure such as bacterial endotoxins, allergens, and fungal glycans; all of which increase the risk of developing a low grade and chronic inflammatory response which creates an imbalance between pro- and anti- inflammatory cytokines (Garrett 1998, Johannessen 2005, Lehmann 2003). Poor air quality conditions associated with subpar housing conditions, such as high concentrations of allergens, may be influencing the immune system to down regulate the Th1 and Th17 immune pathways and upregulating the Th2 immune pathway (Cho 2010). The suboptimal housing conditions First Nations on-reserve have been subjected to may be sanctioning a chronic up-regulation of Th2 immune responses and may be an environmental pressure for the genetic predisposition of variants favouring the Th2 pathway over the Th17 pathway.

5.4 Conclusion

Pathogen and host interactions have evolved together over millennia, each shaping the others ability to attack, defend, and coexist. A growing area of research is that of the role of SNPs in the promoter and coding regions of key immune genes and their effects on the immune system's ability to defend against pathogens. The identification of SNPs that potentially effect the immune response to MTB in Canadian First Nation populations who experience some of the highest rates of TB infection globally is of significant importance (Peoples 2014, WHO 2015). The identification of SNP profiles that differ from those of non-Indigenous people's will help guide the development of therapeutics and vaccines. Biological health determinants however, are not the only issue causing high rates of TB in these populations. Other compounding issues include inequities in the social determinants of health and environmental factors. This study is unable to address all of these issues but contributes to the development of the immunogenic characterization to assist in the advancement of therapeutics and the biological determinants of health.

The hypothesis of this research was developed based on the findings of previous research that found SNPs in the cytokine promoter regions in Manitoba First Nations that may impact Th1 and Th2 immune regulation in this group (Larcombe 2005, Larcombe 2008, Larcombe 2015, Decter 2013). The hypothesis states that in addition to social and environmental factors that influence health, distinct First Nations groups (Dene, Cree and Saulteaux) have different frequencies of SNPs in the key Th17 immunity related genes of IL-17A, IL-17RA, IL-23R, IFN-γR, and the $P2RX_7$ gene, as compared to a non-Indigenous Canadian cohort. These differences could result in differential expression of immune regulators and an altered outcome in the

effective immune response to MTB. The occurrence of SNPs may have a tangible impact on the First Nations rates of infectious diseases through the down regulation of the Th17 immune pathway in response to MTB. The selection of genes included in this study was complimentary to prior work, as the Th17 immune pathway was a major T cell immune pathway not previously investigated in these populations (Larcombe 2005, Larcombe 2008, Larcombe 2015, Decter 2013).

There were two overall goals for this study; First, investigate functional SNPs in the Th17 arm of the human immune response (i.e. IL-17A, IL-17RA, IL-23R, and IFN-γR) as well as in the $P2RX_7$ gene, in a First Nations cohort. Secondly, the application of knowledge translation strategies and techniques within the community and high school students of Lac Brochet, Manitoba was completed in an attempt to intrigue students to pursue post-secondary education and/or a career in health research and science.

This research determined that there is a statistically significant difference between First Nations and non-Aboriginal Manitobans in some, but not all, of the SNP profiles as well as difference between some of the individual First Nations groups in a variety of the SNP profiles. SNPs detected in IL-17A and IL-17RA indicated a statistical difference favouring alleles associated with the overall upregulation of pro-inflammatory events and granuloma formation in the non-Indigenous population. IL-23R portrayed an allelic upregulation which favours the Th17 immune pathway in non-Indigenous, Dene 2, and Cree as compared to one Dene community and Saulteaux. A sub-optimal IL-23R response would reduce the amount of IL-17 available to defend against MTB (Quesniaux 2013). The IFN-γR SNP frequencies are equal in all populations except for the Cree which has an allelic profile suggesting that the receptor may be more highly upregulated in this population as compared to the non-Indigenous population. IFN-γ helps

regulate Th17 immune responses and high levels of the INF-γR equate to higher levels of Th1 cell production and lower levels of Th17 cell production. The similar SNP allele frequencies of the non-Indigenous, Dene, and Saulteaux suggest that these groups may have similar reduced expression of Th17 while the Cree could have an increased production of Th1cells. This information is contradictory to the profiles of IL-17A, IL-17RA, and IL-23R. However, as mentioned, it is likely the interaction of multiple genes and of the SNPs that influence the different immune pathways and not individual SNPs in isolation.

Finally, the $P2RX_7$ gene displayed a novel finding. The Dene may have a compromised immune defensive against MTB as they had statistically different frequencies of alleles compared to other populations, potentially indicating an abrogated P2X cation channel. This abrogated channel is essentially inoperative as a defense mechanism against TB infection. The Cree, Saulteaux, and non-Indigenous cohorts all have statistically similar functioning levels of this cation channel.

This research, along with information from prior studies, suggests that Manitoban First Nations have SNP frequencies that may affect a functional down-regulation of Th17 related genes and an immunoregulatory upregulation of Th2 cytokines (Decter 2013, Larcombe 2005). Preference for SNPs associated with the upregulation of the Th2 immune pathway suggests that the First Nations immune pathways may have a strong functional effect against fungal and parasitic infections found in their pre-contact environments. The upregulation of the Th2 immune response has benefits in situations against helminths and other parasites, however, diseases such as virulent MTB, which arrived post contact with European-descent groups, may have put the First Nations people at a disadvantage.

The second objective of this research, pertaining knowledge translations, was accomplished by way of distributing the research infographic to community health centers. The laboratory tour and DNA extraction tutorial with First Nations youth from two of the partner communities was a unique opportunity for sharing technical details about genomic research with specific demographics. The basic scientific methods used for this research (DNA collection, extraction and analysis in the context of health) are easily transferable to high school students who are familiar with basic concepts of inheritance and are familiar with the complexity of the health issues that exist in their communities.

Evolutionary pressures over generations have influenced the selection of immune response SNP profiles in contemporary First Nations. The First Nation's immunogenetic profile hinder the promotion of Th17 and Th1 immune responses in the favour of the Th2 immune response (Larcombe 2005, Larcombe 2008, Larcombe 2015, Decter 2013). Immunological differences between non-Indigenous Manitobans and Manitoban First Nations cohorts are observable in the variance demonstrated by their immunogenetic libraries. The immune response of pre-contact First Nations was likely adapted to the environment and the exposure to specific microbiological pathogens. The disease environment encouraged the selection of an upregulated Th2 immune response to handle parasitic loads and extracellular infections. However, the arrival of European-descent groups and the spread of intracellular infections (i.e. MTB) resulted in high rates of morbidity and mortality in the Indigenous population.. Improvement in health will occur with the improvement of social, biological, and environmental factors which effect TB epidemiology. Garnering a working immunogenic library of SNPs is one of the ways in which health researchers can assist in the attempt to provide a treatment, and ideally, eradicate the high prevalence TB in First Nations populations.

5.5 Future directions

Successful vaccination construction against the pathogen of MTB has been limited to the Bacille Calmette-Guerin (BCG) vaccine and this lone therapeutic has demonstrated less than desirable effectiveness (Andersen 2005). Vaccinomic investigations ideally craft a vaccine designed for particular populations, as each population responds uniquely to the variant MTB strains based on the pathogens and host genetic profile (Moller 2010, Ovsyannikova 2011). Prior studies have demonstrated that different strains of MTB have increased or decreased pathogenicity and that SNPs in different genes of the host increases and decreases resistant to these specific strains (Caws 2008). This makes establishing a genetic profile for at risk populations an endeavor of paramount importance. One prior vaccinomic study demonstrated IL-12 DNA vaccinations in mice which upregulate the Th1 response, while another exhibited TLR4 adjuvants which activate the innate immune system and potent Th1-inducing properties in cynomolgus monkey model ((Lowrie 1999, Coler 2013). These studies offer promising possibilities for future therapeutic treatments as the treatments have demonstrated a selective promotion of the Th1 immune response.

Canadian Aboriginal populations face distinct health challenges that relate to the social determinants of health and may also be influenced by gene variants that affect the quality of the immune response to infectious diseases like TB. Socio-economic disparities influence differential disease susceptibility and/or resistance and now a growing encyclopedia of the variation in the genomic library and how it influences the immune system is being identified. Population differences in the genomic library, even single variants like SNPs, have eminent importance with regards to the ability to mount an immune response against pathogens. These SNP variants can alter the function of cytokines affecting the individual T cell pathways and

there downstream signaling cascades. SNPs regulating the bias towards one arm or another of the immune system can effectively protect an individual from certain diseases and exploit them against others. If the immune response required is not in favour of the populations immunogenic profile, then a sluggish immune response may ensue. This less than optimal response may lead to an inability to contain or eliminate a pathogen before extensive damage is done to the host. Results of this study suggest that First Nations populations have an inhibited Th17 immune response when contrasted to that of non-Indigenous Manitobans.

Possible future research which could help establish future SNP profiles either related to Th17, or other important immune system genes, could be further genotyping of individual genes related to TB infection or the genotyping of the entire immunogenomic library. Currently First Nations in Manitoba are not prepared to consider genome-wide scans for research due in part to the past misappropriation of biological tissues including DNA and the concern about challenges to rights of territory and resources, and because of possible implications of spiritual fallout within First Nations communities (Mosby 2013, Dukepoo 1998). However, as appropriate conduct of research under the principles of OCAP™, Indigenous self-determination, and the Tri-Agency Council, and with the development of mutually beneficial research with western academics continues the possibility of more thorough genotyping may be an option in the future. This would occur only with explicit and detailed consultation with First Nation research partners for continued genetic research. Examination of SNP frequencies in these, and other, cytokines may provide functional information that could be used to support the optimization of the different T cell pathways in First Nations.

The development of a more thorough understanding of the SNP frequency variances guiding regulation of the different T cell pathways may lead to therapies against diseases having

significant acute and chronic impacts; such as diabetes, hepatitis, and tuberculosis, in Manitoba First Nations communities.

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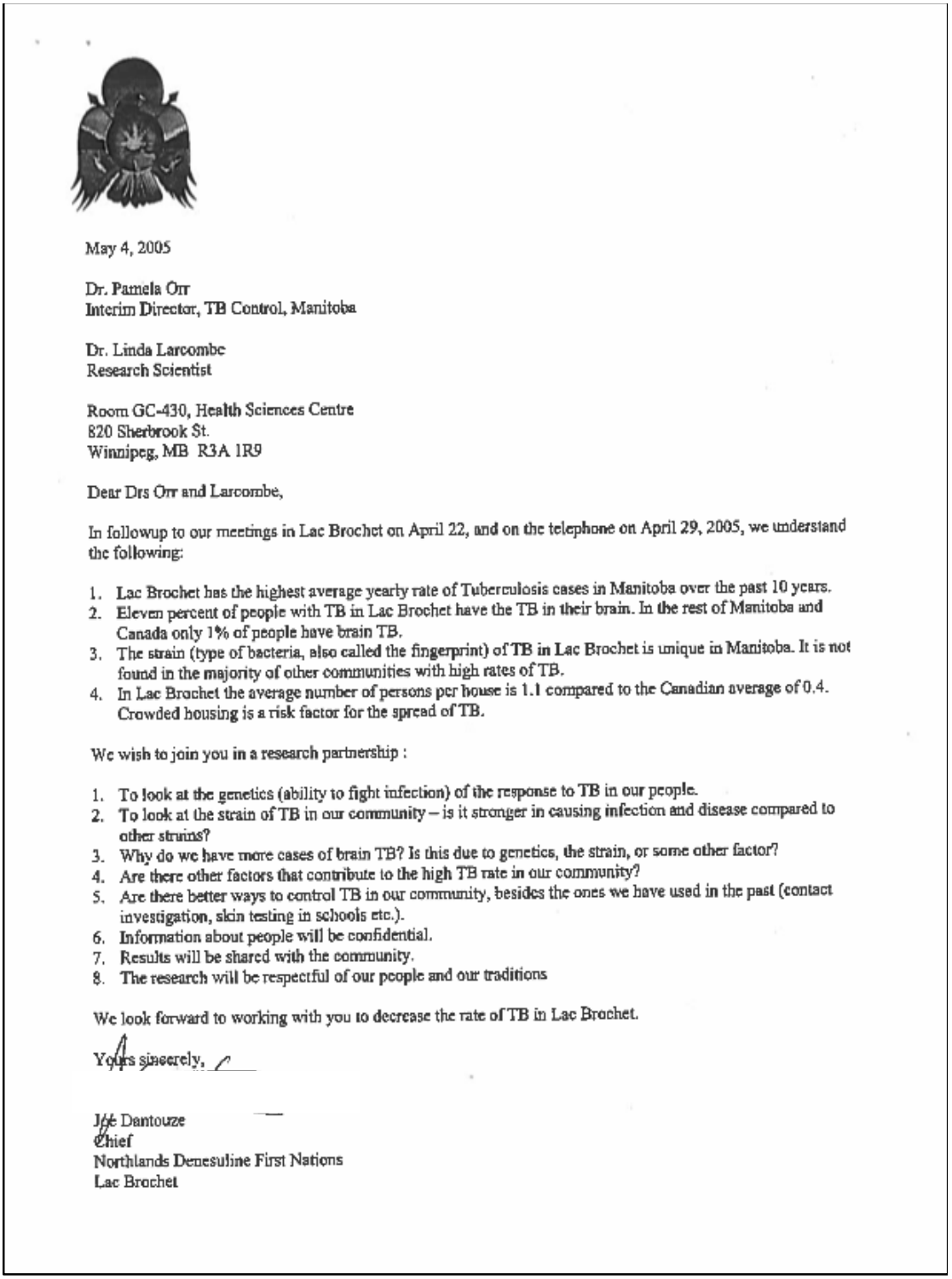
Appendix

Figure A1: Research study outline and participation signatures of Chief: Northlands Denesuline First Nations at Lac Brochet.



May 4, 2005

Dr. Pamela Orr
Interim Director, TB Control, Manitoba

Dr. Linda Larcombe
Research Scientist

Room GC-430, Health Sciences Centre
820 Sherbrook St.
Winnipeg, MB R3A 1R9

Dear Drs Orr and Larcombe,

In followup to our meetings in Lac Brochet on April 22, and on the telephone on April 29, 2005, we understand the following:

1. Lac Brochet has the highest average yearly rate of Tuberculosis cases in Manitoba over the past 10 years.
2. Eleven percent of people with TB in Lac Brochet have the TB in their brain. In the rest of Manitoba and Canada only 1% of people have brain TB.
3. The strain (type of bacteria, also called the fingerprint) of TB in Lac Brochet is unique in Manitoba. It is not found in the majority of other communities with high rates of TB.
4. In Lac Brochet the average number of persons per house is 1.1 compared to the Canadian average of 0.4. Crowded housing is a risk factor for the spread of TB.

We wish to join you in a research partnership :

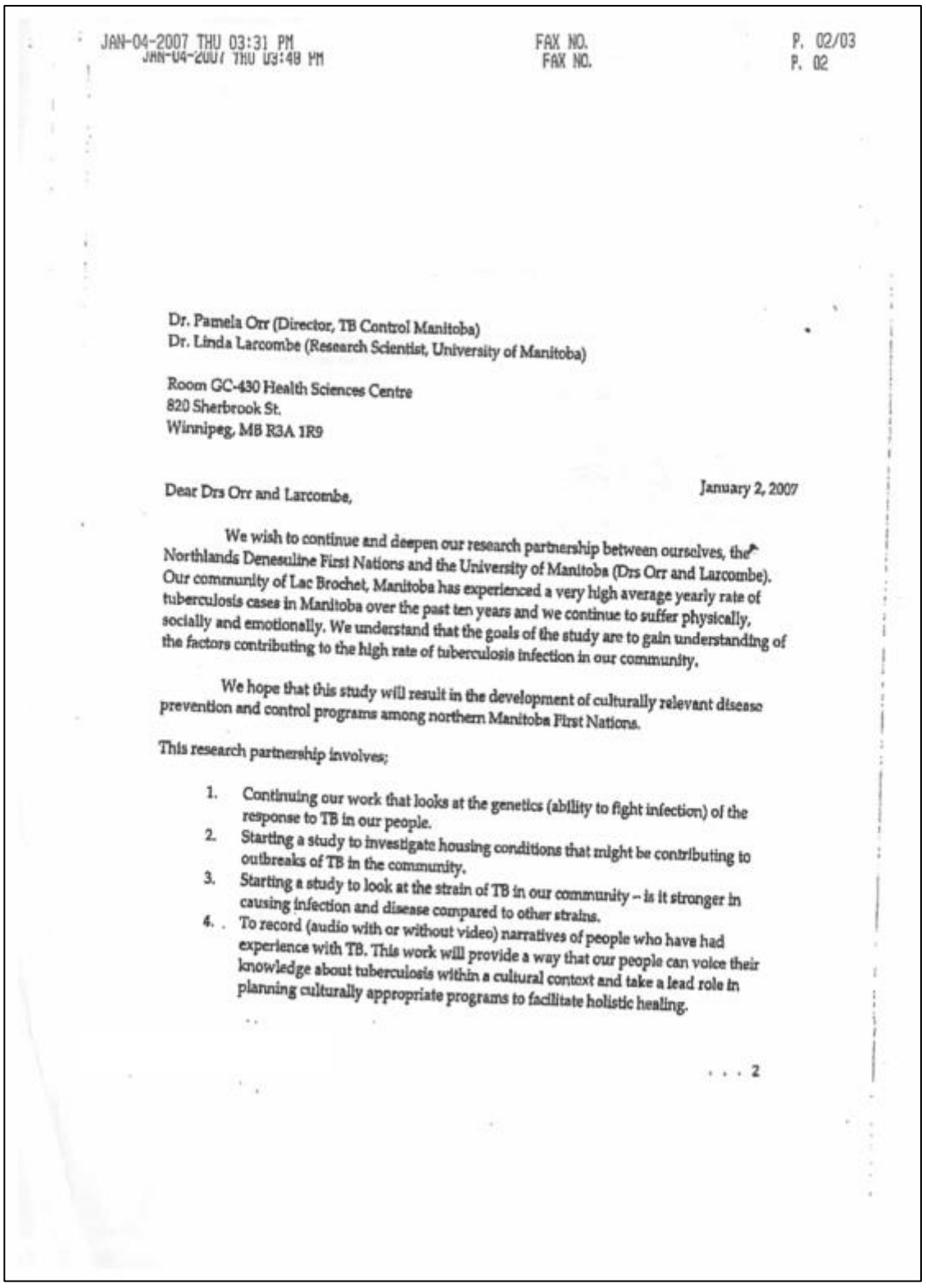
1. To look at the genetics (ability to fight infection) of the response to TB in our people.
2. To look at the strain of TB in our community – is it stronger in causing infection and disease compared to other strains?
3. Why do we have more cases of brain TB? Is this due to genetics, the strain, or some other factor?
4. Are there other factors that contribute to the high TB rate in our community?
5. Are there better ways to control TB in our community, besides the ones we have used in the past (contact investigation, skin testing in schools etc.).
6. Information about people will be confidential.
7. Results will be shared with the community.
8. The research will be respectful of our people and our traditions

We look forward to working with you to decrease the rate of TB in Lac Brochet.

Yours sincerely,

Joe Dantouze
Chief
Northlands Denesuline First Nations
Lac Brochet

Figure A2: Continued Research Approval signature of Chief: Northlands Denesuline First Nations at Lac Brochet.



JAN-04-2007 THU 03:31 PM FAX NO. P. 02/03
JAN-04-2007 THU 03:48 PM FAX NO. P. 02

Dr. Pamela Orr (Director, TB Control Manitoba)
Dr. Linda Larcombe (Research Scientist, University of Manitoba)

Room GC-430 Health Sciences Centre
820 Sherbrook St.
Winnipeg, MB R3A 1R9

Dear Drs Orr and Larcombe, January 2, 2007

We wish to continue and deepen our research partnership between ourselves, the Northlands Denesuline First Nations and the University of Manitoba (Drs Orr and Larcombe). Our community of Lac Brochet, Manitoba has experienced a very high average yearly rate of tuberculosis cases in Manitoba over the past ten years and we continue to suffer physically, socially and emotionally. We understand that the goals of the study are to gain understanding of the factors contributing to the high rate of tuberculosis infection in our community.

We hope that this study will result in the development of culturally relevant disease prevention and control programs among northern Manitoba First Nations.

This research partnership involves:

1. Continuing our work that looks at the genetics (ability to fight infection) of the response to TB in our people.
2. Starting a study to investigate housing conditions that might be contributing to outbreaks of TB in the community.
3. Starting a study to look at the strain of TB in our community – is it stronger in causing infection and disease compared to other strains.
4. To record (audio with or without video) narratives of people who have had experience with TB. This work will provide a way that our people can voice their knowledge about tuberculosis within a cultural context and take a lead role in planning culturally appropriate programs to facilitate holistic healing.

. . . 2

Page 2

Our partnership is based on these principles;

1. The results will be shared with the community
2. Specific information about individuals will be confidential
3. The research will be respectful of our people and our traditions.

We look forward to working together with this study.

Sincerely yours,

Chief J. Dantouze
NORTHLANDS FIRST NATION
Lac Brochet, MB

Figure A3: Research study outline and participation signatures of Chief and Council: Sayisi Dene First Nations at Tadoule Lake.

JUL. 6. 2010 11:19AM SAYISI DENE FN NO. 962 P. 2

SAYISI DENE FIRST NATION
TADOULE LAKE, MAN.

July 6, 2010

University of Manitoba
Dr. Linda Lamcombe
503-Basic Medical Sciences Building
745 Bannytne Ave.
Winnipeg, MB
R3E 0J9

Re: Letter received July 6, 2010

Sayisi Dene First Nation Chief Jimmy Thorassie and Councillors Steven Thorassie, Stewart Yassie, Tony Powderhorn would like to open an dialoge with the following;

1. Housing assessement
2. Collection of blood samples for genetic and vitamin D analyis
3. Collection of stories from people who have illness due to mold and mildew

The 80 houses in this community of Tadoule Lake, Mb, and the state of the people's in those houses to be investigated for health hazards. The survey would involved people's health in those houses, housing conditions, physical structure of the house - the condition of the housing material, ventilation, insulation, heating plumbing, water and the presence of mold), to interview for specific health
Information.

We the Council of Sayisi Dene First Nation request this approval from your department DEAN to move forward with this request as soon as possible.

We will await your response, We request to start immediately, the information will enable the leadership to make improvements for housing on local community of Tadoule Lake, Manitoba settlement.

Thank you for your kind consideration.

SDFN Chief and Councillors

Cc:
SDFN administration
SDFN finance

Figure A4: Research study outline for blood sample collection and participation signatures of Chief and Council: Sayisi Dene First Nations at Tadoule Lake.

JUL. 6. 2010 11:19AM SAYISI DENE FN NO. 962 P. 2

SAYISI DENE FIRST NATION
TADOULE LAKE, MAN.

July 6, 2010

University of Manitoba
Dr. Linda Lamcombe
503-Basic Medical Sciences Building
745 Bannytne Ave.
Winnipeg, MB
R3E 0J9

Re: Letter received July 6, 2010

Sayisi Dene First Nation Chief Jimmy Thorassie and Councillors Steven Thorassie, Stewart Yassie, Tony Powderhorn would like to open an dialoge with the following:

1. Housing assessement
2. Collection of blood samples for genetic and vitamin D analyis
3. Collection of stories from people who have illness due to mold and mildew

The 80 houses in this community of Tadoule Lake, Mb, and the state of the people's in those houses to be investigated for health hazards. The survey would involved people's health in those houses, housing conditions, physical structure of the house - the condition of the housing material, ventilation, insulation, heating plumbing, water and the presence of mold). to interview for specific health
Information.

We the Council of Sayisi Dene First Nation request this approval from your department DEAN to move forward with this request as soon as possible.

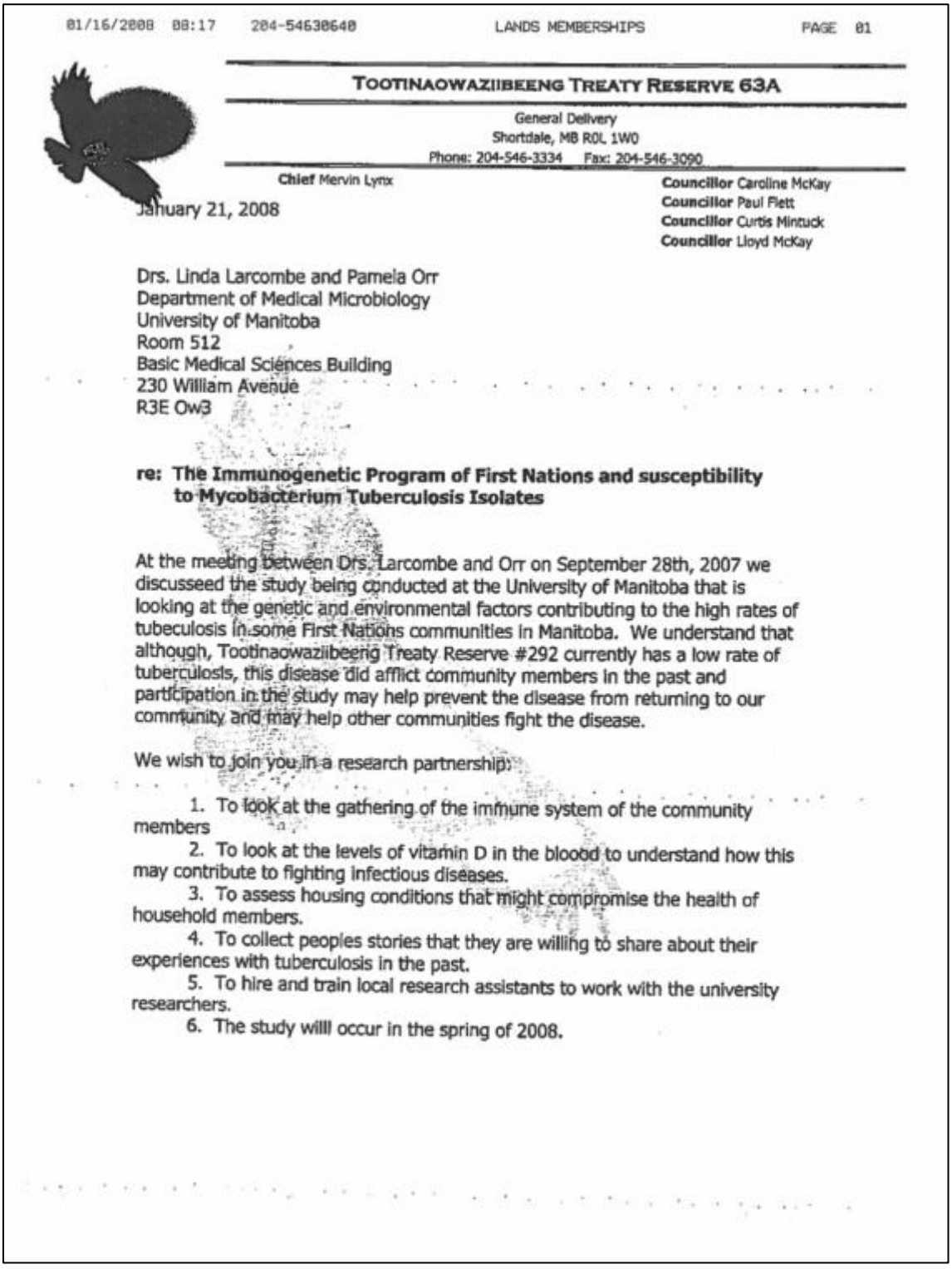
We will await your response, We request to start immediately, the information will enable the leadership to make improvements for housing on local community of Tadoule Lake, Manitoba settlement.

Thank you for your kind consideration.

SDFN Chief and Councillors

Cc:
SDFN administration
SDFN finance

Figure A5: Research study outline and participation signatures of Chief and Council: Tootinaowaziibeeng Treaty Reserve First Nation at Valley River.



01/16/2008 08:17 204-54630640 LANDS MEMBERSHIPS PAGE 01

TOOTINAOWAZIIBEENG TREATY RESERVE 63A

General Delivery
Shortdale, MB R0L 1W0
Phone: 204-546-3334 Fax: 204-546-3090

Chief Mervin Lynx

Councillor Caroline McKay
Councillor Paul Flett
Councillor Curtis Mintuck
Councillor Lloyd McKay

January 21, 2008

Drs. Linda Larcombe and Pamela Orr
Department of Medical Microbiology
University of Manitoba
Room 512
Basic Medical Sciences Building
230 William Avenue
R3E Ow3

re: The Immunogenetic Program of First Nations and susceptibility to Mycobacterium Tuberculosis Isolates

At the meeting between Drs. Larcombe and Orr on September 28th, 2007 we discusseed the study being conducted at the University of Manitoba that is looking at the genetic and environmental factors contributing to the high rates of tubeculosis in some First Nations communities in Manitoba. We understand that although, Tootinaowaziibeeng Treaty Reserve #292 currently has a low rate of tuberculosis, this disease did afflict community members in the past and participation in the study may help prevent the disease from returning to our community and may help other communities fight the disease.

We wish to join you in a research partnership:

1. To look at the gathering of the immune system of the community members
2. To look at the levels of vitamin D in the bloood to understand how this may contribute to fighting infectious diseases.
3. To assess housing conditions that might compromise the health of household members.
4. To collect peoples stories that they are willing to share about their experiences with tuberculosis in the past.
5. To hire and train local research assistants to work with the university researchers.
6. The study willl occur in the spring of 2008.

We understand that:

1. Community meetings will be held to provide open and sincere communication between the university researchers and the community.
2. Results will be shared with community.
3. Informed consent of the individual participants will be obtained.
4. Information about the people will be kept confidential and all data is kept secure.
5. The research will be respectful of our people and our traditions.
6. The university researchers will wish to communicate the results of the study to other academics through scientific presentations and by publishing the results.
   Confidentiality of individuals and the community will be maintained.
7. This research partnership follows the guidelines outlined by the Canadian Institutes of Health Research.

Respectfully yours,

Chief Mervin Lynx

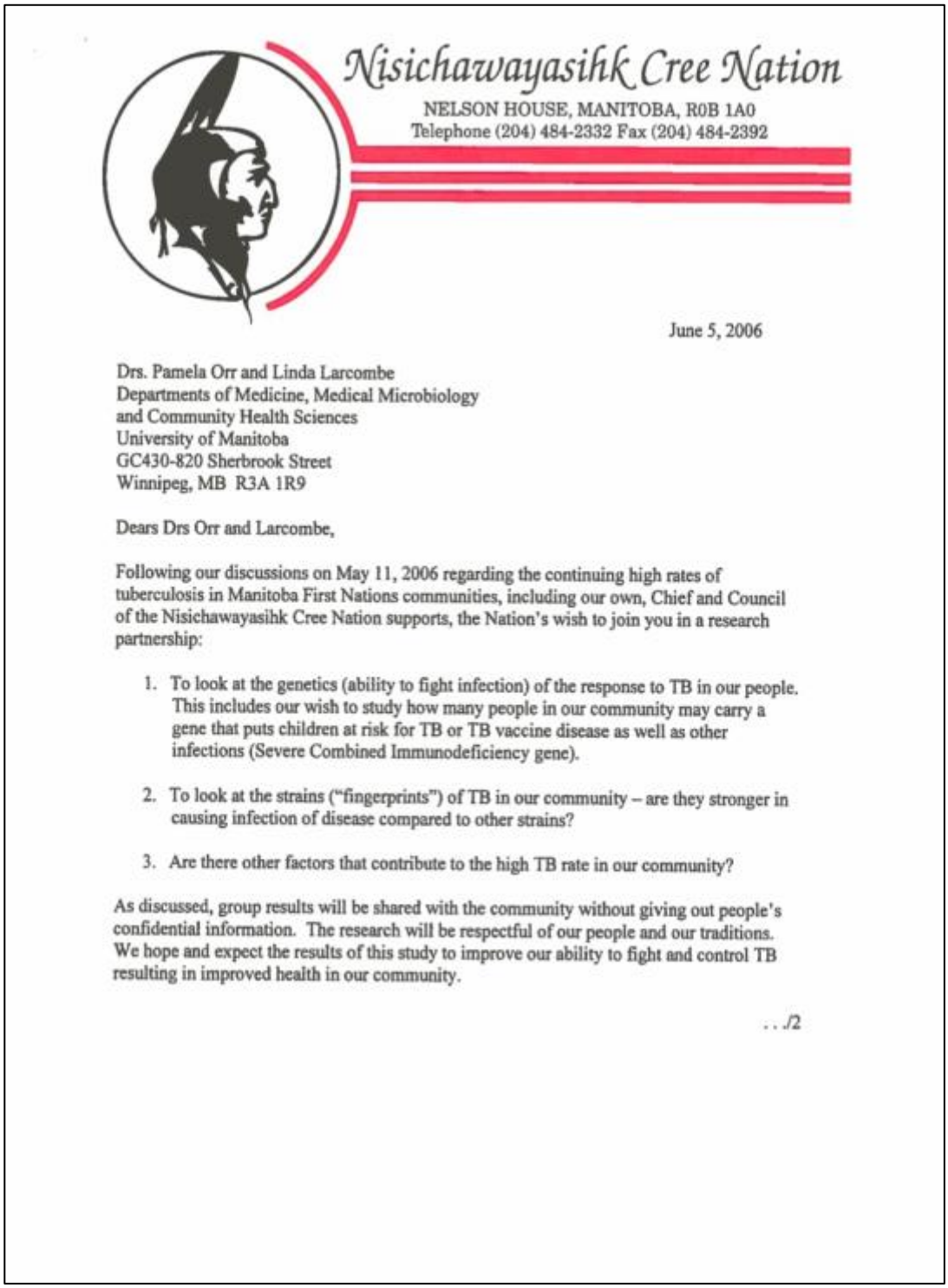
Councillor Paul Flett

Councillor Caroline McKay

Councillor Lloyd McKay

Councillor Curtis Mintuck

Figure A6: Research study outline and participation signatures of Chief and Council: Nisichawayasihk Cree Nation at Nelson House.



Nisichawayasihk Cree Nation

NELSON HOUSE, MANITOBA, R0B 1A0
Telephone (204) 484-2332 Fax (204) 484-2392

June 5, 2006

Drs. Pamela Orr and Linda Larcombe
Departments of Medicine, Medical Microbiology
and Community Health Sciences
University of Manitoba
GC430-820 Sherbrook Street
Winnipeg, MB R3A 1R9

Dears Drs Orr and Larcombe,

Following our discussions on May 11, 2006 regarding the continuing high rates of tuberculosis in Manitoba First Nations communities, including our own, Chief and Council of the Nisichawayasihk Cree Nation supports, the Nation's wish to join you in a research partnership:

1. To look at the genetics (ability to fight infection) of the response to TB in our people. This includes our wish to study how many people in our community may carry a gene that puts children at risk for TB or TB vaccine disease as well as other infections (Severe Combined Immunodeficiency gene).

2. To look at the strains ("fingerprints") of TB in our community – are they stronger in causing infection of disease compared to other strains?

3. Are there other factors that contribute to the high TB rate in our community?

As discussed, group results will be shared with the community without giving out people's confidential information. The research will be respectful of our people and our traditions. We hope and expect the results of this study to improve our ability to fight and control TB resulting in improved health in our community.

../2

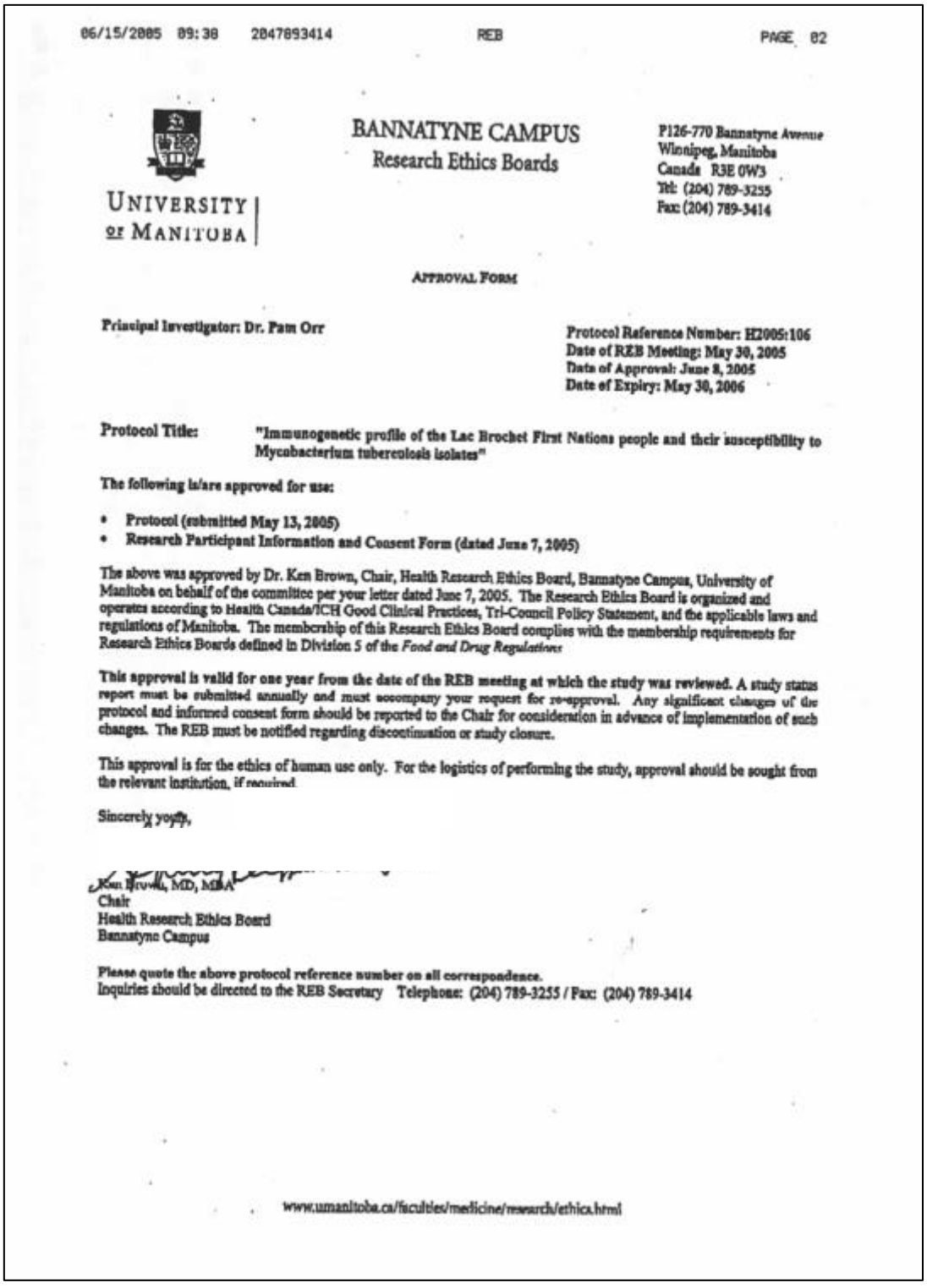
We look forward to working with you in this research partnership.

Yours sincerely,

Theresa Yetman, I/Chief Executive Officer
Nisichawayasihk Cree Nation

Cc Nisichawayasihk Cree Nation Chief and Council

Figure A7: Health Research Ethics Board of University of Manitoba guidelines for "Immunogenetic profile of the Lac Brochet First Nations people and their susceptibility to Mycobacterium tuberculosis isolates".



06/15/2005 09:38 2047893414 REB PAGE 02

UNIVERSITY of MANITOBA

BANNATYNE CAMPUS
Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. Pam Orr

Protocol Reference Number: H2005:106
Date of REB Meeting: May 30, 2005
Date of Approval: June 8, 2005
Date of Expiry: May 30, 2006

Protocol Title: "Immunogenetic profile of the Lac Brochet First Nations people and their susceptibility to Mycobacterium tuberculosis isolates"

The following is/are approved for use:

- Protocol (submitted May 13, 2005)
- Research Participant Information and Consent Form (dated June 7, 2005)

The above was approved by Dr. Ken Brown, Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated June 7, 2005. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations*

This approval is valid for one year from the date of the REB meeting at which the study was reviewed. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

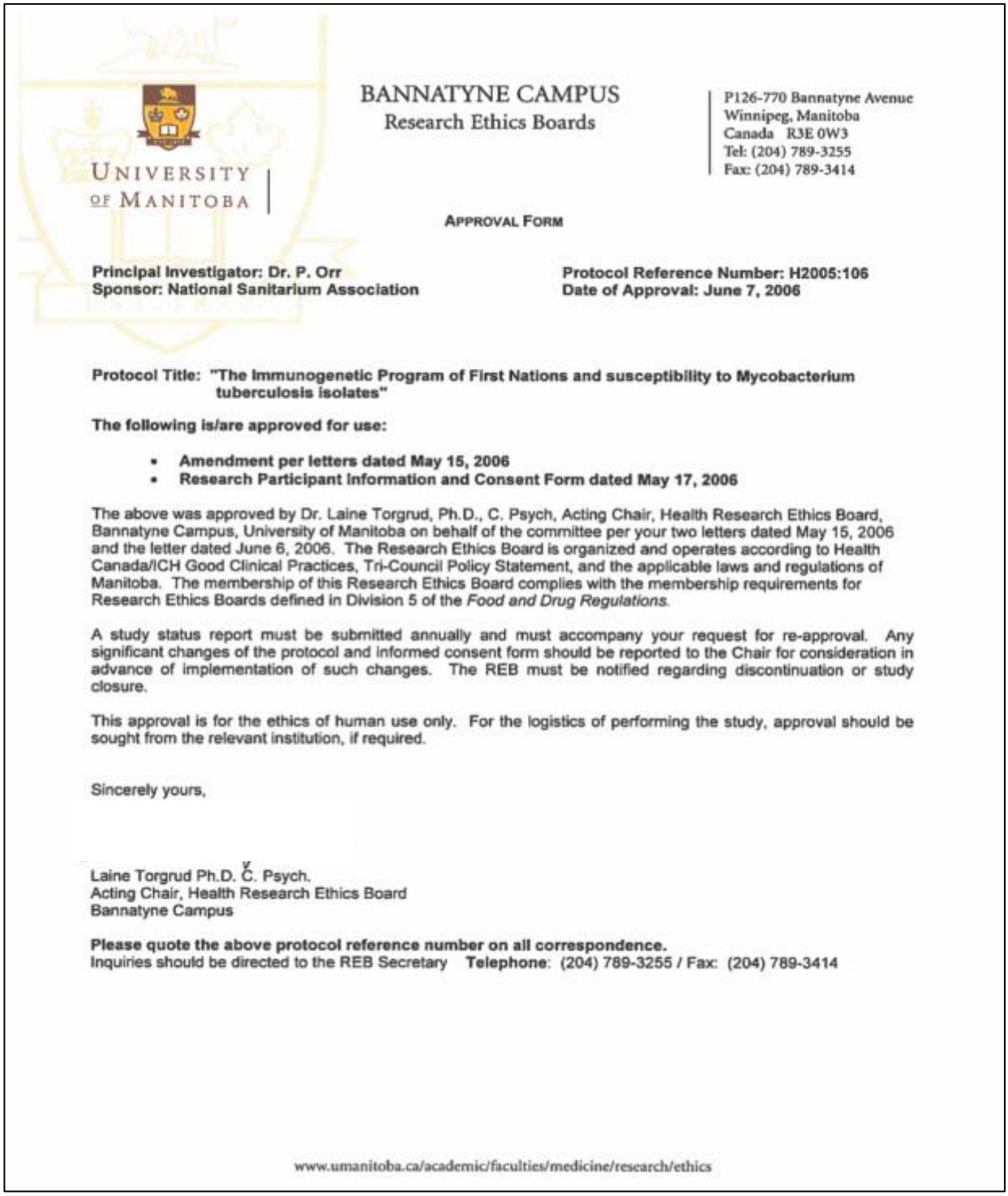
Sincerely yours,

Ken Brown, MD, MBA
Chair
Health Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary Telephone: (204) 789-3255 / Fax: (204) 789-3414

www.umanitoba.ca/faculties/medicine/research/ethics.html

Figure A8: Health Research Ethics Board of University of Manitoba 2008 amendment guidelines for "Immunogenetic profile of First Nations people and their susceptibility to Mycobacterium tuberculosis isolates".



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BANNATYNE CAMPUS
Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. P. Orr
Sponsor: National Sanitarium Association

Protocol Reference Number: H2005:106
Date of Approval: June 7, 2006

Protocol Title: "The Immunogenetic Program of First Nations and susceptibility to Mycobacterium tuberculosis isolates"

The following is/are approved for use:

- **Amendment per letters dated May 15, 2006**
- **Research Participant Information and Consent Form dated May 17, 2006**

The above was approved by Dr. Laine Torgrud, Ph.D., C. Psych, Acting Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your two letters dated May 15, 2006 and the letter dated June 6, 2006. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations*.

A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

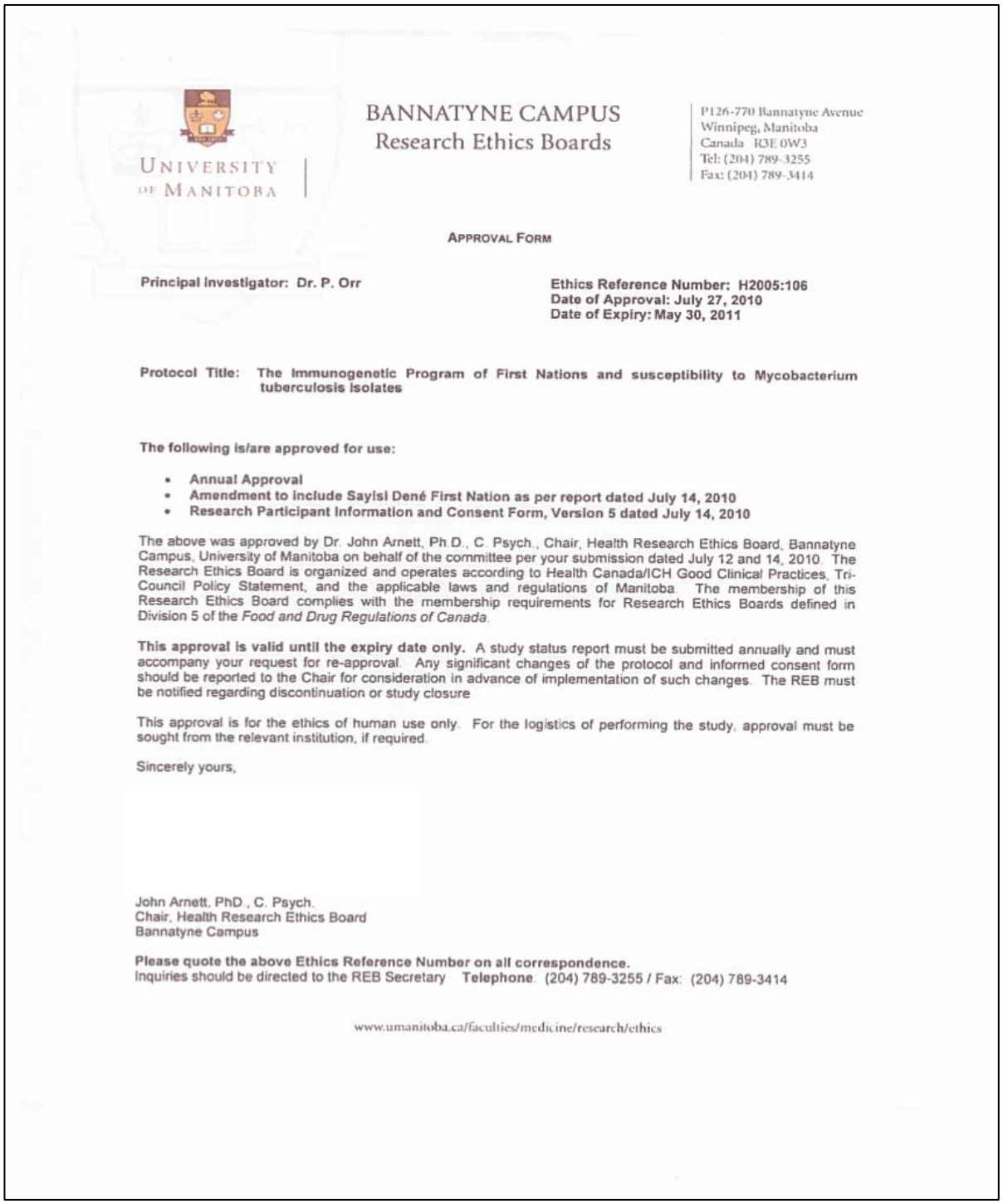
Sincerely yours,

Laine Torgrud Ph.D. C. Psych.
Acting Chair, Health Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone**: (204) 789-3255 / Fax: (204) 789-3414

www.umanitoba.ca/academic/faculties/medicine/research/ethics

Figure A9: Health Research Ethics Board of University of Manitoba 2010 amendment guidelines for "Immunogenetic profile of First Nations people and their susceptibility to Mycobacterium tuberculosis isolates".



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BANNATYNE CAMPUS
Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. P. Orr

Ethics Reference Number: H2005:106
Date of Approval: July 27, 2010
Date of Expiry: May 30, 2011

Protocol Title: The Immunogenetic Program of First Nations and susceptibility to Mycobacterium tuberculosis isolates

The following is/are approved for use:

- **Annual Approval**
- **Amendment to include Sayisi Dené First Nation as per report dated July 14, 2010**
- **Research Participant Information and Consent Form, Version 5 dated July 14, 2010**

The above was approved by Dr. John Arnett, Ph.D., C. Psych., Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your submission dated July 12 and 14, 2010. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations of Canada*.

This approval is valid until the expiry date only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval must be sought from the relevant institution, if required.

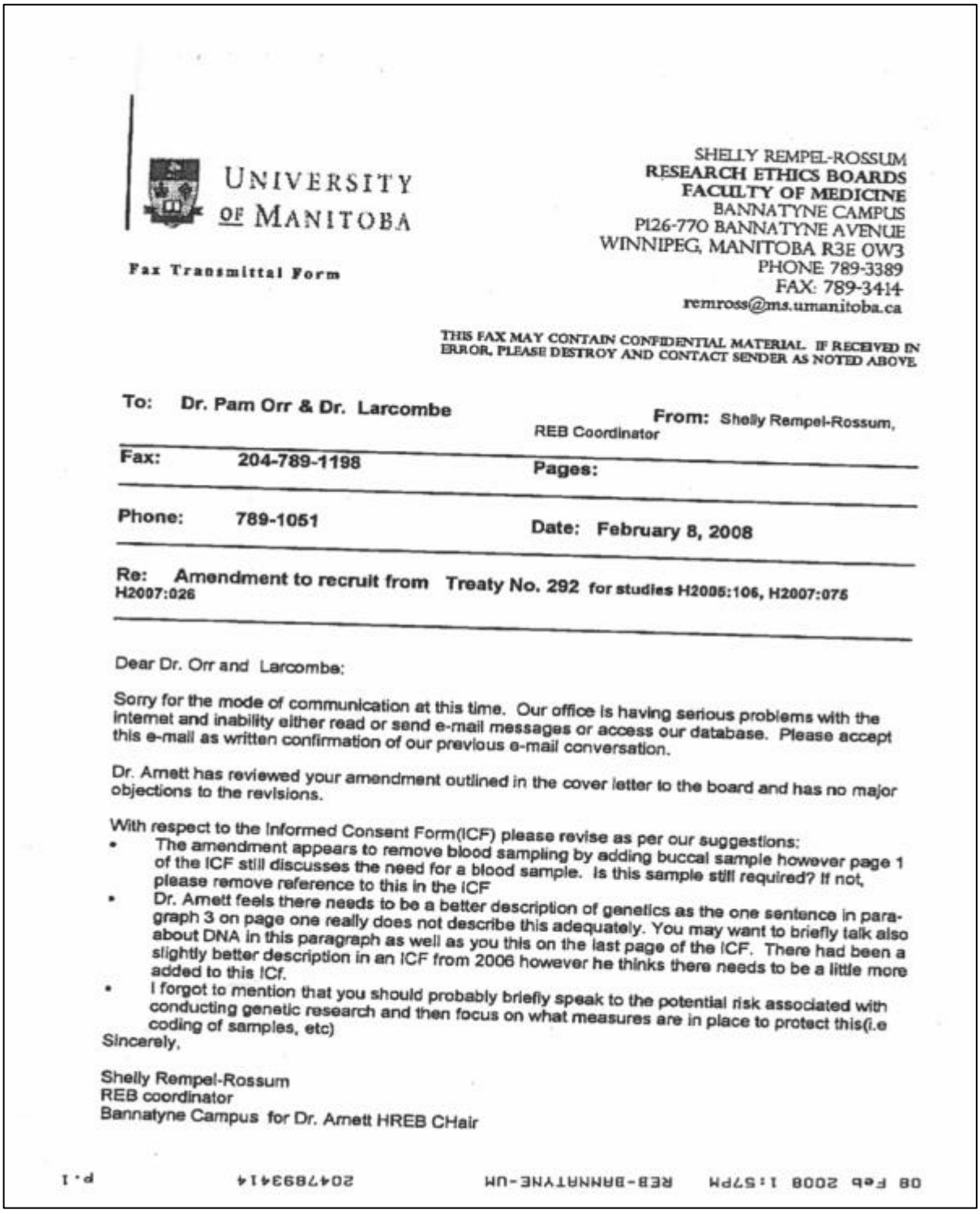
Sincerely yours,

John Arnett, PhD., C. Psych.
Chair, Health Research Ethics Board
Bannatyne Campus

Please quote the above Ethics Reference Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone**: (204) 789-3255 / Fax: (204) 789-3414

www.umanitoba.ca/faculties/medicine/research/ethics

Figure A10: Approval for Health Research Ethics Board of University of Manitoba amendment guidelines for "Immunogenetic profile of First Nations people and their susceptibility to Mycobacterium tuberculosis isolates".



UNIVERSITY OF MANITOBA

Fax Transmittal Form

SHELLY REMPEL-ROSSUM
RESEARCH ETHICS BOARDS
FACULTY OF MEDICINE
BANNATYNE CAMPUS
P126-770 BANNATYNE AVENUE
WINNIPEG, MANITOBA R3E 0W3
PHONE 789-3389
FAX: 789-3414
remross@ms.umanitoba.ca

To: Dr. Pam Orr & Dr. Larcombe **From:** Shelly Rempel-Rossum, REB Coordinator

Fax: 204-789-1198 **Pages:**

Phone: 789-1051 **Date:** February 8, 2008

Re: Amendment to recruit from Treaty No. 292 for studies H2005:106, H2007:075 H2007:026

Dear Dr. Orr and Larcombe:

Sorry for the mode of communication at this time. Our office is having serious problems with the internet and inability either read or send e-mail messages or access our database. Please accept this e-mail as written confirmation of our previous e-mail conversation.

Dr. Arnett has reviewed your amendment outlined in the cover letter to the board and has no major objections to the revisions.

With respect to the Informed Consent Form(ICF) please revise as per our suggestions:

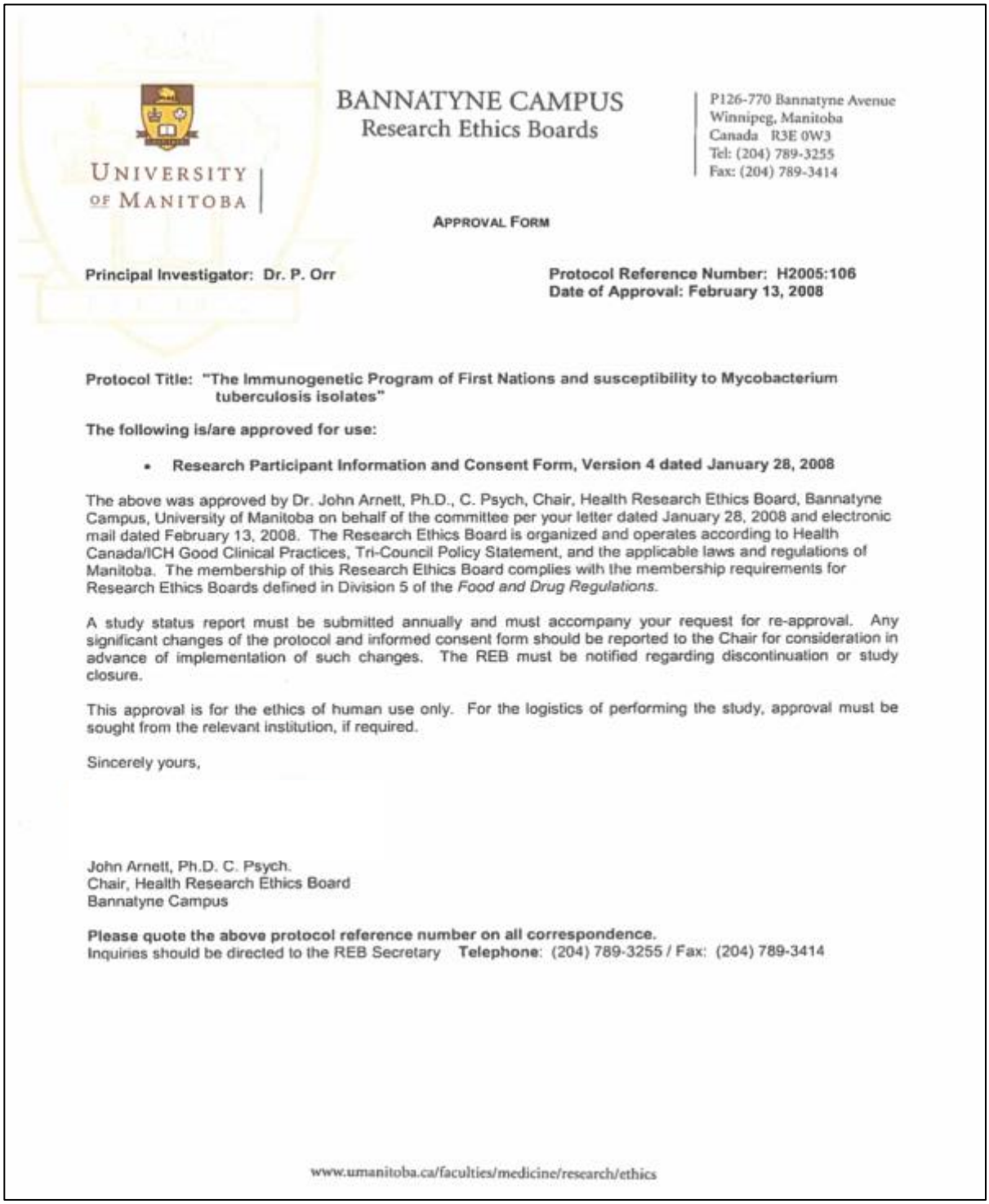
- The amendment appears to remove blood sampling by adding buccal sample however page 1 of the ICF still discusses the need for a blood sample. Is this sample still required? If not, please remove reference to this in the ICF
- Dr. Arnett feels there needs to be a better description of genetics as the one sentence in paragraph 3 on page one really does not describe this adequately. You may want to briefly talk also about DNA in this paragraph as well as you this on the last page of the ICF. There had been a slightly better description in an ICF from 2006 however he thinks there needs to be a little more added to this ICf.
- I forgot to mention that you should probably briefly speak to the potential risk associated with conducting genetic research and then focus on what measures are in place to protect this(i.e coding of samples, etc)

Sincerely,

Shelly Rempel-Rossum
REB coordinator
Bannatyne Campus for Dr. Arnett HREB CHair

08 Feb 2008 1:57PM REB-BANNATYNE-UM 2047893414 p.1

Figure A11: Health Research Ethics Board of University of Manitoba 2008 annual re-approval form for "Immunogenetic profile of First Nations people and their susceptibility to Mycobacterium tuberculosis isolates".



UNIVERSITY OF MANITOBA

BANNATYNE CAMPUS
Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. P. Orr

Protocol Reference Number: H2005:106
Date of Approval: February 13, 2008

Protocol Title: "The Immunogenetic Program of First Nations and susceptibility to Mycobacterium tuberculosis isolates"

The following is/are approved for use:

- **Research Participant Information and Consent Form, Version 4 dated January 28, 2008**

The above was approved by Dr. John Arnett, Ph.D., C. Psych, Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated January 28, 2008 and electronic mail dated February 13, 2008. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations*.

A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval must be sought from the relevant institution, if required.

Sincerely yours,

John Arnett, Ph.D. C. Psych.
Chair, Health Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone**: (204) 789-3255 / Fax: (204) 789-3414

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Figure A12: Health Research Ethics Board of University of Manitoba 2014 Certificate for annual approval for "Immunogenetic profile of First Nations people and their susceptibility to Mycobacterium tuberculosis isolates".

UNIVERSITY of MANITOBA | BANNATYNE CAMPUS Research Ethics Board

P126 - 770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Telephone 204-789-3255
Fax 204-789-3414

HEALTH RESEARCH ETHICS BOARD (HREB)

CERTIFICATE OF ANNUAL APPROVAL

PRINCIPAL INVESTIGATOR: Dr. P. Orr	INSTITUTION/DEPARTMENT: UofM / Internal Medicine	ETHICS #: HS14459 (H2005:106)
HREB MEETING DATE (if applicable):	APPROVAL DATE: April 2, 2014	EXPIRY DATE: May 30, 2015
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (if applicable):		

PROTOCOL NUMBER: NA	PROJECT OR PROTOCOL TITLE: The Immunogenetic Program of First Nations and susceptibility to Mycobacterium tuberculosis isolates
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: National Sanitarium Association	

Submission Date of Investigator Documents: April 1 and 2, 2014	HREB Receipt Date of Documents: April 1 and 2, 2014

REVIEW CATEGORY OF ANNUAL REVIEW: Full Board Review ☐ Delegated Review ☒

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name(if applicable)	Version(if applicable)	Date

Annual approval

Annual approval implies that the most recent HREB approved versions of the protocol, investigator Brochures, advertisements, letters of initial contact or questionnaires, and recruitment methods, etc. are approved.

Consent and Assent Form(s):

CERTIFICATION

The University of Manitoba (UM) Health Research Board (HREB) has reviewed the annual study status report for the research study/project named on this ***Certificate of Annual Approval*** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. Annual approval was granted by the Chair or Acting Chair, UM HREB, per the response to the conditions of approval outlined during the initial review (full board or delegated) of the annual study status report.

HREB ATTESTATION

The University of Manitoba (UM) Health Research Board (HREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulations of Manitoba. In respect to clinical trials, the HREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

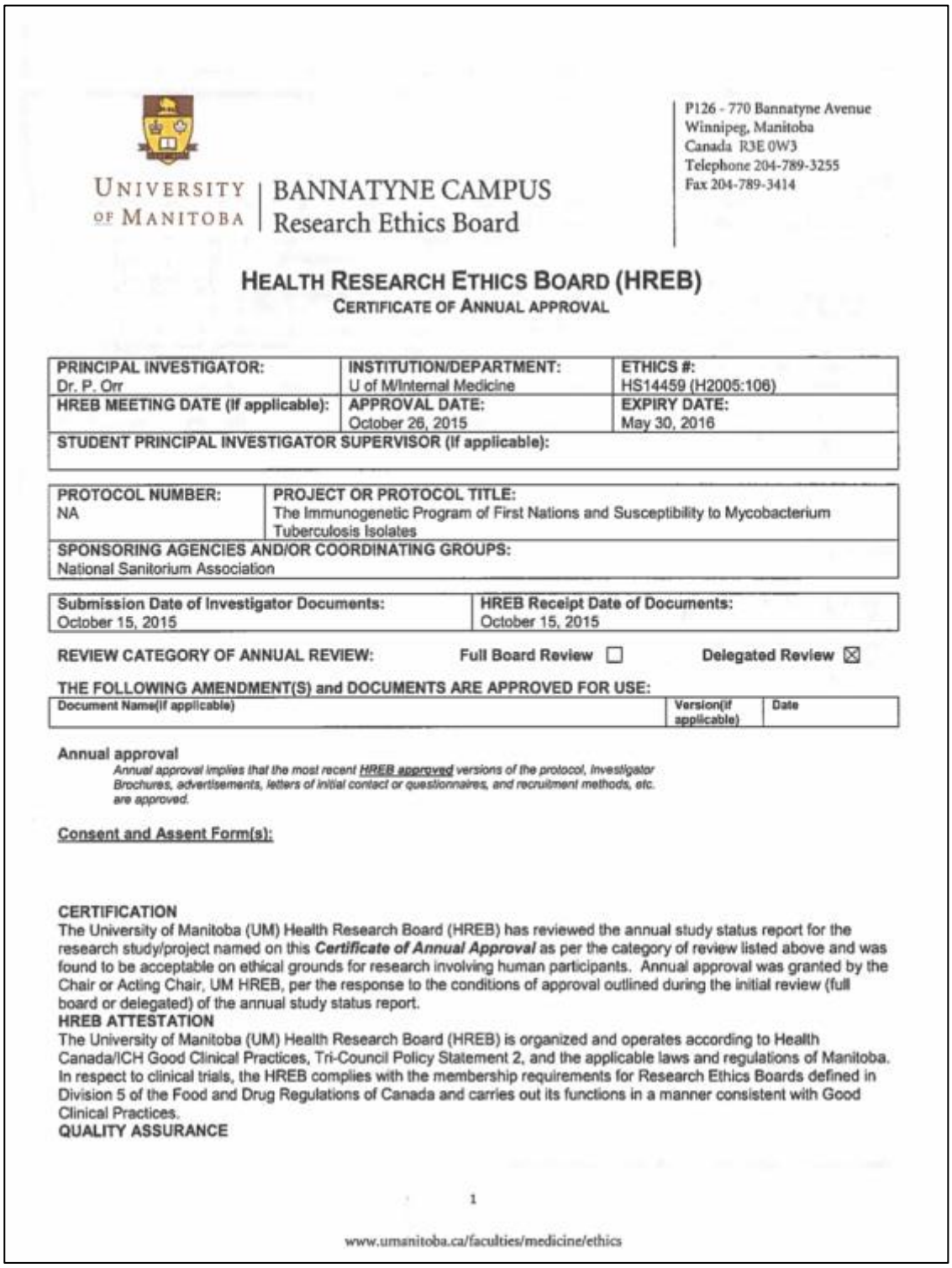
QUALITY ASSURANCE

The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

1

www.umanitoba.ca/faculties/medicine/ethics

Figure A13: Health Research Ethics Board of University of Manitoba 2015 Certificate for annual approval for "Immunogenetic profile of First Nations people and their susceptibility to Mycobacterium tuberculosis isolates".



UNIVERSITY OF MANITOBA | BANNATYNE CAMPUS Research Ethics Board

P126 - 770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Telephone 204-789-3255
Fax 204-789-3414

HEALTH RESEARCH ETHICS BOARD (HREB)
CERTIFICATE OF ANNUAL APPROVAL

PRINCIPAL INVESTIGATOR: Dr. P. Orr	INSTITUTION/DEPARTMENT: U of M/Internal Medicine	ETHICS #: HS14459 (H2005:106)
HREB MEETING DATE (If applicable):	APPROVAL DATE: October 26, 2015	EXPIRY DATE: May 30, 2016
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER: NA	PROJECT OR PROTOCOL TITLE: The Immunogenetic Program of First Nations and Susceptibility to Mycobacterium Tuberculosis Isolates
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: National Sanitorium Association	

Submission Date of Investigator Documents: October 15, 2015	HREB Receipt Date of Documents: October 15, 2015

REVIEW CATEGORY OF ANNUAL REVIEW: Full Board Review ☐ Delegated Review ☒

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name(If applicable)	Version(if applicable)	Date

Annual approval

Annual approval implies that the most recent HREB approved versions of the protocol, investigator Brochures, advertisements, letters of initial contact or questionnaires, and recruitment methods, etc. are approved.

Consent and Assent Form(s):

CERTIFICATION

The University of Manitoba (UM) Health Research Board (HREB) has reviewed the annual study status report for the research study/project named on this ***Certificate of Annual Approval*** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. Annual approval was granted by the Chair or Acting Chair, UM HREB, per the response to the conditions of approval outlined during the initial review (full board or delegated) of the annual study status report.

HREB ATTESTATION

The University of Manitoba (UM) Health Research Board (HREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulations of Manitoba. In respect to clinical trials, the HREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

QUALITY ASSURANCE

1

www.umanitoba.ca/faculties/medicine/ethics

The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

CONDITIONS OF APPROVAL:

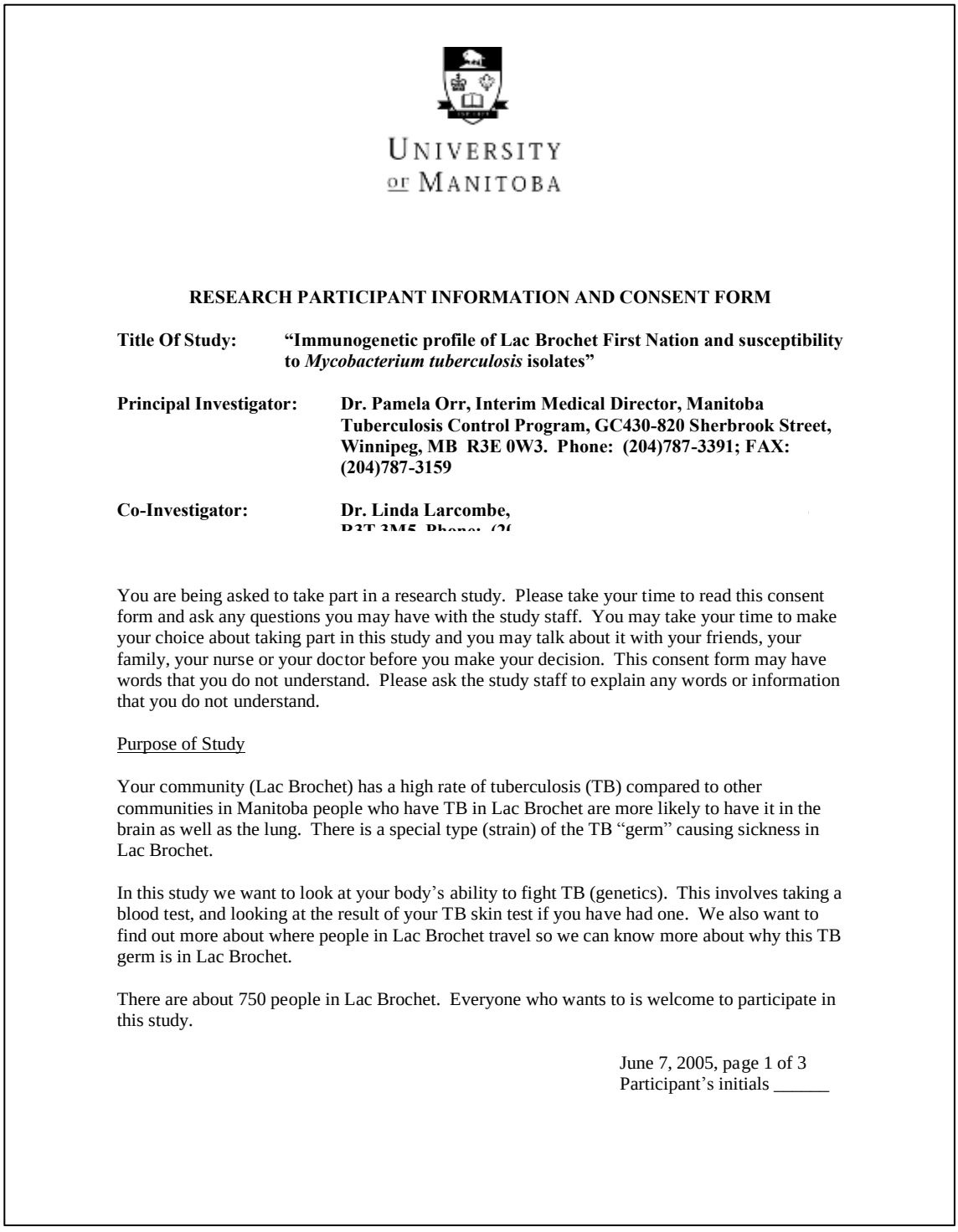
1. The study is acceptable on scientific and ethical grounds for the ethics of human use only. ***For logistics of performing the study, approval must be sought from the relevant institution(s).***
2. This research study/project is to be conducted by the local principal investigator listed on this certificate of approval.
3. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to the research study/project, and for ensuring that the authorized research is carried out according to governing law.
4. **This approval is valid until the expiry date noted on this certificate of annual approval. A Bannatyne Campus Annual Study Status Report** must be submitted to the REB within 15-30 days of this expiry date.
5. Any changes of the protocol (including recruitment procedures, etc.), informed consent form(s) or documents must be reported to the HREB for consideration in advance of implementation of such changes on the **Bannatyne Campus Research Amendment Form.**
6. Adverse events and unanticipated problems must be reported to the REB as per Bannatyne Campus Research Boards Standard Operating procedures.
7. The UM HREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report**.

Sincerely,

John Arnett, PhD., C. Pysch.
Chair, Health Research Ethics Board
Bannatyne Campus

Please quote the above Human Ethics Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone:** (204) 789-3255/ **Fax:** (204) 789-3414

Figure A14: Research participant information and consent form: Lac Brochet, Manitoba.



UNIVERSITY
of MANITOBA

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title Of Study: **"Immunogenetic profile of Lac Brochet First Nation and susceptibility to *Mycobacterium tuberculosis* isolates"**

Principal Investigator: **Dr. Pamela Orr, Interim Medical Director, Manitoba Tuberculosis Control Program, GC430-820 Sherbrook Street, Winnipeg, MB R3E 0W3. Phone: (204)787-3391; FAX: (204)787-3159**

Co-Investigator: **Dr. Linda Larcombe,**
R3T 3M5. Phone: (2(

You are being asked to take part in a research study. Please take your time to read this consent form and ask any questions you may have with the study staff. You may take your time to make your choice about taking part in this study and you may talk about it with your friends, your family, your nurse or your doctor before you make your decision. This consent form may have words that you do not understand. Please ask the study staff to explain any words or information that you do not understand.

Purpose of Study

Your community (Lac Brochet) has a high rate of tuberculosis (TB) compared to other communities in Manitoba people who have TB in Lac Brochet are more likely to have it in the brain as well as the lung. There is a special type (strain) of the TB "germ" causing sickness in Lac Brochet.

In this study we want to look at your body's ability to fight TB (genetics). This involves taking a blood test, and looking at the result of your TB skin test if you have had one. We also want to find out more about where people in Lac Brochet travel so we can know more about why this TB germ is in Lac Brochet.

There are about 750 people in Lac Brochet. Everyone who wants to is welcome to participate in this study.

June 7, 2005, page 1 of 3
Participant's initials _____

"Immunogenetic profile of Lac Brochet First Nation and susceptibility to *Mycobacterium tuberculosis isolates"*

Study Procedure

If you take part in this study you will have blood taken from you:

1½ tablespoons (14 cc) from you if you are an adult, or 1 teaspoon (5 cc) if you are a child.

This blood will be taken to Winnipeg where it will be examined. We will test the blood for ways in which the body can fight the TB germ.

When we take your blood we will ask you a few questions about your date of birth and where you regularly travel.

We will also look in your chart to see what the result of your TB skin test is if you have had one. The result of your skin test will be compared to the result of your blood test.

Risks and Discomforts

Taking blood causes a little pain where the needle goes into the skin. Sometimes the skin gets bruised where we take the blood. For children we can use a cream to decrease any pain the child may feel.

Benefits

There may or may not be any direct benefit to you from participating in this study. We hope the information learned from this study will help us in our fight against TB in Lac Brochet.

Cost

There is no cost to you to participate in the study.

Alternatives

You do not have to participate in the study. The staff at the Nursing Station and the visiting doctors (Dr. Lyons and Dr. Orr) provide regular medical care for people who have TB, have been in contact with TB, or who have questions.

Confidentiality

What we learn from this study will be shared with the people of Lac Brochet, but not in a way that identifies you. What we mean is that we will explain our results in a general way, not using names or any information that can identify a person.

We will keep your information confidential. When we do our research we use numbers as a code, not names. We keep our study results in a safe locked office.

Participant's initials _____

"Immunogenetic profile of Lac Brochet First Nation and susceptibility to *Mycobacterium tuberculosis isolates"*

Voluntary Participation/Withdrawal

Your decision to take part in this study is up to you. You can refuse to take part or decide later not to take part. If you do not take part in the study you will still get medical care at the Nursing Station the same as anyone else.

Questions

You are free to ask any questions you want, at any time. Dr. Orr can be reached at 1-204-787-3391, and Dr. Larcombe at 1-204-261-0654. Do not sign this form until all your questions have been answered in a satisfactory way.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with Dr. Orr and or her study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I have been able to make my own free choice about participating or not participating. I will be given a copy of this consent form after signing it. I understand that taking part in this study is my own choice and that I can leave at any time. I freely agree to participate in this research study.

I understand that information about me will be kept confidential, but that The University of Manitoba Research Ethics Board can check study records to make sure the study is being done in the right ethical way.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

We will keep the blood for 10 years. If a new test of the body's strength to fight TB becomes available, do you allow us to use your blood for testing?

☐ No ☐ Yes

Participant signature _____ Date _____

Participant printed name _____

Parent/legal guardian's signature _____ Date _____

Parent/legal guardian's printed name _____

Child's signature _____ Date _____

Figure A15: Research participant information and consent form: Tadoule Lake, Manitoba.

UNIVERSITY
of MANITOBA

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title Of Study: **"*The immunogenetic program of First Nations and susceptibility to Mycobacterium tuberculosis isolates*"**

Principal Investigator: **Dr. Pamela Orr, Medical Director, Manitoba Tuberculosis Control Program, GC 430-820 Sherbrook Street, Winnipeg, MB R3E 0W3. Phone: (204)787-3391; FAX: (204)787-3159**

Co-Investigator: **Dr. Linda Larcombe, Department of Medical Microbiology Rm 512 Basic Medical Sciences Building, University of Manitoba Phone: (204)789-1051; FAX: (204)789-1198**

DNA Consent Form (Sayisi Dene First Nation)

You are being asked to take part in a research study. Please take your time to read this consent form and ask any questions you may have with the study staff. You may take your time to make your choice about taking part in this study and you may talk about it with your friends, your family, your nurse or your doctor before you make your decision. This consent form may have words that you do not understand. Please ask the study staff to explain any words or information that you do not understand.

Purpose of Study

Your community (Sayisi Dene First Nation) currently has a low rate of tuberculosis (TB) compared to other First Nations communities in Manitoba. Other First Nations communities have some of the highest rates of TB in the world. In this study we want to look at your body's ability to fight TB (genetics) to better understand this variability. This study involves taking a cheek swab from participants to collect DNA. The DNA holds the genetic information that this study is interested in examining. We will examine the unique genetics of the First Nations people to help understand why some First Nations groups are at a higher risk of becoming infected with TB.

There are about 375 people at Tadoule Lake. Everyone who wants to is welcome to participate in this study.

Study Procedure

If you take part in this study you will have a buccal swabs rubbed against the inside of each cheek to collect skin cells.

We will ask you a few questions at the time we collect the samples including your date of birth

Participant's initials _____

and where you regularly travel.

The samples will be taken to Winnipeg where it will be examined. We will test the samples for ways in which the body can fight the TB germ.

When we take the samples we will ask you a few questions about your date of birth, home address and your general health status.

We will also look in your chart to see what the result of your TB skin test is if you have had one. The result of your skin test will be compared to the result of your blood test.

Risks and Discomforts

Taking a buccal swab from the inside of the cheek can cause some discomfort.

Benefits

There may or may not be any direct benefit to you from participating in this study. We hope the information learned from this study will help us in our fight against TB in First Nations groups.

Cost

There is no cost to you to participate in the study.

Alternatives

You do not have to participate in the study.

Confidentiality

What we learn from this study will be shared with the people of Sayisi Dené First Nation, but not in a way that identifies you. What we mean is that we will explain our results in a general way, not using names or any information that can identify a person.

We will keep your information confidential. When we do our research we use numbers as a code, not names. The genetic information from your cheek swab and the DNA will be kept in a secure laboratory facility and will be identified using a code, not your name.

We keep our study results in a safe locked office.

Voluntary Participation/Withdrawal

Your decision to take part in this study is up to you. You can refuse to take part or decide later not to take part. If you do not take part in the study you will still get medical care at the Nursing Station the same as anyone else.

Participant's initials _____

"*The immunogenetic program of First Nations and susceptibility to Mycobacterium tuberculosis isolates"*

Questions

You are free to ask any questions you want, at any time. Dr. Orr can be reached at 1-204-787-3391, and Dr. Larcombe at 1-204-789-1051. Do not sign this form until all your questions have been answered in a satisfactory way.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with Dr. Orr and or her study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I have been able to make my own free choice about participating or not participating. I will be given a copy of this consent form after signing it. I understand that taking part in this study is my own choice and that I can leave at any time. I freely agree to participate in this research study.

I understand that information about me will be kept confidential, but that The University of Manitoba Research Ethics Board can check study records to make sure the study is being done in the right ethical way.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

We will keep the DNA for 10 years. If a new test of the body's strength to fight TB becomes available, do you allow us to use your DNA for testing.

No ☐ Yes ☐

Participant signature _____ Date _____

Participant printed name _____ _____

Parent/legal guardian's signature _____ Date _____

Parent/legal guardian's printed name _____

Child's signature _____ Date _____

Participant's initials _____

Figure A16: Research participant information and consent form: Valley River, Manitoba.

UNIVERSITY
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RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title Of Study: **"*The immunogenetic program of First Nations and susceptibility to Mycobacterium tuberculosis isolates*"**

Principal Investigator: **Dr. Pamela Orr, Medical Director, Manitoba Tuberculosis Control Program, GC 430-820 Sherbrook Street, Winnipeg, MB R3E 0W3. Phone: (204)787-3391; FAX: (204)787-3159**

Co-Investigator: **Dr. Linda Larcombe, Department of Medical Microbiology Rm 512 Basic Medical Sciences Building, University of Manitoba Phone: (204)789-1051; FAX: (204)789-1198**

You are being asked to take part in a research study. Please take your time to read this consent form and ask any questions you may have with the study staff. You may take your time to make your choice about taking part in this study and you may talk about it with your friends, your family, your nurse or your doctor before you make your decision. This consent form may have words that you do not understand. Please ask the study staff to explain any words or information that you do not understand.

Purpose of Study

Your community (Valley River) currently has a low rate of tuberculosis (TB) compared to other First Nations communities in Manitoba. Other First Nations communities have some of the highest rates of TB in the world. In this study we want to look at your body's ability to fight TB (genetics) to better understand this variability. This study involves taking a cheek swab from participants to collect DNA. The DNA holds the genetic information that this study is interested in examining. We will examine the unique genetics of the First Nations people to help understand why some First Nations groups are at a higher risk of becoming infected with TB.

There are about 600 people in Valley River. Everyone who wants to is welcome to participate in this study.

Study Procedure

If you take part in this study you will have a buccal swabs rubbed against the inside of each cheek to collect skin cells.

We will ask you a few questions at the time we collect the samples including your date of birth and where you regularly travel.

Participant's initials _____

The samples will be taken to Winnipeg where it will be examined. We will test the samples for ways in which the body can fight the TB germ.

When we take the samples we will ask you a few questions about your date of birth, home address and your general health status.

We will also look in your chart to see what the result of your TB skin test is if you have had one. The result of your skin test will be compared to the result of your blood test.

Risks and Discomforts

Taking a buccal swab from the inside of the cheek can cause some discomfort.

Benefits

There may or may not be any direct benefit to you from participating in this study. We hope the information learned from this study will help us in our fight against TB in First Nations groups.

Cost

There is no cost to you to participate in the study.

Alternatives

You do not have to participate in the study.

Confidentiality

What we learn from this study will be shared with the people of Valley River, but not in a way that identifies you. What we mean is that we will explain our results in a general way, not using names or any information that can identify a person.

We will keep your information confidential. When we do our research we use numbers as a code, not names. The genetic information from your cheek swab and the DNA will be kept in a secure laboratory facility and will be identified using a code, not your name.

We keep our study results in a safe locked office.

Voluntary Participation/Withdrawal

Your decision to take part in this study is up to you. You can refuse to take part or decide later not to take part. If you do not take part in the study you will still get medical care at the Nursing Station the same as anyone else.

Participant's initials _____

"*The immunogenetic program of First Nations and susceptibility to Mycobacterium tuberculosis isolates"*

Questions

You are free to ask any questions you want, at any time. Dr. Orr can be reached at 1-204-787-3391, and Dr. Larcombe at 1-204-789-1051. Do not sign this form until all your questions have been answered in a satisfactory way.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with Dr. Orr and or her study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I have been able to make my own free choice about participating or not participating. I will be given a copy of this consent form after signing it. I understand that taking part in this study is my own choice and that I can leave at any time. I freely agree to participate in this research study.

I understand that information about me will be kept confidential, but that The University of Manitoba Research Ethics Board can check study records to make sure the study is being done in the right ethical way.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

We will keep the DNA for 10 years. If a new test of the body's strength to fight TB becomes available, do you allow us to use your DNA for testing.

☐ No ☐ ☐ Ye ☐

Participant signature _____ Date _____

Participant printed name _____

Parent/legal guardian's signature _____ Date _____

Parent/legal guardian's printed name _____

Child's signature _____ Date _____

Participant's initials _____

Figure A17: Research participant information and consent form: Nelson House, Manitoba.

UNIVERSITY
of MANITOBA

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title Of Study: **"The immunogenetic program of First Nations and susceptibility to Mycobacterium tuberculosis isolates"**

Principal Investigator: **Dr. Pamela Orr, Interim Medical Director, Manitoba Tuberculosis Control Program, GC430-820 Sherbrook Street, Winnipeg, MB R3E 0W3. Phone: (204) 787-3391; FAX: (204) 787-3159**

Co-Investigator: **Dr. Linda Larcombe,**

You are being asked to take part in a research study. Please take your time to read this consent form and ask any questions you may have with the study staff. You may take your time to make your choice about taking part in this study and you may talk about it with your friends, your family, your nurse or your doctor before you make your decision. This consent form may have words that you do not understand. Please ask the study staff to explain any words or information that you do not understand.

Purpose of Study

First Nations people in Manitoba have a high rate of TB although the prevalence varies between communities. In this study we want to look at your body's ability to fight TB (genetics) to better understand this variability. This involves taking a blood test, and looking at the result of your TB skin test if you have had one. This study will examine the unique genetics of the First Nations people to help understand why this group is at a higher risk of becoming infected with TB. We also want to find out more about where people in Nelson House travel so we can know more about the distribution of the TB germ.

There are about 2500 people at Nelson House. Everyone who wants to is welcome to participate in this study.

 Participant's initials _____

"*The immunogenetic program of First Nations and susceptibility to Mycobacterium tuberculosis isolates"*

Study Procedure
If you take part in this study you will have blood taken from you:

1½ tablespoons (14 cc) from you if you are an adult, or 1 teaspoon (7 cc) if you are a child.

This blood will be taken to Winnipeg where it will be examined. We will test the blood for ways in which the body can fight the TB germ.

When we take your blood we will ask you your date of birth and where you regularly travel.

We will look in your chart to see what the result of TB skin test is if you have had one. The result of your skin test will be compared to the result of you blood test. If you have had TB we will ask the National Microbiology Laboratory in Winnipeg for the "DNA fingerprint" of the germ or the specific strain of TB.

Risks and Discomforts
Taking blood causes a little pain where the needle goes into the skin. Sometimes the skin gets bruised where we take the blood. For children we can use a cream to decrease any pain the child may feel.

Benefits
There may or may not be any direct benefit to you from participating in this study. We hope the information learned from this study will help us in our fight against TB among First Nations in Manitoba.

Cost

There is no cost to you to participate in the study.

Alternatives
You do not have to participate in the study. The staff at the Nursing Station and the visiting doctors provide regular medical care for people who have TB, have been in contact with TB, or who have questions.

Confidentiality
What we learn from this study will be shared with the people of Nelson House, but not in a way that identifies you. What we mean is that we will explain our results in a general way, not using names or any information that can identify a person.

We will keep your information confidential. When we do our research we use numbers as a code, not names. We keep our study results in a safe locked office.

 Participant's initials _____

"*The immunogenetic program of First Nations and susceptibility to Mycobacterium tuberculosis isolates"*

Voluntary Participation/Withdrawal

Your decision to take part in this study is up to you. You can refuse to take part or decide later not to take part. If you do not take part in the study you will still get medical care at the Nursing Station the same as anyone else.

Questions

You are free to ask any questions you want, at any time. Dr. Orr can be reached at 1-204-787-3391, and Dr. Larcombe at 1-204-789-1148. Do not sign this form until all your questions have been answered in a satisfactory way.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with Dr. Orr and or her study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I have been able to make my own free choice about participating or not participating. I will be given a copy of this consent form after signing it. I understand that taking part in this study is my own choice and that I can leave at any time. I freely agree to participate in this research study.

I understand that information about me will be kept confidential, but that The University of Manitoba Research Ethics Board can check study records to make sure the study is being done in the right ethical way.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

We will keep the blood for 10 years. If a new test of the body's ability to fight TB becomes available do you allow us to use you blood for testing?
Yes or No.

At the community's request we have been asked to store blood for the future analysis of the severe combined immunodeficiency (SCIDs). Do you allow us to store the blood for analysis in the future. Yes or No.

☐ No ☐ Yes

Participant signature _____ Date _____

Participant printed name _____

Parent/legal guardian's signature _____ Date _____

Parent/legal guardian's printed name _____

Child's signature _____ Date _____

Participant's initials _____

Figure A18: Research participant information and consent form: non-Indigenous.

DIAGNOSTIC SERVICES OF MANITOBA | SERVICES DE DIAGNOSTIC DU MANITOBA

Transplant Immunology Laboratory

Consent Form

Transplant Immunology Laboratory

Voluntary Cheek Swab Collection:

I, _____, hereby volunteer and consent to provide a cheek swab to the Transplant Immunology Laboratory staff and to donate that cheek swab to the Transplant Immunology Laboratory. My cheek swab may only be used in tests or procedures performed by the Transplant Immunology Laboratory.

I further understand that in accordance with PHIA regulations, my identity and any results arising from the testing of my donated cheek swab for such purposes will remain anonymous to all other than those specifically requiring this information for the performance and interpretation of the actual test or procedure.

Signed and witnessed this _____day of_____, 20_____.

_____ _____

Signature Witness to Signature

For Lab Use Only:	
Accession #:	

Manual Use TI.100.011 20Oct2006

Table A1: Pearson chi square tests and associated p-values for comparing individual population alleles. Corrected for multiple populations using Bonferroni correction. $P \leq 0.0045$ is significant and indicated in bold type.

Population	IL-17A χ^2 (p value)	IL-17RA χ^2 (p value)	IL-23R χ^2 (p value)	P2RX7 χ^2 (p value)	IFN-γR χ^2 (p value)
First Nation: Non-Indigenous	29.107 **(0.0000)**	26.707 **(0.0000)**	18.519 **(0.0000)**	26.448 **(0.0000)**	5.424 (0.0199)
Dene 1: Non-Indigenous	8.174 **(0.0043)**	20.517 **(0.0000)**	13.676 **(0.0002)**	60.385 **(0.0000)**	1.023 (0.3119)
Dene 1: Dene 2	2.884 (0.0895)	1.359 (0.2437)	0.900 (0.3427)	3.329 (0.0681)	0.484 (0.4865)
Dene 1: Cree	2.061 (0.1511)	3.578 (0.0586)	2.527 (0.1119)	44.050 **(0.0000)**	4.254 (0.0391)
Dene 1: Saulteaux	2.065 (0.1507)	2.978 (0.0844)	0.077 (0.7815)	29.485 **(0.0000)**	2.828 (0.0927)
Dene 2: Non-Indigenous	18.886 **(0.0000)**	8.916 (**0.0028**)	6.070 (0.0137)	28.540 **(0.0000)**	0.038 (0.8453)
Dene 2: Cree	0.037 (0.8478)	8.073 **(0.0045)**	0.407 (0.5236)	23.111 **(0.0000)**	6.674 (0.0098)
Dene 2: Saulteaux	0.081 (0.7760)	0.280 (0.5968)	1.347 (0.2459)	11.806 **(0.0006)**	5.042 (0.0247)
Cree: Non-Indigenous	15.727 **(0.0001)**	37.306 **(0.0000)**	2.723 (0.0989)	0.428 (0.5131)	9.055 **(0.0026)**

Cree: Saulteaux	0.006 (0.9359)	11.241 **(0.0008)**	3.099 (0.0783)	2.906 (0.0883)	0.211 (0.6547)
Saulteaux: Non-Indigenous	17.271 **(0.0000)**	5.681 (0.0171)	13.685 **(0.0002)**	1.884 (0.1699)	7.233 (0.0072)

Table A2: Pearson chi square/Fisher exact tests and associated p-values for comparing individual population genotypes. * = Fisher exact test. Corrected for multiple populations using Bonferroni correction. $P \leq 0.0045$ is significant and indicated in bold type.

Population	IL-17A χ^2 (p value)	IL-17AR χ^2 (p value)	IL-23R χ^2 (p value)	P2X7 χ^2 (p value)	IFN-γR χ^2 (p value)
First Nation: Non-Indigenous	29.85 **(0.0000)**	30.80 **(0.0000)**	18.01 **(0.0001)**	18.81 **(0.0001)**	4.86 (0.0880)
Dene 1: Non-Indigenous	12.65 **(0.0018)**	23.34 **(0.0000)**	14.16 **(0.0008)**	42.28 **(0.0000)**	0.96 (0.6188)
Dene 1: Dene 2	12.23 **(0.0022)**	1.82 (0.4025)	* (0.6441)	2.54 (0.2808)	0.43 (0.8065)
Dene 1: Cree	8.84 (0.0120)	4.89 (0.0867)	* (0.3012)	28.03 **(0.0000)**	3.85 (0.1459)
Dene 1: Saulteaux	11.66 **(0.0029)**	4.39 (0.1114)	* (0.3229)	24.16 **(0.0000)**	3.52 (0.1720)
Dene 2: Non-Indigenous	19.91 **(0.0000)**	* **(0.0031)**	7.12 (0.0284)	21.6 **(0.0000)**	0.13 (0.9371)
Dene 2: Cree	0.16 (0.9231)	9.98 (0.0068)	* (0.7798)	15.85 **(0.0004)**	5.74 (0.0567)
Dene 2: Saulteaux	0.07 (0.9656)	* (0.6482)	* (0.1508)	11.39 **(0.0034)**	5.62 (0.0602)
Cree: Non-Indigenous	16.16 **(0.0003)**	36.69 **(0.0000)**	3.29 (0.1930)	0.35 (0.8395)	8.48 (0.0144)
Cree: Saulteaux	0.10 (0.9512)	12.17 **(0.0023)**	* (0.0703)	* (0.2520)	* (0.4541)
Saulteaux: Non-Indigenous	18.23 **(0.0001)**	* (0.0415)	6.21 (0.0448)	2.3 (0.3166)	7.08 (0.0290)