Association of Killer Immunoglobulin-like Receptor (KIR) Genes with Tuberculosis Disease in Two Canadian Cohorts

By

Kali Braun

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

Department of Medical Microbiology and Infectious Diseases

Faculty of Medicine

University of Manitoba

Winnipeg, Manitoba, Canada

Abstract

Tuberculosis (TB) is a respiratory disease caused by *Mycobacterium tuberculosis.* TB is responsible for more deaths than any other single pathogen. In Canada, and more specifically in Canadian-born Aboriginals and foreign born populations, high incidence of TB causes significant morbidity and mortality. Genetic susceptibility or resistance to infectious disease such as tuberculosis has been correlated with ethnicity, which in conjunction with environmental and host risk factors, can ultimately determine TB disease progression.

Killer immunoglobulin-like receptors (KIR) on natural killer (NK) cells interact with other immune cells to monitor the immune system and combat infectious diseases, such as tuberculosis. The balance of activating and inhibiting KIR interactions helps determine the NK cell response. The presence or absence of specific KIR genes, individually or in conjunction, may be associated with tuberculosis (active, latent, or uninfected disease status) as well as ethnicity of an individual. It is hypothesized that the differences in KIR profiles, gene frequencies, and/or haplotypes in Canadian born Aboriginal, Canadian born non-Aboriginal, and foreign born individuals elicits a differential activation or inhibition profile, resulting in differential cytokine expression and eventually contributes to the outcome of TB infection. Identification of ethnic specific genes that confer host susceptibility to TB infection will help to better understand the interaction of host genetics and the immune system. The outcome of the human immune response to tuberculosis may determine the type of infection or disease that develops. In this study we examined the enrichment or depletion of KIR genes in different ethnic populations in Manitoba with special focus on active, latent, and

uninfected TB status. In addition, we sought to explore the statistical correlation between TB status and inhibitory/stimulatory KIR haplotypes.

We found significant differences in KIR genes frequencies, not only between different population groups, but within subgroups as well. Active, latent, and tuberculosis uninfected individuals also showed differences in specific KIR gene frequencies. Analysis of KIR profiles revealed diversity among population and TB status groups. Lastly, significant differences were seen in KIR haplotypes across population groups. The skewed distribution of A-containing centromeric haplotypes (containing fewer activating genes) along with the increased presence of TB disease within these same haplotypes suggests a correlation. Further work, such as sequence analysis of target regions, KIR-HLA disease association studies, and cytokine response studies are needed to further characterize the relationship between *Mycobacterium tuberculosis* and KIRs in individuals with tuberculosis.

Acknowledgements

I would firstly like to thank my supervisor Dr. Meenu Sharma for her advice and support throughout my project. My time in her lab has allowed me to grow professionally and personally.

I would like to thank the members of my committee, Drs. Meenu Sharma, Linda Larcombe, T. Blake Ball, and Ann Karen Brassinga, for their guidance during my project. I would also like to thank Sara Christianson and the staff of the NRCM as well as Kate Decter for their technical assistance. Funding support was provided through a stipend from the University of Manitoba.

Thank you to all my grad student friends: a tight-knit family who can understand the pains (and triumphs) of day-to-day grad student life.

Thanks to my parents for moral support and unconditional love throughout my University career thus far, and for wherever the path may take me next. Also, thank you Mom for proof reading this thesis.

Lastly, I would like to thank my best friend, and husband, Mike for being so supportive, loving, and understanding. He and the boys (Forty, Logan, and Steve) brighten every day. I look forward to the adventures of parenthood which we are about to embark on in a few short weeks.

TABLE OF CONTENTS

List of Tables

List of Figures

List of Abbreviations

AFB	Acid-fast bacilli
ATS	American Thoracic Society
ApaI	Restriction enzyme
BCG	Bacille Calette-Guérin
BsmI	Restriction enzyme
CDC	Centers for Disease Control and Prevention
CFP-10	*M. tuberculosis*-specific antigen
CMV	Cytomegalovirus
CTLD	C-type lectin domain family
DAP12	Signalling adaptor molecule
DNA	Deoxyribonucleic acid
DOT	Directly observed therapy
ELISA	Enzyme-linked immunosorbent assay
EMB	Ethambutol
ESAT-6	*M. tuberculosis*-specific antigen
FokI	Restriction enzyme
HCV	Herpes simplex virus
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
IDSA	Infectious Disease Society of America
IFN	Interferon
IGRA	Interferon-gamma release assay
Ig-SF	Immunoglobulin superfamily
IL	Interleukin
INH	Isoniazid
ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibition motif
KIR	Killer immunoglobulin-like receptor
KLR	Killer cell lectin-like receptor
LAIR	Leukocyte-associated inhibitory receptor
LIR/ILT	Leukocyte immunoglobulin-like receptor/immunoglobulin-like transcript
LRC	Leukocyte receptor complex
LTBI	Latent tuberculosis infection
MDA	Multiple displacement amplification
MGIT	Mycobacterial growth indicator tube
MHC	Major histocompatibility complex
NCR	Natural cytotoxicity receptor
NK	Natural killer
NKG2	Killer cell lectin-like receptor
NKp44/46	Natural cytotoxicity receptors
NRAMP1	Natural resistance associated macrophage protein 1
NTM	Non-tuberculous mycobacteria
PCR	Polymerase chain reaction
PPD	Purified protein derivative

PTPN6	Phosphatase
PTPN22	Intracellular lymphoid-specific phosphatase
PZA	Pyrazinamide
Rab	Ras-associated small GTP-ases
RIF	Rifampin
RNA	Ribonucleic acid
RPT	Rifapentine
SLC11A1	Solute carrier family 11 member 1
SSP	Sequence specific primers
TaqI	Restriction enzyme
TB	Tuberculosis
TB7.7	*M. tuberculosis*-specific antigen
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TST	Tuberculin skin test
UPGMA	Unweighted pair group method with arithmetic mean
VDR	Vitamin D receptor
WHO	World Health Organization

List of Copyrighted Material for which Permission was Obtained

Figure 2: KIR gene organization. Center portion of figure adapted from *The KIR Gene Cluster* (Carrington and Norman, 2003). Publication in public domain, no permission required via National Center for Biotechnology Information (NCBI) Bookshelf.

Figure 4: Compilation of KIR disease association studies (Bashirova et al., 2006). Permission obtained via Copyright Clearance Center on June 10, 2013.

Figure 5: Traditional areas of the Dene, Cree and Ojibwa First Nations. Unpublished figured used with permission via personal communication with committee member Dr. Linda Larcombe, 2013.

1. Introduction

1.1. Mycobacteria

The genus *Mycobacterium* is the only genus in the family *Mycobacteriaceae*. Mycobacteria are aerobic, non-spore-forming, non-motile rods which may branch upon multiplication (130). They range in size from 0.2 - 0.6 μm by 1 – 10 μm. The colony morphology of *Mycobacterium* spp. can range from smooth to rough, and may or may not be pigmented. Certain species of Mycobacteria are slow-growing compared to other bacteria, with generations times up to 20 hours (130). They have a typical cell wall structure; however, the presence of mycolic acid provides a hydrophobic permeability barrier that requires specialized staining techniques (see section 1.1.3.3).

Mycobacteria can be sub classified into *M. tuberculosis* complex and non-tuberculous mycobacteria (NTM). The *M. tuberculosis* complex includes *M. tuberculosis, M. bovis, M. bovis* bacilli Calette-Guérin (BCG) vaccine strain, *M. africanum, M. caprae, M. microti, M. canettii,* and *M. pinnipedii.* All of these species belong to the same complex based on genetic homogeneity (130). Only 0.01 - 0.03% nucleotide variation exists between these species (16, 54).

1.1.1. Tuberculosis Disease Burden

Of all the species in the genus, the most pathogenic organisms belong to the *Mycobacterium tuberculosis* complex. The World Health Organization estimated the global prevalence of *M. tuberculosis* infection was 33%, with 9.2 million new cases of

tuberculosis (TB) in 2010 (128/100,000 population) (141). In the same year, 1.1 million people died of TB. When looking at the national scale, Canada reported 1577 new active cases of TB (4.6/100,000) in 2010 (126). The incidence of active TB disease in Manitoba in 2010 was 10.7/100,000 population, with a disproportionate amount of TB in Canadian-born Aboriginal[1] peoples (39.8/100,000) compared with Canadian-born non-Aboriginal peoples (1.6/100,000) (126). Furthermore, the overall rate of TB on First Nations reserves in Manitoba reached 58.3/100,000 (126). Rates of TB in certain Manitoba First Nations communities can exceed 400/100,000 (100, 116). Foreign born individuals living in Manitoba had an incidence of 20.7/100,000 (126). Overall, different population groups in the province of Manitoba have largely elevated rates of TB disease, contributing to a provincial rate more than double that of the national rate.

[1]*Aboriginal – persons who have identified themselves as being North American Indian (First Nations), Inuit or Métis, according to Section 35 of the Constitution Act of 1982.*

1.1.2. Tuberculosis Disease

Pulmonary tuberculosis is a respiratory illness caused by *Mycobacterium tuberculosis* (130). Approximately 90% of infected non-immunosuppressed individuals never develop active disease, while 10% develop active disease at some point during their lifetime (101, 130). Latent TB infection (LTBI) refers to the condition in which *M. tuberculosis* remains viable in the macrophage (Section 1.2) but retains a small amount of metabolic activity (129). At this stage, clinical signs and symptoms of active TB (coughing, weight loss, night sweats) are absent. In addition, LTBI has historically been

described as exposed or unexposed, as compared to a gradient or degree of exposure (123). Current evidence suggests LTBI may be better explained as a spectrum of disease correlating to degree, duration, and proximity of exposure (123).

The pathogenesis and transmission of TB are interrelated. How *M. tuberculosis* interacts with the human host determines its survival (17). Following ingestion of the bacilli by alveolar macrophages, the organism may or may not be destroyed, depending on the degree to which phagocytizing cells are activated, on host genetic factors, and on resistance mechanisms of the bacteria (17). *M. tuberculosis* infected macrophages appear to be diminished in their ability to present antigens to $CD4^+$ T-cells, leading to persistent infection (103). Additionally, antigen presenting cells contribute to defective T-cell proliferation/function by the production of cytokines (see Section 1.4.1 for more detail).

A proportion of those who are recently infected are unable to contain the infection despite the stimulation of the cell-mediated immune response and delayed-type hypersensitivity. These individuals progress to active disease within a matter of months (17). Those newly infected people in whom active TB does not develop within this period of time are left with LTBI. These individuals will either never experience disease, or later develop active TB disease. In LTBI, it is presumed that a combination of local conditions, a properly functioning cell-mediated immune system, and the presence of inhibitors result in conditions unfavorable for replication of *M. tuberculosis* (17).

1.1.3. Diagnosis and Screening

A limited number of tests are available for diagnosing and screening for *M. tuberculosis*, including the tuberculin skin test (TST), the interferon-gamma release assay (IGRA), the acid-fast smear, and the gold standard, *Mycobacterium tuberculosis* culture.

1.1.3.1.TST

After the tubercle bacillus was discovered by Koch in 1890, tuberculin, a preparation of heat-killed bacilli was modified and introduced for human use by von Pirquet in 1907 (109, 134). The TST was the first immunodiagnostic test for TB, in which purified protein derivative (PPD, tuberculin) is intradermally injected and read 48-72 hours later (135). A positive result (induration) is caused by tuberculin reactivity. Induration is due to a delayed type hypersensitivity reaction. Sensitized T-cells circulating in the blood following a previous *M. tuberculosis* infection are stimulated, causing vasodilation, edema, and infiltration of lymphocytes, basophils, monocytes, and neutrophils to the injection site suggesting previous exposure to the pathogen (59).

The TST cannot distinguish a positive result due to active TB infection from a response due to immune memory resulting from BCG vaccination, cross-reactivity with a NTM, or false positives (130). The subjective nature of both placing and reading the TST can greatly affect the sensitivity and specificity of this test (9, 59). In addition to the above reasons, variability between tuberculin suppliers (133), diagnostic induration size cut-offs, and the need to return 48-72 hours later to have results measured limits its

usefulness (109).

The effects of the shortcomings of this test are two-fold: those that are actively infected but have a false-negative TST may not be treated; those that are not infected but have a false-positive TST may be unnecessarily treated with LTBI prophylaxis. Multiple studies have shown that 20% or more of patients with TB had a false-negative TST (56, 59, 108, 119). Likewise, studies exist showing induration in uninfected individuals (26, 35). Yet over 100 years since its introduction, the TST remains a widely used screening test for TB infection.

1.1.3.2.IGRA

Since *M. tuberculosis* is an intracellular pathogen residing in the macrophage, TB antigens are presented by major histocompatibility complex (MHC) class I and class II molecules (61). The resulting type-1 T-cell response (cell-mediated immune response) is characterized by interferon (IFN)-gamma production (11). Upon progression to active TB, the cell-mediated immune response additionally produces interleukin (IL)-4, IL-5, and IL-10 (36). During latent TB infection the mycobacteria remain replicatively dormant in the macrophage but only retain a small amount of metabolic activity. Because of this, unprocessed *M. tuberculosis* antigens are unlikely to leave the macrophage and be picked up and processed by B-cells involved in the humoral immune response and antibody production. Therefore, the ideal LTBI diagnostic test needs to measure cell-mediated immune responses rather than antibody responses (109).

IGRA is a more recently developed immunodiagnostic test. Unlike the TST, IGRA is not affected by BCG vaccination, does not cross-react with the majority of NTMs, and is less prone to variability in reading results. The test measures the amount of IFN-γ released from memory T-cells in blood when they are incubated with the *M. tuberculosis*-specific antigens ESAT-6, CFP-10, and TB7.7 (117, 130). These antigens are found in *M. tuberculosis* complex organisms (*M. tuberculosis, M. bovis, M. africanum, M. microti,* and *M. canetti*) but are absent in the BCG vaccine strain and many NTMs (117). The test uses both a negative saline control as well as a mitogen (phytohaemagglutinin) positive control that measures the immune status of the individual being tested. IFN-γ responses are measured by an enzyme-linked immunosorbent assay (ELISA) (109). Although IGRA is unable to distinguish active TB from latent TB disease, it is a much more accurate indicator of latent TB than the TST (30). The optimal strategy for diagnosing latent TB may be a TST followed by an IGRA. The downside to wide spread use of IGRAs in developing countries is its high cost compared to the TST. In addition, the altered immune response in immunosuppressed individuals makes interpreting the IGRA results difficult at times (130).

1.1.3.3.Acid-fast Smear

Special stains are used to microscopically detect mycobacteria in a patient sample. The high mycolic acid content of their cell walls makes them waxy and therefore unable to be stained using the conventional Gram staining procedure. Mycobacteria can be

stained with the fluorescent dye auramine O, which fluoresces when bound to RNA or DNA, or a carbol fuchsin-based method such as Ziehl-Neelson (130). The acid-fast smear is less sensitive, but is much quicker than culture. To perform an acid-fast smear, a clinical specimen is fixed to a glass slide, stained, and examined using microscopy. Both fluorescent as well as colorimetric stains are recommended. The test results are reported as acid-fast bacilli (AFB)/ml. The amount of AFB needed to result in a smear-positive result can be as high as to 10^6AFB/ml (130).

1.1.3.4.Culture

Mycobacterium tuberculosis culture is the gold standard for the diagnosis of active TB disease. As few as 10^1 to 10^2 viable organisms/ml are required for a positive result. Sputum samples, the principle specimen obtained, contains normal bacterial flora and mucin, which bind mycobacteria, making isolation difficult. Hence, specimens from non-sterile body sites must be decontaminated before being cultured. Mucolytic and decontaminating agents are used to destroy everything but the potential *M. tuberculosis* contained in the sample prior to culturing (135).

The MGIT (mycobacterial growth indicator tube) 960 system (Becton Dickson Microbiology Systems) is an automated mycobacterial liquid culture system. The MGIT system detects mycobacterial growth in broth medium using a fluorescence-quenching-based oxygen sensor (130). As the mycobacteria grow, the oxygen in the media depletes, causing the indicator to fluoresce.

The optimum incubation temperature for most cultures is 35-37ºC. *M. tuberculosis* cultures obtained from skin and soft tissue should be incubated at 25-33ºC (130). Cultures of smear negative samples grown on solid media are held for 6-8 weeks before being reported as negative. Smear-positive culture-negative samples grown on solid media should be held onto for an additional 4 weeks before reporting negative (130).

1.1.4. Vaccine

The BCG vaccine strain was created by Albert Calmette and Camille Guérin between 1908 and 1919 when they passed *M. bovis* through bile-containing medium 231 times, effectively attenuating the strain (118). The vaccine was first used in humans in 1921. The BCG vaccine provides good protection against disseminated forms of childhood TB (military TB, TB meningitis) by reducing hematogenous (bloodborne) spread from the site of primary infection (67). Vaccine-induced protection against pulmonary TB in adults however is variable. Of all human vaccines in routine use today, the BCG vaccine has the greatest variation in protective efficacy (130). The current World Health Organization (WHO) recommendations are to vaccinate infants as soon as possible after birth in countries with a high burden of TB. The BCG vaccine is currently given soon after birth to children in over 100 countries, including routine administration to Aboriginal newborns in Canada (67). The high cost, benefit ratio, adverse reactions associated, and ability to hinder TB investigations through TST, has led to the

discontinuation of vaccination in the Atlantic Provinces (67).

1.1.5. Treatment

In treating TB, the American Thoracic Society (ATS), Centers for Disease Control and Prevention (CDC), and the Infectious Diseases Society of America (IDSA) set goals to firstly cure the patient, and secondly minimize transmission of *M. tuberculosis* to others (124). These goals keep both the safety of the individual as well as the community in the forefront. This patient-centred approach often utilizes directly observed therapy (DOT), where patients are observed ingesting each dose of anti-tuberculosis medications by a healthcare worker. First line TB medications include isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB).

1.1.5.1.Latent TB

For individuals with latent TB, the need for INH therapy is determined, based on factors such as age, recent conversion to TST positivity, and probability of future immunosuppression potentially leading to active disease (129). Depending on the treatment regimen, INH may be taken daily for many months and there is concern regarding toxicity related side effects. The full treatment recommendations can be found at http://www.thoracic.org/statements/resources/mtpi/rr5211.pdf. Recent evidence suggests that a combination regimen of INH with rifapentine (RPT) for 12 weeks with DOT is as effective as the previous lengthy treatment strategy (105, 113, 120). These new

guidelines are recommended for those patients that are ≥12 years of age who have LTBI and have factors predictive of developing active TB (105). Completion of therapy is based on the actual number of doses of medication(s) taken within a specific time period (124).

1.1.5.2.Active TB

Determining the need to initiate treatment should be based on epidemiologic information, radiographic findings (chest x-ray), microscopic findings (acid fast smear), and mycobacterial culture and susceptibility testing results (124). Treatment for active TB (caused by drug-susceptible organism) includes a four drug regimen of anti-tuberculosis chemotherapy including INH, RIF, PZA, and EMB. The first phase of treatment last for 2 months, followed by 4-7 months of continuation therapy. The intense initial phase aims to kill actively replicating *M. tuberculosis*, while the continuation phase targets any persisting bacilli (42, 127). The full recommendations can be found at http://www.thoracic.org/statements/resources/mtpi/rr5211.pdf. Again, completion of therapy is based on the actual number of doses of medications taken within a specific time period. The assessment of cure is based on clinical response, AFB smear, and culture at completion of treatment (124).

1.2. The Host Immune Response: Cells and Cytokines

The immune system functions to prevent or limit infections caused by microorganisms. The human (mammalian) immune system can be broadly divided into the innate and acquired arms. The innate arm is the first line of defense against a microorganism once it breaches the skin or mucous membranes (70). The acquired arm of the immune system is much more specific than the innate arm, and takes several days to become fully functional (71). The acquired arm can be further divided into cell-mediated immunity and antibody-mediated (humoral) immunity.

The cell-mediated immune system includes several cell types: macrophages, which present antigens to T-cells; helper ($CD4^+$) T-cells, which participate in antigen recognition and regulation; natural killer (NK) cells, which can inactivate pathogens; and cytotoxic ($CD8^+$) T-cells, which can kill infected cells. Both macrophages and $CD4^+$ T-cells produce cytokines that activate killing of the pathogen by $CD8^+$ T-cells (71). NK cells share several functions with $CD8^+$ T-cells including killing mechanisms and cytokine production (104). The cells of the immune system can be seen in Figure 1.

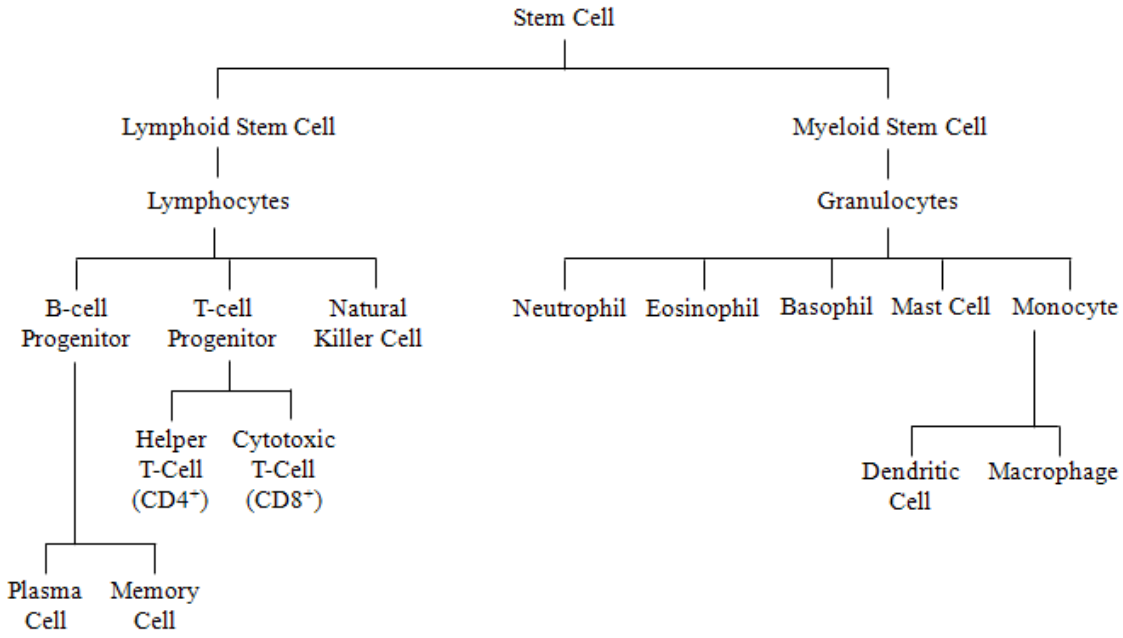


Figure 1: Cells of the immune system

Cytokines are secreted proteins that generate a potent innate immune response and provide signals to initiate the aquired immune repsonse (23). Their function involves numerous aspects of cell growth, differentiation, and activation. The nature of the immune response determines which cytokines are produced and whether the response is cytotoxic, humoral, cell-mediated, or allergic (23). There are many cytokine families, including interleukins (IL), interferons (INF), and the tumor necrosis factor (TNF) superfamily, all which will be highlighted later in this paper.

When a bacterial pathogen enters the body, it is ingested by a macrophage and broken down into antigens, which appear on the macrophage's surface along with MHC proteins. The interaction of MHC with $CD4^+$ T-cells results in production of interleukins (71). Interleukins are glycoproteins produced by leukocytes for regulating immune responses. They have immunomodulatory effects on a wide variety of responses in cells and tissues (15).

Natural killer cells make up approximately 15% of peripheral blood lymphocytes and are critical in bridging the innate and adaptive immune response to infection by production of cytokines (82). They were named "natural" killers as they are active without prior exposure to the pathogen. NK cells mediate their protective effect in two ways: via perforin/granzymes (cytotoxicity carried out by granule exocytosis of membrane pore-forming molecules/proteases), or by death ligand interactions (Fas-FasL) (79). Regulation of NK cells is achieved through a number of receptors expressed on the cell surface, belonging either to the immunoglobulin superfamily (Ig-SF) domain or those

resembling C-type lectins (C-type lectin domain family, CTLD) (84). Ig-SF domain receptors include killer immunoglobulin-like receptors (KIR), leukocyte immunoglobulin-like receptors/immunoglobulin-like transcripts (LIR/ILT), leukocyte-associated inhibitory receptors (LAIR), and natural cytotoxicity receptors (NCR) such as NKp44, and NKp46 (84). CTLD family receptors include NKG2/CD94 (killer cell lectin-like receptor, KLR) and other NKG2 (KLR) subclasses. Whether the presence or absence of a particular NK cell receptor gene is advantageous or deleterious to an individual with respect to immunity to pathogens remains largely unknown. Genetic diversity of the human immune response plays a role in host survival. Differences in the human genome may impact the quality of the immune response generated against a given pathogen (68).

1.3. Killer Immunoglobulin-like Receptors

KIRs are receptors on NK cells. There are 15 KIR genes and 2 KIR pseudogenes in humans, all of which are located within 150 kb in the leukocyte receptor complex (LRC) on chromosome 19, at the position 19q13.4 (see Figure 2) (19).

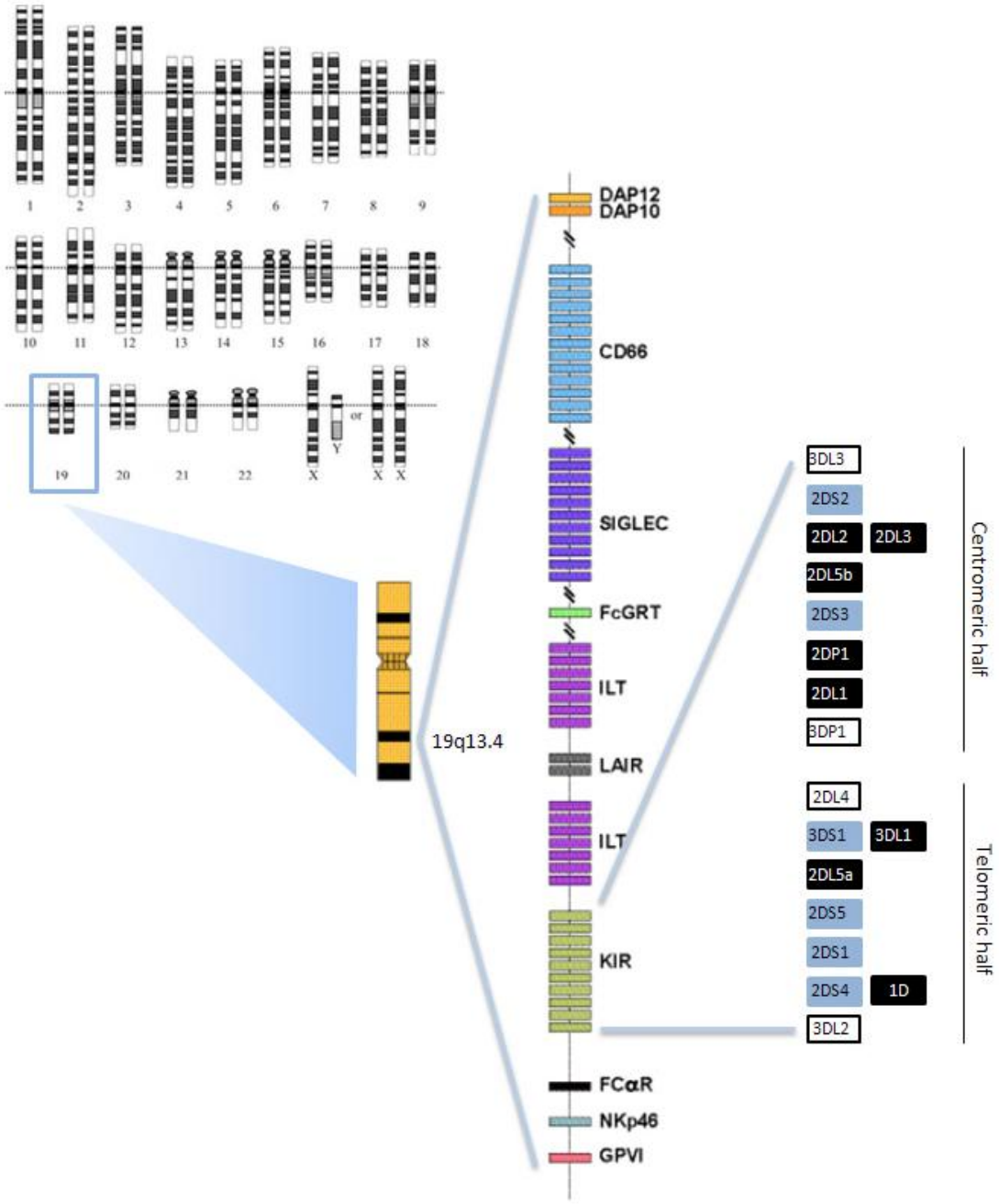


Figure 2: KIR gene organization; left – human karyotype, centre – leukocyte receptor complex located on chromosome 19 containing the killer immunoglobulin-like receptor (KIR) genes (19), right – organization of KIR framework genes (white), stimulatory genes (grey), and inhibitory genes (black). Note that genes side-by-side represent the same locus on the chromosome.

A given individual may not have all KIR genes; however, they will possess all four framework genes in their DNA. Each KIR gene encodes for a receptor on the surface of a NK cell. The activity of NK cells is controlled by a balance of inhibitory and stimulatory signals generated when a ligand binds to a receptor on the cells surface. KIRs are these inhibitory and activating regulatory molecules found on NK cells. KIR control the response of NK cells by delivering inhibitory or activating signals upon recognition of MHC class I ligands on the surface of potential target cells (132). This highly specific recognition system is arbitrated by the integration of signals triggered by a multitude of inhibitory and activating receptors, which trigger cytotoxicity and secretion of cytokines (104). KIR recognize specific human leukocyte antigen (HLA) class I molecules, which are products of the MHC. KIR can distinguish an array of polymorphic HLA class I to mount an effective immune response against pathogens. The genes that encode HLA ligands are located on many different chromosomes, resulting in variable KIR-HLA combinations which may contribute to susceptibility of an individual's health status or outcome of disease (6, 64). The interaction of KIR and HLA class I allow NK cells to identify and inhibit immune responses targeted toward normal cells. Conversely, an infected cell with down-regulated HLA leads to activation of NK cells via stimulatory KIR interaction, causing an immune response. These ligands for activating receptors could be either "induced-self", "altered-self" (HLA class I loaded with a foreign peptide), or "non-self" (pathogen-encoded molecules) (104). When the stimulatory signals overcome the inhibitory signals, a molecular cascade leads to NK cell cytotoxicity (62,

82). More specifically, inhibitory KIR contain an immunoreceptor tyrosine-based inhibition motif (ITIM) which interacts with a phosphatase (PTPN6), preventing phosphorylation of the cascade leading to activation (cytotoxicity and cytokine release) (62). Activating KIR lack ITIMs but interact with a signaling adaptor (DAP12) that contains an immunoreceptor tyrosine-based activation motif (ITAM) that interacts with a kinase (such as Src kinase), which in turn phosphorylates multiple molecules in the activation cascade leading to cytokine release (62).

KIR genes have a high level of sequence similarity leading to a predisposition for homologous recombination, explaining the expansion and contraction of the KIR locus (64). KIRs possess either 2 or 3 extracellular immunoglobulin-like domains (involved in ligand binding), and either a long or short cytoplasmic tail (involved in signaling). KIR are named taking into account the number of extracellular domains (2D or 3D) and long (L) or short (S) cytoplasmic tail or (P) pseudogene (see Figure 3) (6). Inhibitory KIRs have long tails, while activating KIRs have short tails. The final digit indicates the number of the gene encoding a protein with this structure (104). KIR genes are highly variable in nature due to both polygenic as well as multi-allelic polymorphisms (82). Two-domain KIRs recognize HLA-C allotypes while three-domain KIRs recognize HLA-B allotypes (44). Although KIRs with specificity for HLA-A (31), -B (53), -C (91), and –G (84) have been defined.

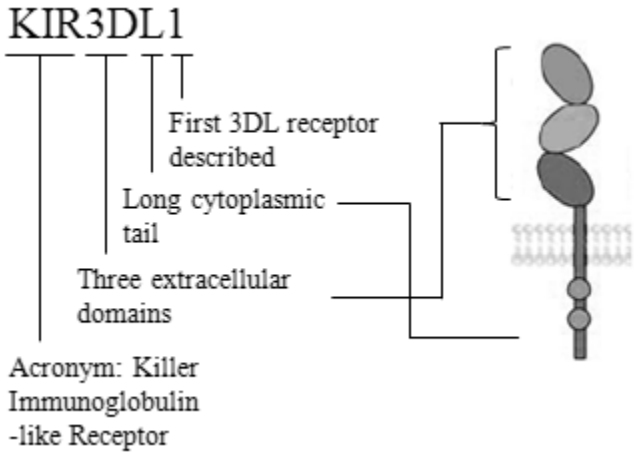


Figure 3: KIR nomenclature

1.3.1. Role of KIR in Disease

There is extensive genome diversity of both KIR and HLA in humans. It is believed that this variation may affect resistance or susceptibility to a number of pathogens through ligand-receptor interactions and the downstream signaling and/or cytokine release that follows (6, 19). Genetic susceptibility or resistance to infectious disease has been correlated with ethnicity (see Section 1.3.2.), which in conjunction with environmental and host risk factors, can ultimately determine disease progression (28, 89, 92). The discovery of an unexpected level of diversity within the KIR genes has led to a search for their role in human disease, making them an attractive target for disease association studies (64). A collection of disease association studies involving inflammatory conditions, infectious diseases, cancer, and reproductive disorders can be seen in Figure 4.

In human immunodeficiency virus (HIV), presence of KIR3DS1 with HLA-Bw4 motif was associated with slower disease progression (20). Studies searching for the role of KIR and HLA in hepatitis C virus (HCV) infection compared clearance versus persistence of the virus. Similar to HIV, KIR3DS1 with HLA-Bw4 has a protective effect (65). In another viral example, activating KIR may be protective against cytomegalovirus (CMV) (24) and inhibitory KIR2DL1 may be detrimental (45).

Less is known for the role of KIR in bacterial diseases. Susceptibility to viral infections tend to be associated with inhibitory genotypes while susceptibility to autoimmune diseases and diseases with inflammatory pathology such as bacterial lung

infections and bronchiectasis are often associated with activating genotypes (13). It is however noted that activating and inhibiting KIR repertoires along with differential HLA ligands may lead to an inherent potential for NK cell activation (12). A study of KIR in syphilis patients found that homozygous KIR1D was more prevalent in patients compared to controls (144). In *Listeria monocytogenes* infection, rats with depleted Ly49 (equivalent to human KIR) containing NK cells had increased bacterial loads. Increasing NK cell population resulted in more resistance to infection (94). There also exists a small number of tuberculosis disease association studies (4, 14, 75, 78). One such study, found that inhibitory KIR, specifically KIR2DL1 and KIR2DL2 were found more frequently among TB patients suggesting a possible inhibition of NK cell activity against infected target cells (4). Another study found that centromeric-AA status, which contains no stimulatory genes, is associated with TB status (14). A third study found that KIR2DS1, KIR2DS3, and KIR3DS1 frequencies were significantly higher in TB patients than controls, and speculated they may have a role in susceptibility to disease (74). Lastly, one study found that KIR2DL3 was more prevalent among TB patients, and along with the higher prevalence of haplotype A, suggested greater inhibition of killer function of NK cells in a TB setting (78). As many of these studies point out, population studied, KIR polymorphisms, environmental, cultural, and gene-gene interactions all play a part in interpreting the role that KIRs play in disease. The above presented research rounds out the majority of the literature available for KIR disease association studies involving bacteria.

Disease	Association	Effect
Autoimmune/Inflammatory		
1. Psoriatic arthritis	2DS1/2DS2; HLA-Cw group homozygosity	Susceptibility
2. Psoriasis vulgaris	2DS1/HLA-Cw*06	Susceptibility
	2DS1;2DL5; KIR haplotype B	Susceptibility
3. Guttate psoriasis	Weak association with 2DS1	Susceptibility (not significant)
4. Rheumatoid vasculitis	2DS2/HLA-Cw*03	Susceptibility
5. Scleroderma	2DS2+/KIR2DL2-	Susceptibility
6. IDDM	2DS2/HLA-C1	Susceptibility
	2DS2/2DL2	Susceptibility
7. Behçet's disease	Abnormal 3DL1 expression	Associated with severe eye disease
8. Idiopathic bronchiectasis	2DS1 &/or 2DS2/HLA-C1 homozygosity	Susceptibility
9. Spondylarthritides	Increased expression of 3DL2	May contribute to disease pathogenesis
10. Acute coronary syndromes	De novo expression of 2DS2/DAP12 in CD4+ T cells	May contribute to disease pathogenesis
Infectious		
1. HIV-1	3DS1/HLA-B-Bw4 80I	Slows progression
	B*57 supertype with Bw4-80I epitope/3DL1	Slows progression
2. HCV	i. 2DL3/HLA-C1 homozygosity	Resolution of infection
	ii. 3DS1/HLA-Bw4	Resolution of infection
	3DS1/HLA-Bw4 80I	Protection from development of hepatocellular carcinoma
3. Reactivation of CMV following bone marrow transplantation	>1 activating KIR in donor	Reduced risk of CMV reactivation in recipient.
4. *P. Falciparum*	3DL2*002	High response to iRBC
Cancer		
1. Malignant melanoma	*2DL2/2DL3; HLA-C1*	Susceptibility
2. Leukemia	i. 2DL2	Susceptibility
	ii. AB1 and AB9 KIR phenotypes	
3. Cervical cancer	3DS1/absence of HLA-C2 &/or HLA-Bw4	Susceptibility
	i. Genotype 10	Susceptibility Protection
	ii. 2DL5*002	
4. Nasopharyngeal carcinoma	≥5 activating KIR	Susceptibility
5. Lymphoproliferative disease of large granular lymphocytes	Abnormal KIR expression	May contribute to disease pathogenesis
	KIR/HLA class I mismatch	May contribute to disease pathogenesis
6. Cutaneous T-cell lymphoma	Expression of 3DL2 on malignant cells	May contribute to disease pathogenesis; useful diagnostic marker.
Reproduction/uterine disorders		
1. Preeclampsia	Mothers with AA KIR genotype; Fetus with HLA-C2	Susceptibility
2. Recurrent spontaneous abortion	Mothers with fewer inhibitory KIR	Susceptibility
3. Pelvic endometriosis/adenomyosis	Abnormal KIR expression	May contribute to disease pathogenesis

Figure 4: Compilation of KIR disease association studies from several reviews (18, 98, 137) as assembled by Bashirova et al. (6). Used with permission.

1.3.2. KIR by Ethnicity

The diversity in KIR genes contributes to distinct disease outcomes between ethnic populations. Killer immunoglobulin-like receptors are one subset of genes that have been shown to be associated with many different diseases (64, 98) across many different ethnic groups (29, 60, 66, 95, 96, 136, 142). Distinct outcomes of immune-regulated diseases are primarily due to differential expression of cytokines between different populations such as Caucasians, Aboriginals, and other ethnicities. The KIR diversity, as well as activating/inhibiting balance of KIR genes, contributes to distinct disease outcomes between these ethnically diverse populations.

Work by Rempel, *et al* found that haplotype B centromeric KIR genes were reduced while haplotype B telomeric KIR genes (greater immune activating phenotype) were prominent in Manitoba First Nations compared with Caucasians (107). A recent publication by La, *et al* found that presence of specific KIR3DL1/KIR3DS1 and KIR2DL2/KIR2DL3 genes were associated with NK cell dysfunction, leading to a severe disease outcome in Manitoba First Nations with H1N1 (66). A study from India reported KIR3DL1 to be positively associated with malaria severity (73). Additionally, presence of four activating genes protected from frequent malaria infection while six activating genes negatively influenced disease outcome.

1.4. Human Immune Response to Tuberculosis

1.4.1. Immunology

M. tuberculosis is spread by airborne transmission via droplet nuclei which are formed when particles of moisture containing viable bacteria begin to evaporate, concentrating the microbes (135). Droplet nuclei are produced when a person with active TB coughs or talks (121). Only nuclei containing 1-3 bacilli are small enough to reach the alveolar spaces. The first line of alveolar defense is the microbicidal activity of the alveolar macrophages (135). It is the inability of the infected macrophage to contain the *M. tuberculosis* that is fundamental to the pathogenesis of TB. Macrophages that contain multiplying *M. tuberculosis* may die, leading to release of the bacilli, and attraction of monocytes (immature macrophages). Cell-mediated immunity is triggered approximately 21 days after initial infection. As a result, the alveolar macrophages demonstrate an increased ability to destroy the intracellular bacilli (27, 76). However, macrophage death also occurs at this stage, resulting in a granuloma, a characteristic pathologic finding of TB (135). Macrophages then can become highly activated and destroy the bacilli, or poorly activated, ingest but fail to destroy the bacilli. Macrophages and neutrophils are considered the first line of defence against *M. tuberculosis* infection. Once the infection establishes in the lungs, the alveolar macrophages phagocytize the bacilli (38, 140). The extent of this activation influences whether the immune response results in clearance or containment of the pathogen; with the end result of latent infection or the development of active disease (43).

Granulomas that form in the lung are composed of a mass of *M. tuberculosis* infected macrophages, stimulated macrophages that have differentiated into multinucleated giant cells, epithelioid cells and foamy macrophages loaded with lipid droplets and neutrophils (110). This granuloma then becomes surrounded by lymphocytes (mostly $CD4^+$ T-cells, with some $CD8^+$ T-cells and B-cells) (99). Classically, the granuloma contains a necrotic caseous core caused by cell lysis, resulting in a hypoxic centre. It has been hypothesized that latent *M. tuberculosis* reside in the central hypoxic core in an altered metabolic state, while the active *M. tuberculosis* replicate in the oxygenated areas (5).

TB is a chronic disease requiring constant expression of cellular immunity to limit bacterial growth (63). Specific cytokines, namely IL-2, INF-γ, and TNF are all fundamental in the control of *M. tuberculosis* (25, 41). INF-γ is secreted by $CD4^+$ T-cells and functions to activate macrophages and secrete products that can lyse *M. tuberculosis* (40). Induction of a protective INF-γ response against *M. tuberculosis* is dependent on IL-12, which is secreted by dendritic cells (40). Additionally, the interaction between IL-12 and INF-γ is needed to maintain immune responses in the lungs to control the chronic infection (111). TNF is produced by multiple immune cells (macrophages, neutrophils, dendritic cells, T-cells, and NK cells) and functions to induce chemokine production, maintain integrity of the granuloma, and boost intracellular killing (21, 90).

Additional cytokines also play a role in TB infection, such as IL-6, IL-10, IL-17, and IL-23. IL-6 is produced mainly by phagocytic cells and mediates T-cell activation,

growth, and differentiation (23). During *M. tuberculosis* infection, IL-6 interferes with INF-γ signalling, specifically inhibiting INF-γ induced autophagy (34). Autophagy is needed to eliminate intracellular bacteria. IL-10 is produced by macrophages, neutrophils, dendritic cells, B-cells, and T-cells and functions as an immunosuppressive cytokine that dampens the immune response to intracellular bacteria by inhibiting production of specific cytokines (112). When overproduced, IL-10 contributes to chronic infection. IL-17 impairs neutrophil survival and decreases lung inflammation and improved disease outcome (93). IL-23 is an inducer of INF-γ and is essential to the expression of IL-17 response to mycobacterial infection (63). Regardless of what is known about the immune response to TB, the factors that allow one individual to be protected from *M. tuberculosis* infection while others develop active disease is not understood.

NK cells are important in early infection as they are capable of activating phagocytic cells at the site of infection (103). NK cells may directly lyse the *M. tuberculosis*, or can lyse infected monocytes. *In vitro* culture of NK cells with live *M. tuberculosis* brings about the expansion of NK cells, further supporting their important role as responders to TB infection (37). Additionally, NK cells are producers of IFN-γ, which plays many roles in TB infection as described above.

1.4.2. Host Genetics

In addition to the immunological aspects to control of TB disease, host genetics also plays a role in combating the pathogen. Several host genes involved in the process of antigen presentation, macrophage activation, and granuloma formation have been identified (77).

HLA is involved in presentation of *M. tuberculosis* antigens. Many studies have been performed to show an association of specific HLA genotypes with TB susceptibility/resistance, most notable at the HLA-DR and HLA-DQB1 loci, which determine which mycobacterial antigens are presented to helper T-cells (33, 48, 77, 87).

Non-HLA genes have also been implicated in TB disease. The PTPN22 gene codes for an intracellular lymphoid-specific phosphatase involved in the inflammatory response by the down-regulation of NK cell, neutrophil, and T-cell activation (49). The R620W (C1858T) polymorphism in PTPN22 may be a genetic factor predisposing an individual to the development of TB. T-allele carriers were found to be protected against disease while C-allele carriers were prone to development of TB disease (49).

Toll-like receptors (TLRs) are a family of mammalian cell-surface proteins that stimulate pro-inflammatory cytokine gene transcripts in response to various microbial ligands (77). TLR-2 is involved in the recognition of *M. tuberculosis* (85). Macrophages detect mycobacteria through activation of TLR-2, leading to intracellular killing (122). An Arg753Gln polymorphism in TLR-2 may influence TB susceptibility by leading to decreased response of macrophages to bacterial peptides, resulting in an attenuated

immune response (72, 97).

The polymorphic variants of the vitamin D receptor (VDR) gene have been emphasized with susceptibility/resistance to pulmonary tuberculosis (10). VDR is a ligand activated transcription factor, which upon binding vitamin D3 (an immunomodulatory hormone), interacts with vitamin D response elements and alters their transcription (81). Polymorphisms in the VDR gene are identified by allelic variation in restriction enzyme sites including BsmI (T to G), ApaI (T to G), TaqI (T to C), and FokI (C to T), and are associated with susceptibility/resistance to TB disease (2, 77).

Solute carrier family 11 member 1 protein (SLC11A1), formerly NRAMP1 (natural resistance associated macrophage protein 1) is a pH-dependant proton/divalent cation antiporter involved in macrophage function. Polymorphisms in SLC11A1 have been associated with susceptibility to inflammatory diseases and intracellular bacteria including *M. tuberculosis* (3, 131).

Macrophages play a dual role in the antimycobacterial host response: they restrict the growth of engulfed mycobacteria while at the same time they can shield the pathogen from being fully eradicated (61). A critical element of mycobacterial survival involves the blockade of phagosomal maturation, which would normally lead to the death of the engulfed microbes. Ras-associated small GTP-ases (Rab) molecules are mediators of these fusion events. There are more than 60 Rab family members. Polymorphisms in Rab5, 7, 13, 24, 27A, and 33 are associated with susceptibility/resistance to TB (77).

The release of cytokines in response to *M. tuberculosis* infection is an immunologic response; however, the genes encoding cytokines can be viewed as the host genetic component. Polymorphisms in IL-6, IL-10, IFN-γ, TNF-α, and others cytokines have been associated with susceptibility/resistance to TB disease (1, 8, 47, 77).

The development of TB disease is the result of a complex interaction between the host and pathogen. Numerous host genes are likely to be involved in this process. Lastly, KIRs, the focus of this research, are the receptors which interact with HLA, genes which already have well established connections to TB disease outcomes. The extensive diversity of KIR, and its role in NK cell function, recruitment to the site of infected macrophages, and cytotoxicity/cytokine release highlight the importance in studying this host gene for its role in TB disease.

1.5. Role of KIR in Tuberculosis Disease

The natural resistance to tuberculosis is reflected in the ability of the macrophage to control intracellular growth of the organism. A resistant host shows a chronic infection generally affecting the lungs, whereas a highly susceptible host shows a rapidly progressing, possibly fatal illness with potential to spread to many organs (7). The study of tuberculosis requires an understanding of the nature and duration of TB disease as well as the particular population under study.

Studies have demonstrated that the outcome of TB infection is affected not only by infection with more or less virulent strains of *M. tuberculosis,* but also the genetic

composition of the host, specifically the presence or absence of genes that regulate the immune system (28, 92, 100, 114, 116, 128).

Genes involved in susceptibility to mycobacterial diseases have been previously explored in populations around the world, and more recently in Canadian Aboriginal groups that have had epidemic and endemic TB (3, 52, 69). KIR2DL3 has been found to be significantly more prevalent in Lebanese and Mexican TB patients compared to control populations without TB (78, 86). A study in a Lur population in Iran found an association between KIR3DS1 and susceptibility to TB (115). Other studies have shown KIR3DL1 to be implicated in more severe disease outcomes, and KIR2DL3 to be more prevalent among TB patients (78). Inhibitory genes KIR3DL1, KIR2DL3 and activating genes KIR2DS1, KIR2DS5 conferred susceptibility towards TB either individually or in haplotype combinations in a population in south India (102). A study from China found frequencies of KIR2DS1, KIR2DS3, and KIR3DS1 were significantly higher in TB patients compared to controls (75). In conjunction, these studies demonstrate there is a genetic component to TB disease, and that they may play a role in altered TB disease progression and disease outcomes.

2. Hypotheses

Genetic diversity of the genes encoding the human immune response plays a critical role in the ability of the host to interact with the pathogen by mounting an appropriate immune response and hence impacting host survival (68). The discovery of an unexpected level of diversity within the KIR genes has led to a search for their role in human disease (64). The presence or absence of specific KIR genes, individually or in conjunction, may be associated with tuberculosis status (active disease, latent disease, not infected) as well as ethnicity of an individual (78, 86, 115). It is hypothesized that the differences in KIR profiles, gene frequencies, and/or haplotypes in Canadian-born Aboriginal, Canadian-born non-Aboriginal, and foreign born individuals elicits a differential activation or inhibition profile, resulting in differential cytokine expression and eventually contributes to the outcome to TB infection. Identification of ethnic specific genes that confer host susceptibility to TB infection will help to better understand the interaction of host genetics and the immune system. The outcome of the human immune response to tuberculosis may result in the type of infection or disease that develops. In this study we examined the enrichment or depletion of KIR genes in different ethnic populations in Manitoba with special focus on patients with active, latent, or uninfected TB status. In addition, we sought to explore the statistical correlation between TB status and inhibitory/stimulatory KIR haplotypes.

The specific hypotheses for this study are as follows:

i. KIR gene frequencies will differ between ethnically diverse groups: Canadian-born Aboriginal, Canadian-born non-Aboriginal, and foreign born individuals, as well as between subjects falling within specific tuberculosis disease status groups: active infection, latent infection, and uninfected.

ii. Within each of the above mentioned groups, a predominant KIR profile(s) will be identified.

iii. Inhibitory KIR haplotypes (immune-dampening) will be more prevalent in those with active TB, while activating KIR haplotypes (immune-stimulating) will be more prevalent in the uninfected group.

3. Specific Objectives

i. Determine the presence or absence of all possible KIR genes with a polymerase chain reaction (PCR) assay in order to compare gene frequencies between the specified groups

ii. Assemble each individual's KIR genes into a KIR profile and group identical profiles together in order to compare specified groups by statistical comparison

iii. Categorize individuals into haplotypes using a defined scheme in order to compare the distribution of TB cases

4. Materials and Methods

4.1. Study Populations

The study population was comprised of two subsets of samples that were part of larger studies. Subset A contained Dene First Nations, Cree First Nations and Ojibwa First Nations participants from three Manitoba Reserve communities with active, latent, or uninfected TB status, as well as uninfected Caucasian controls from Winnipeg, Manitoba. Subset B contained individuals from three population groups (Canadian-born non-Aboriginal, Canadian-born Aboriginal, and foreign born individuals) living in Manitoba with active, latent, or uninfected TB status.

4.1.1. Subset A: Northern Manitoba First Nations

This study group was comprised of a total of 168 study subjects with known TB status. The samples consisted of whole blood and/or buccal swabs from 93 adult First Nations individuals who resided in a First Nations community and self-identified as either Dene (n=63), Cree (n=19), or Ojibwa (n=11), as well as uninfected Caucasian controls (n=75) from Winnipeg, Manitoba. Within each First Nations subgroup, there were internal uninfected controls. All First Nations population groups had approximately equal distribution of males and females. There were more females than males in the Caucasian group. In all groups, the majority of individuals were over 40 years of age. The samples were obtained with informed written consent, in addition to approval by the University of Manitoba Bannatyne Campus Research Ethics Board (ethics reference

number: H2005-106) and the First Nations communities involved. Interviews and questionnaires were conducted probing for evidence of investigation and treatment of active or latent tuberculosis, including history of TST and treatment regimens. The study comprised of 59 individuals with no TB, 14 individuals with latent TB, and 20 individuals with active TB. Sample demographics can be seen in Table 1. Although most First Nations individuals involved received bacilli Calmette-Guérin (BCG) vaccination at birth, the interval of greater than 18 years between BCG and TST prevents any significant number of false-positives due to cross-reactivity (39, 106). Therefore history of a positive TST result was assumed to be from a delayed-type hypersensitivity reaction to the tuberculin, and not a cross reaction from potential previous BCG vaccination.

First Nations subgroups differ from each other in their language (Dene – Athapaskan language family, Cree and Ojibwa – Algonkian language family), culture (political and social), and geographic separation (Figure 5). The Athapaskan Dene and the Algonkian Cree and Ojibwa groups each have distinct histories, experiences with disease (including immunogenic differences), and differing degrees of isolation from each other and from early European explorers (68, 69). The extent to which these unique histories have influenced their susceptibility or resistance to infectious diseases and their immunogenetic profile is unclear, but is important to analyze not just Aboriginal vs. non-Aboriginal when ethnocultural subgroup is known.

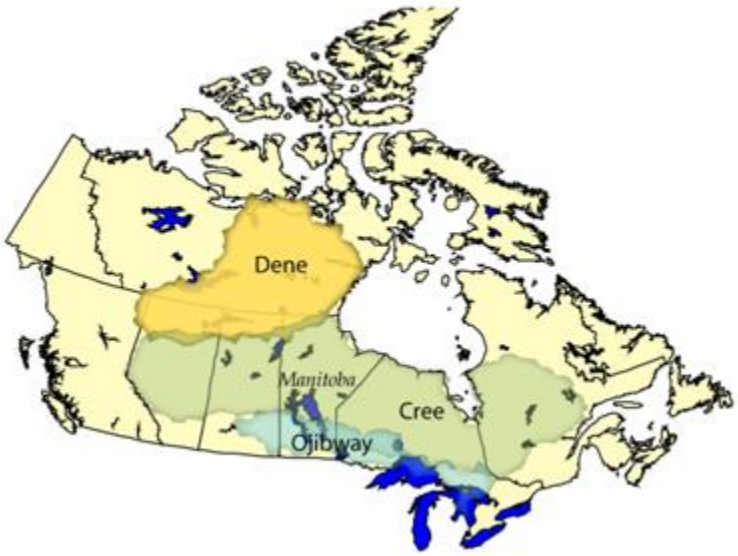


Figure 5: Traditional areas of the Dene, Cree and Ojibwa First Nations. Used with permission, L. Larcombe, 2013.

Table 1: Subset A study population demographics

Parameter	Value	Individuals (%)			
		Caucasian n = 75	Dene n = 63	Cree n = 19	Ojibwa n = 11
Gender	Male	25 (33.3)	34 (54.0)	8 (42.1)	6 (54.5)
	Female	50 (67.7)	29 (46.0)	11 (57.9)	5 (45.5)
Age	≤ 19	0 (0.0)	3 (4.8)	0 (0.0)	0 (0.0)
	20 - 39	0 (0.0)	19 (30.1)	1 (5.3)	2 (18.2)
	40 - 59	44 (58.7)	24 (38.1)	14 (73.7)	6 (54.5)
	≥ 60	31 (41.3)	17 (27.0)	5 (26.3)	3 (27.3)
Disease Status	No TB	75 (100.0)	38 (60.3)	11 (57.9)	10 (90.9)
	Latent	-	10 (15.9)	4 (21.1)	0 (0.0)
	Active	-	15 (23.8)	4 (21.1)	1 (9.1)

4.1.2. Subset B: Ethnically Diverse Manitoban Cohort

This study group was comprised of 209 ethnically diverse individuals living in Manitoba with known TB status. The samples consisted of whole blood from Canadian-born non-Aboriginal (n=77), Canadian-born Aboriginal (n=54) [First Nations (n=52), Metis (n=1), Inuit (n=1)], and foreign born (n=78) individuals from Manitoba. This group did not self-identify their affiliation with any specific First Nations group. Aboriginal and foreign born population groups had approximately equal distribution of males and females. There were more females than males in the non-Aboriginal group. In all groups, the majority of individuals were aged 20-59 years of age. The sampling was performed at TB clinics in Winnipeg, Manitoba. The samples were obtained with approval from the University of Manitoba Bannatyne Campus Research Ethics Board (ethics reference number: H2008-301). Active TB infection (n=59) was confirmed by culture and/or clinical diagnosis including prior TST and AFB testing. LTBI (n=46) was determined using the following criteria: positive TST and positive confirmatory IGRA without clinical features/presence of replicating AFB. Healthy non-TB exposed individuals (n=104) were used as uninfected controls. These individuals were defined by positive or negative TST with a negative confirmatory IGRA. Sample demographics can be seen in Table 2.

Table 2: Subset B study population demographics

Parameter	Value	Individuals (%)		
		non-Aboriginal n = 77	Aboriginal n = 54	Foreign-born n = 78
Gender	Male	24 (31.2)	30 (55.6)	37 (47.4)
	Female	53 (68.8)	24 (44.4)	41 (52.6)
Age	≤ 19	0 (0.0)	0 (0.0)	0 (0.0)
	20 - 39	29 (37.7)	21 (38.9)	38 (48.7)
	40 - 59	43 (55.8)	32 (59.3)	30 (38.5)
	≥ 60	5 (7.1)	1 (1.9)	10 (12.8)
Disease Status	Uninfected control	58 (75.3)	14 (25.9)	32 (41.0)
	Latent	7 (9.1)	5 (9.3)	34 (43.6)
	Active	12 (15.6)	35 (64.8)	12 (15.4)

4.2. DNA Extraction and Replication Procedure

Genomic DNA was extracted using Qiagen DNA Mini Kit as per manufacturer's instructions (Qiagen, Louisville, KY). Genomic DNA was eluted from the provided silica-membrane-based nucleic acid purification column with 200µL of elution buffer (Qiagen Buffer AE) containing 10mM Tris-Cl and 0.5mM EDTA, pH 9.0, and stored at -20˚C until analysis. Due to the low sample volume/concentration available for testing, along with the need for 1.875 – 3.125 ug of genomic DNA, all samples were subjected to whole genome replication by the multiple displacement amplification (MDA) method using the Qiagen Repli-G Mini Kit as per manufacturer's instructions. This method uses the bacteriophage Phi29 DNA polymerase to increase DNA concentration of the testing sample without compromising sequence (57). The concentration of DNA was normalized to 100µg/mL at 260nm using the SmartSpec Plus spectrophotometer (Bio-Rad, Mississauga, ON).

4.3. KIR Genotyping Procedure

KIR genotyping was performed by sequence-specific primer polymerase chain reaction (SSP-PCR) using the Miltenyi Biotec KIR Typing Kit (Auburn, CA) according to the manufacturer's directions with the following adjustments: the initial PCR denaturation step was extended from one minute to two minutes in order to ensure proper amplification of the internal beta-actin control in each reaction well. Following this change, the recommended 28 cycles of denaturation (20s at 94°C), annealing (20s at

63°C), and extension (90s at 72°C) were adhered to. Additionally, TAE buffer was replaced by TBE buffer for gel electrophoresis (Invitrogen, Burlington, ON). Those samples in which the internal beta-actin control did not amplify were repeated.

In order to ensure that the technique was accurately detecting KIR genes in the samples, proficiency and competency testing were carried out using samples with known KIR genotype from the proficiency providers: Fred Hutchinson Cancer Research Center, International Histocompatibility Working Group Cell and Gene Bank (Seattle, WA).

The kit tested for the presence or absence of the following genes: KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5all (A and B), KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4del (KIR1D), KIR2DS4ins (full length), KIR2DS5, KIR3DL1, KIR3DL2, KIR3DL3, KIR3DS1, KIR2DP1, and KIR3DP1.

The amplicons were visualized with UV light (Bio-Rad Gel Doc EZ Imager, Mississauga, ON) following gel electrophoresis at 13V/cm on a 2% agarose gel (Invitrogen) containing ethidium bromide (Invitrogen). The presence or absence of a gene was converted to a numerical designation: [1] presence, [0] absence. Additionally, the assemblage of KIR genes in a given individual makes up their KIR profile. A schematic of the work flow can be seen in Figure 6.

Stock samples:

	Active TB	Latent TB	Uninfected	Total
Canadian born Aboriginal	35	5	14	**54**
Canadian born non-Aboriginal	12	7	58	77
Foreign born	12	34	32	**78**
Total	**59**	**46**	**104**	**209**

↓

Whole genome replication (Qiagen DNA Mini Kit)

↓

Normalize DNA concentration to 100µg/mL using spectrophotometry

↓

KIR typing (Miltenyi Biotec KIR Typing Kit)

↓

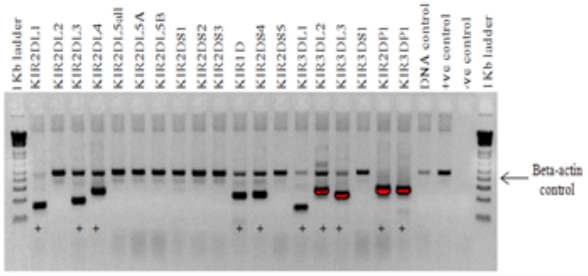
Polymerase chain reaction:

	No. of Cycles	Temp (°C)	Time (s)
Initial denaturation	1	95	120
Denature	28	94	20
Anneal		63	20
Extend		72	90
Hold		4	∞

Visualize via gel electrophoresis: 13V/cm, 2% agarose gel containing ethidium bromide

↓

Banding pattern:



↓

BioNumerics KIR profile:

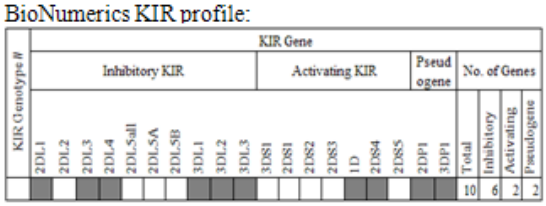


Figure 6: Schematic representation of workflow from stock sample to generation of a KIR profile

4.4. Haplotype Determination

Haplotype designation was determined as previously described (55, 58, 107). The order of KIR genes within the telomeric and centromeric half as well as haplotype A/B distinction is seen in Figure 7. Briefly, (i) framework genes (KIR3DL3, KIR3DP1, KIR2DL4, and KIR3DL2) are recognized as homozygous; (ii) in the centromeric portion, KIR2DL2/KIR2DL3 are considered alleles of the same locus: presence of only one indicates homozygous BB or AA, respectively, and presence of both indicates a heterozygous AB centromeric haplotype; (iii) in the telomeric portion, KIR3DL1/KIR3DS1 are considered alleles of the same locus: presence of only one indicates homozygous AA or BB, respectively, and presence of both indicates heterozygous AB telomeric haplotype. Haplotype A contains only one activating KIR, KIR2DS4. Haplotype B is a more wide-ranging haplotype, encompassing 1-5 activating KIR, including KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5, and KIR3DS1 (18).

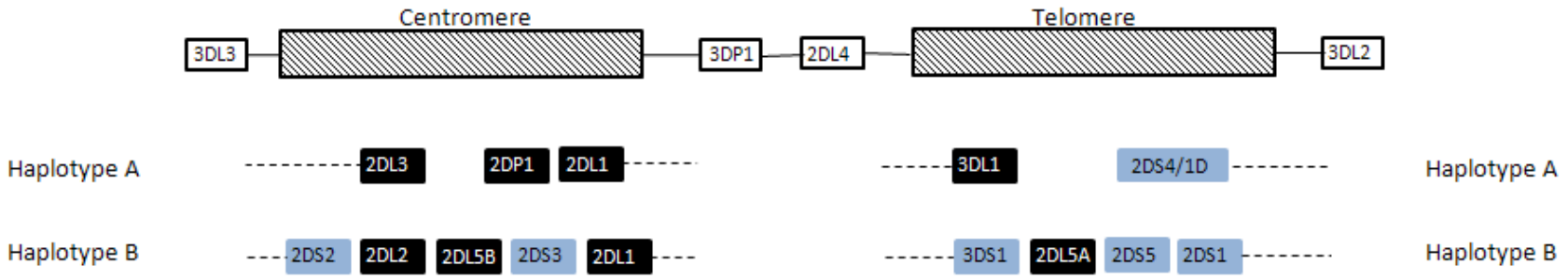


Figure 7: Schematic of KIR gene haplotypes A and B; white – framework genes, grey – activating KIR, black – inhibitory KIR; note that KIR2DP1 and KIR3DP1 are pseudogenes, and that KIR2DL2/2DL3 as well as KIR3DL1/3DS1 represent the same locus.

4.5. Data Analysis

Data for each individual was entered into BioNumerics software version 5.0 (Applied Maths, Belgium) as binary character data. All KIR genes were combined into a single KIR profile for each individual and clustered to identify prevalent profiles among specified groups using the categorical co-efficient and unweighted pair group method with arithmetic mean (UPGMA) (22). KIR gene frequencies were tabulated by direct counts from the clustered profiles to determine frequency within a defined group. Differences between population groups and TB status groups were estimated using the two-tailed Fisher's exact test (GraphPad Software, La Jolla, CA). A *P*-value ≤ 0.05 was considered statistically significant.

5. Results

5.1. Subset A: Northern Manitoba First Nations

5.1.1. KIR Gene Frequencies

In order to determine the differences in KIR gene frequencies between different population groups, subgroups (Caucasians, First Nations – Dene, Cree, and Ojibwa) and disease status groups (active, latent, and uninfected TB status), the KIR gene frequency data obtained was analyzed and compared (Specific Objective i.). All 168 samples consistently contained the framework genes KIR2DL4, KIR3DL2, KIR3DL3, and the pseudogenes KIR2DP1 and KIR3DP1. There were many significant differences (P-value $\leq$0.05, two-tailed Fischer's Exact Test) in the frequencies of KIR genes between Caucasians and First Nations. The following KIR genes were lower in First Nations vs. Caucasians: KIR2DL2 (18.28% vs. 60.00%, $P<0.0001$), KIR2DS2 (18.28% vs. 61.33%, $P<0.0001$), KIR2DS3 (8.42% vs. 29.33%, $P=0.0009$), KIR1D (38.71% vs. 85.33%, $P<0.0001$), and KIR3DL1 (82.80% vs. 97.33%, $P=0.0022$). In contrast, the following KIR genes were higher in First Nations compared to Caucasians: KIR2DL5A (52.69% vs. 34.67%, $P=0.0258$), KIR2DS1 (53.76 vs. 36.00%, $P=0.0290$), KIR2DS4 (94.62% vs. 76.00%, $P=0.0006$), KIR2DS5 (49.46% vs. 18.67%, $P<0.0001$), and KIR3DS1 (55.91% vs. 34.67%, $P=0.0081$) (Table 3).

Table 3: Comparison of killer immunoglobulin-like receptor (KIR) gene frequencies in Caucasians, First Nations and First Nations ethnocultural subgroups (Subset A)

Frequency (%)	KIRs																		
	2DL1	2DL2	2DL3	2DL4	2DL5all	2DL5A	2DL5B	2DS1	2DS2	2DS3	1D	2DS4	2DS5	3DL1	3DL2	3DL3	3DS1	2DP1	3DP1
Caucasian (n = 75)	74 (98.67)	45 (60.00)	67 (89.33)	75 (100.00)	32 (42.67)	26 (34.67)	31 (41.33)	27 (36.00)	46 (61.33)	22 (29.33)	64 (85.33)	57 (76.00)	14 (18.67)	73 (97.33)	75 (100.00)	75 (100.00)	26 (34.67)	75 (100.00)	75 (100.00)
First Nations																			
All (n = 93)	89 (95.70)	17 (18.28)	89 (95.70)	93 (100.00)	50 (53.76)	49 (52.69)	49 (52.69)	50 (53.76)	17 (18.28)	8 (8.42)	36 (38.71)	88 (94.62)	46 (49.46)	77 (82.80)	93 (100.00)	93 (100.00)	52 (55.91)	93 (100.00)	93 (100.00)
Dene (n = 63)	61 (96.82)	10 (15.87)	61 (96.82)	63 (100.00)	29 (46.03)	29 (46.03)	29 (46.03)	29 (46.03)	10 (15.87)	6 (9.52)	19 (30.16)	62 (98.41)	26 (41.27)	53 (84.13)	63 (100.00)	63 (100.00)	30 (47.62)	63 (100.00)	63 (100.00)
Cree (n = 19)	19 (100.00)	3 (15.79)	19 (100.00)	19 (100.00)	15 (78.95)	15 (78.95)	15 (78.95)	15 (78.95)	3 (15.79)	2 (10.53)	10 (52.63)	18 (94.74)	14 (73.68)	14 (73.68)	19 (100.00)	19 (100.00)	15 (78.95)	19 (100.00)	19 (100.00)
Ojibwa (n = 11)	9 (81.82)	4 (36.36)	9 (81.82)	11 (100.00)	6 (54.55)	5 (45.45)	5 (45.45)	6 (54.55)	4 (36.36)	0 (0.00)	7 (63.64)	8 (72.73)	6 (54.55)	10 (90.91)	11 (100.00)	11 (100.00)	7 (63.64)	11 (100.00)	11 (100.00)
P-value																			
First Nations vs Caucasians	0.3821	**<0.0001**	0.1374	1.0000	0.1653	**0.0285**	0.1635	**0.0290**	**<0.0001**	**0.0009**	**<0.0001**	**0.0006**	**<0.0001**	**0.0022**	1.0000	1.0000	**0.0081**	1.0000	1.0000
Dené vs Caucasians	0.5920	**<0.0001**	0.1099	1.0000	0.7327	0.2220	0.6084	0.2965	**<0.0001**	**0.0052**	**<0.0001**	**<0.0001**	**0.0046**	**0.0121**	1.0000	1.0000	0.1637	1.0000	1.0000
Cree vs Caucasians	1.0000	**0.0007**	0.3519	1.0000	**0.0090**	**0.0007**	**0.0044**	**0.0014**	**0.0006**	0.1404	**0.0040**	0.1077	**<0.0001**	**0.0034**	1.0000	1.0000	**0.0007**	1.0000	1.0000
Ojibwa vs Caucasians	**0.0419**	0.1945	0.6098	1.0000	0.5261	0.5153	1.0000	0.3218	0.1892	0.0587	0.0947	1.0000	**0.0167**	0.3402	1.0000	1.0000	0.0962	1.0000	1.0000
Dené vs Cree	1.0000	1.0000	1.0000	1.0000	**0.0172**	**0.0172**	**0.0172**	**0.0172**	1.0000	1.0000	0.1008	0.4119	**0.0182**	0.3215	1.0000	1.0000	**0.0191**	1.0000	1.0000
Dené vs Ojibwa	0.1027	0.2028	0.1027	1.0000	0.7466	1.0000	1.0000	1.0000	0.2028	0.5826	**0.0434**	**0.0093**	0.5152	1.0000	1.0000	1.0000	0.5151	1.0000	1.0000
Cree vs Ojibwa	0.1264	0.3717	0.1264	1.0000	0.2252	0.1081	0.1081	0.1081	0.3717	0.5195	0.7084	0.1264	0.4253	0.3717	1.0000	1.0000	0.4172	1.0000	1.0000

Significant P-values (≤ 0.05) are bolded

More specifically, when examining gene frequencies within the First Nations subgroups, KIR2DL2 (18.28% vs. 60.00%, $P<0.0001$), KIR2DS2 (15.87% vs. 61.33%, $P<0.0001$), KIR2DS3 (9.52% vs. 29.33%, $P=0.0052$), KIR1D (30.16% vs. 85.33%, $P<0.0001$), and KIR3DL1 (84.13% vs. 97.33%) were lower while KIR2DS4 (98.41% vs. 76.00%, $P<0.0001$) and KIR2DS5 (41.27% vs. 18.67%, $P=0.0046$) were present in higher frequencies in Dene First Nations compared to Caucasians. Gene frequencies of KIR2DL2 (15.79% vs. 60.00%, $P=0.0007$), KIR2DS2 (15.79% vs. 61.33%, $P=0.0006$), KIR1D (52.63% vs. 85.33%, $P=0.0040$), and KIR3DL1 (73.68% vs. 97.33%, $P=0.0034$) were lower while frequencies of KIR2DS5all (78.95% vs. 42.67%, $P=0.0090$), KIR2DL5A (78.95% vs. 34.67%, $P=0.0007$), KIR2DL5B (78.95% vs. 41.33%, $P=0.0044$), KIR2DS1 (78.95% vs. 36.00%, $P=0.0014$), KIR2DS5 (73.68% vs. 18.67%, $P<0.0001$), and KIR3DS1 (78.95% vs. 34.67%, $P=0.0007$) were higher in Cree First Nations compared to Caucasians. Only two genes, KIR2DL1 (81.82% vs. 98.67%, $P=0.0419$) and KIR2DS5 (54.55% vs. 18.67%, $P=0.0167$) were significantly different between Ojibwa First Nations and Caucasians. The presence of KIR1D (allele of KIR2DS4) was significantly lower in First Nations (Dene and Cree but not Ojibwa) individuals compared to Caucasians (38.71% vs. 85.33%, $P<0.0001$). Overall, there were differences seen between not only First Nations and Caucasians, but also between the First Nations ethnocultural subgroups.

To determine differences in KIR gene frequencies between TB disease status groups, active, latent, and uninfected TB status groups were compared. When comparing

KIR gene frequencies in First Nations individuals with any TB status (active and latent) to those with an uninfected TB status, the only gene approaching statistical significance was KIR1D, found in 45.75% of First Nations with no disease but only 26.47% in First Nations with TB status (P=0.0795; Table 4). No significant differences were seen in KIR gene frequencies between latent and active TB groups.

Table 4: Comparison of killer immunoglobulin-like receptor (KIR) gene frequencies in First Nations by tuberculosis status (Subset A)

Frequency (%)	KIRs																		
	2DL1	2DL2	2DL3	2DL4	2DL5all	2DL5A	2DL5B	2DS1	2DS2	2DS3	1D	2DS4	2DS5	3DL1	3DL2	3DL3	3DS1	2DP1	3DP1
Tuberculosis (TB)																			
All (n = 34)	34 (100.00)	4 (11.76)	34 (100.00)	34 (100.00)	18 (52.94)	18 (52.94)	18 (52.94)	18 (52.94)	4 (11.76)	2 (5.88)	9 (26.47)	34 (100.00)	17 (50.00)	28 (82.35)	34 (100.00)	34 (100.00)	19 (55.88)	34 (100.00)	34 (100.00)
Latent (n = 14)	14 (100.00)	2 (14.29)	14 (100.00)	14 (100.00)	9 (64.29)	9 (64.29)	9 (64.29)	9 (64.29)	2 (14.29)	0 (0.00)	4 (28.57)	14 (100.00)	9 (64.29)	11 (78.57)	14 (100.00)	14 (100.00)	9 (64.29)	14 (100.00)	14 (100.00)
Active (n = 20)	20 (100.00)	2 (10.00)	20 (100.00)	20 (100.00)	9 (45.00)	9 (45.00)	9 (45.00)	9 (45.00)	2 (10.00)	2 (10.00)	5 (25.00)	20 (100.00)	8 (40.00)	17 (85.00)	20 (100.00)	20 (100.00)	10 (50.00)	20 (100.00)	20 (100.00)
No Tuberculosis (n = 59)	55 (93.22)	13 (22.03)	55 (93.22)	59 (100.00)	32 (54.23)	31 (52.54)	31 (52.54)	32 (54.23)	13 (22.03)	6 (10.17)	27 (45.76)	54 (91.53)	29 (49.15)	49 (83.05)	59 (100.00)	59 (100.00)	33 (55.93)	59 (100.00)	59 (100.00)
P-value																			
TB vs No TB	0.2927	0.2730	0.2927	1.0000	1.0000	1.0000	1.0000	1.0000	0.2730	0.7058	0.0795	0.1543	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Latent TB vs No TB	1.0000	1.0000	1.0000	1.0000	0.7762	0.5542	0.5542	0.7762	1.0000	0.5883	0.3682	0.5758	0.3800	0.7048	1.0000	1.0000	0.7650	1.0000	1.0000
Active TB vs No TB	0.5670	1.0000	0.5673	1.0000	0.6059	0.6120	0.6120	0.6059	1.0000	1.0000	0.1208	0.3221	0.6059	1.0000	1.0000	1.0000	0.7958	1.0000	1.0000
Actve TB vs Latent TB	1.0000	1.0000	1.0000	1.0000	0.4998	0.3151	0.3151	0.4998	1.0000	0.5009	1.0000	1.0000	0.2960	0.6722	1.0000	1.0000	0.4953	1.0000	1.0000

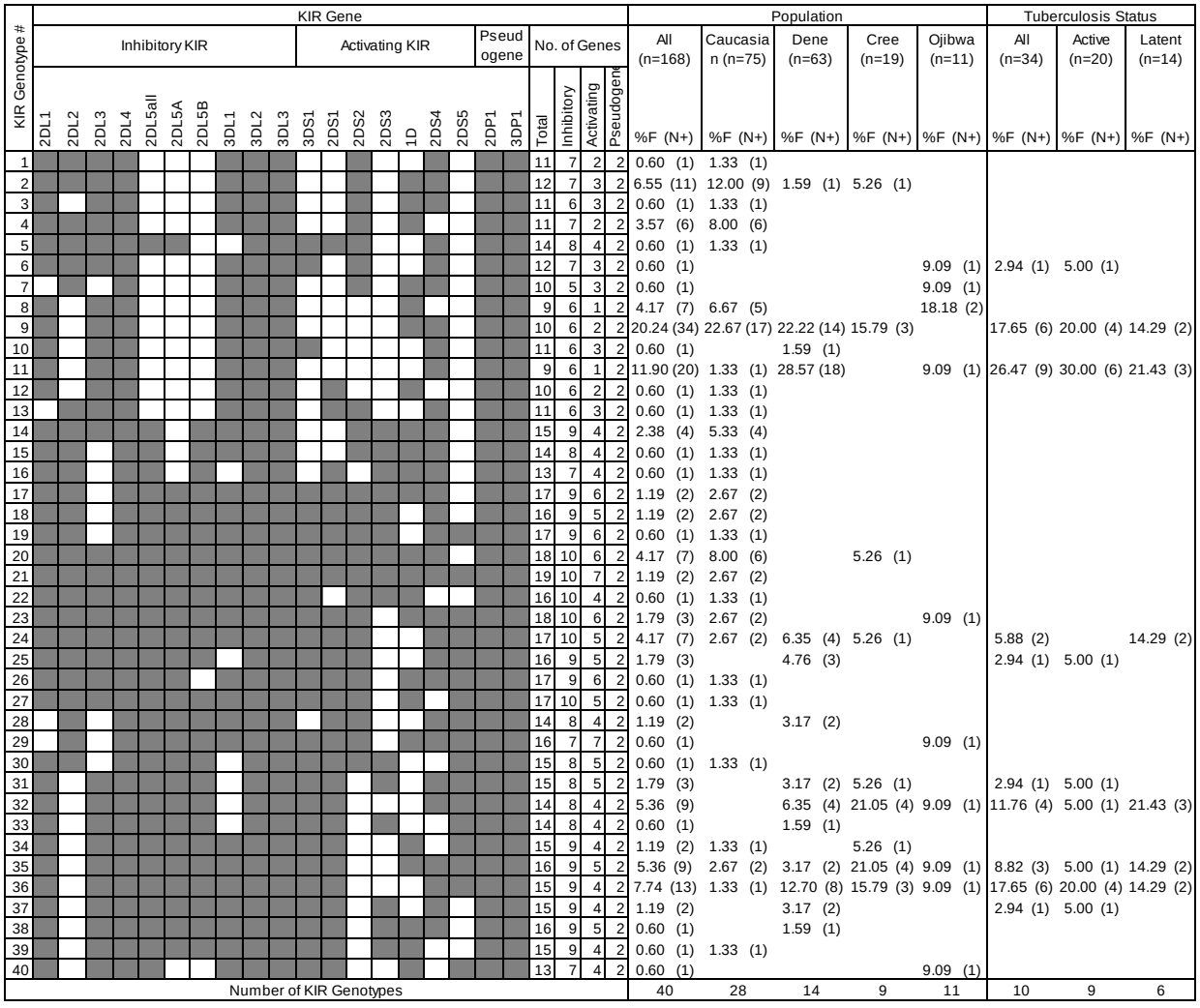
Significant P-values (≤ 0.05) are bolded

5.1.2. KIR Gene Profiles

In order to determine the differences in KIR gene frequencies between population groups as well as disease status groups, gene frequencies were assembled into KIR profiles (Specific Objective ii.). As described in the material and methods, a profile is the presence/absence of 19 possible KIR genes. Forty different profiles (genotypes) were identified in the 168 study participants (Figure 8). These profiles ranged in their distribution from as high as 20.24% (34/168) to as low as 0.60% (1/168). Half of the profiles identified were unique to single individuals. The 4 most predominant genotypes were #9 (34/168, 20.2%), #11 (20/168, 11.9%), #36 (13/168, 7.7%), and #2 (11/168, 6.5%). Of the 40 genotypes, 28 of them were seen in Caucasian individuals and 22 of them were seen in First Nations individuals (10 genotypes overlapping; Figure 9). Within the First Nations subgroups, there were 14 genotypes in Dene, 9 genotypes in Cree, and 11 genotypes in Ojibwa individuals. Although there was overlap between the First Nations groups, 6 genotypes were unique to Dene First Nations (genotypes #10, 25, 28, 33, 37, and 38), and 4 genotypes were unique to Ojibwa First Nations (genotypes #6, 7, 29, and 40). No genotypes were unique to Cree First Nations (Figure 9). Again, we see differences between Caucasians and First Nations, as well as within First Nations subgroups.

Individuals with any TB status (active and latent) were found within 10 of the 40 genotypes (genotypes #6, 9, 11, 24, 25, 31, 32, 35, 36, and 37), most predominantly in genotypes #11 (9/34, 26.5%), #9 (6/34, 17.6%), #36 (6/34, 17.6%), and #31 (4/34,

11.8%). Over seventy-three percent of TB cases (25/34, 73.53%) were found in these four genotypes. Of the 10 genotypes associated with TB cases, 9 of them (all but genotype #24) were found in individuals from the active TB group. Six of the 10 genotypes associated with TB cases (genotypes #9, 11, 24, 32, 35, and 36) were found in individuals from the latent TB group. Only 1 of the 10 genotypes did not include members of the active groups (#24), while 4 of the 10 genotypes did not include members of the latent group (#6, 25, 31, and 37). Overall, the KIR gene profiles identified in individuals with latent and active TB status show less diversity in number of genotypes represented compared to the total number of genotypes identified in the study population.



KIR Genotype #	KIR Gene: Inhibitory KIR 2DL1	2DL2	2DL3	2DL4	2DL5all	2DL5A	2DL5B	3DL1	3DL2	3DL3	3DS1	2DS1	2DS2	2DS3	1D	2DS4	2DS5	Pseudogene 2DP1	3DP1	No. of Genes: Total	Inhibitory	Activating	Pseudogene	Population: All (n=168) %F (N+)	Caucasian (n=75) %F (N+)	Dene (n=63) %F (N+)	Cree (n=19) %F (N+)	Ojibwa (n=11) %F (N+)	Tuberculosis Status: All (n=34) %F (N+)	Active (n=20) %F (N+)	Latent (n=14) %F (N+)
1	■	■	■	■	□	□	□	■	■	■	□	□	■	□	□	■	□	■	■	11	7	2	2	0.60 (1)	1.33 (1)						
2	■	■	■	■	□	□	□	■	■	■	□	□	■	□	■	■	□	■	■	12	7	3	2	6.55 (11)	12.00 (9)	1.59 (1)	5.26 (1)				
3	■	□	■	■	□	□	□	■	■	■	□	□	■	□	■	■	□	■	■	11	6	3	2	0.60 (1)	1.33 (1)						
4	■	■	■	■	□	□	□	■	■	■	□	□	■	□	■	□	□	■	■	11	7	2	2	3.57 (6)	8.00 (6)						
5	■	■	■	■	■	■	□	□	■	■	■	■	■	□	□	■	□	■	■	14	8	4	2	0.60 (1)	1.33 (1)						
6	■	■	■	■	□	□	□	■	■	■	■	□	■	□	□	■	□	■	■	12	7	3	2	0.60 (1)				9.09 (1)	2.94 (1)	5.00 (1)	
7	□	■	□	■	□	□	□	■	■	■	□	□	■	□	■	■	□	■	■	10	5	3	2	0.60 (1)				9.09 (1)			
8	■	□	■	■	□	□	□	■	■	■	□	□	□	□	■	□	□	■	■	9	6	1	2	4.17 (7)	6.67 (5)			18.18 (2)			
9	■	■	□	■	□	□	□	■	■	■	□	□	□	□	■	■	□	■	■	10	6	2	2	20.24 (34)	22.67 (17)	22.22 (14)	15.79 (3)		17.65 (6)	20.00 (4)	14.29 (2)
10	■	□	■	■	□	□	□	■	■	■	■	□	□	□	□	■	□	■	■	11	6	3	2	0.60 (1)		1.59 (1)					
11	■	□	■	■	□	□	□	■	■	■	□	■	□	□	□	■	□	■	■	9	6	1	2	11.90 (20)	1.33 (1)	28.57 (18)		9.09 (1)	26.47 (9)	30.00 (6)	21.43 (3)
12	■	□	■	■	□	□	□	■	■	■	□	■	□	□	■	■	□	■	■	10	6	2	2	0.60 (1)	1.33 (1)						
13	□	■	■	■	□	□	□	■	■	■	□	■	■	□	□	■	□	■	■	11	6	3	2	0.60 (1)	1.33 (1)						
14	■	■	■	■	■	□	■	■	■	■	□	□	■	■	■	■	□	■	■	15	9	4	2	2.38 (4)	5.33 (4)						
15	■	■	□	■	■	□	■	■	■	■	□	□	■	■	■	■	□	■	■	14	8	4	2	0.60 (1)	1.33 (1)						
16	■	■	□	■	■	□	■	□	■	■	□	■	□	■	■	■	□	■	■	13	7	4	2	0.60 (1)	1.33 (1)						
17	■	■	□	■	■	■	■	■	■	■	■	■	■	■	■	■	□	■	■	17	9	6	2	1.19 (2)	2.67 (2)						
18	■	■	□	■	■	■	■	■	■	■	■	■	■	■	□	■	□	■	■	16	9	5	2	1.19 (2)	2.67 (2)						
19	■	■	□	■	■	■	■	■	■	■	■	■	■	■	□	■	■	■	■	17	9	6	2	0.60 (1)	1.33 (1)						
20	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	□	■	■	18	10	6	2	4.17 (7)	8.00 (6)		5.26 (1)				
21	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	19	10	7	2	1.19 (2)	2.67 (2)						
22	■	■	■	■	■	■	■	■	■	■	■	□	■	■	■	□	■	■	■	16	10	4	2	0.60 (1)	1.33 (1)						
23	■	■	■	■	■	■	■	■	■	■	■	■	■	□	■	■	■	■	■	18	10	6	2	1.79 (3)	2.67 (2)			9.09 (1)			
24	■	■	■	■	■	■	■	■	■	■	■	■	■	□	□	■	■	■	■	17	10	5	2	4.17 (7)	2.67 (2)	6.35 (4)	5.26 (1)		5.88 (2)		14.29 (2)
25	■	■	■	■	■	■	■	□	■	■	■	■	■	□	□	■	■	■	■	16	9	5	2	1.79 (3)		4.76 (3)			2.94 (1)	5.00 (1)	
26	■	■	■	■	■	■	□	■	■	■	■	■	■	□	■	■	■	■	■	17	9	6	2	0.60 (1)	1.33 (1)						
27	■	■	■	■	■	■	■	■	■	■	■	■	■	□	■	□	■	■	■	17	10	5	2	0.60 (1)	1.33 (1)						
28	□	■	□	■	■	■	■	■	■	■	□	■	■	□	□	■	■	■	■	14	8	4	2	1.19 (2)		3.17 (2)					
29	□	■	□	■	■	■	■	■	■	■	■	■	■	■	□	■	■	■	■	16	7	7	2	0.60 (1)				9.09 (1)			
30	■	■	□	■	■	■	■	□	■	■	■	■	■	■	□	□	■	■	■	15	8	5	2	0.60 (1)	1.33 (1)						
31	■	□	■	■	■	■	■	□	■	■	■	■	□	■	□	■	■	■	■	15	8	5	2	1.79 (3)		3.17 (2)	5.26 (1)		2.94 (1)	5.00 (1)	
32	■	□	■	■	■	■	■	□	■	■	■	■	□	□	□	■	■	■	■	14	8	4	2	5.36 (9)		6.35 (4)	21.05 (4)	9.09 (1)	11.76 (4)	5.00 (1)	21.43 (3)
33	■	□	■	■	■	■	■	□	■	■	■	■	□	■	□	■	■	■	■	14	8	4	2	0.60 (1)		1.59 (1)					
34	■	□	■	■	■	■	■	■	■	■	□	□	□	□	■	□	■	■	■	15	9	4	2	1.19 (2)	1.33 (1)		5.26 (1)				
35	■	□	■	■	■	■	■	■	■	■	■	■	□	□	■	■	■	■	■	16	9	5	2	5.36 (9)	2.67 (2)	3.17 (2)	21.05 (4)	9.09 (1)	8.82 (3)	5.00 (1)	14.29 (2)
36	■	□	■	■	■	■	■	■	■	■	■	■	□	□	□	■	■	■	■	15	9	4	2	7.74 (13)	1.33 (1)	12.70 (8)	15.79 (3)	9.09 (1)	17.65 (6)	20.00 (4)	14.29 (2)
37	■	□	■	■	■	■	■	■	■	■	■	■	□	■	□	■	□	■	■	15	9	4	2	1.19 (2)		3.17 (2)			2.94 (1)	5.00 (1)	
38	■	□	■	■	■	■	■	■	■	■	■	■	□	■	■	■	□	■	■	16	9	5	2	0.60 (1)		1.59 (1)					
39	■	□	■	■	■	■	■	■	■	■	■	■	□	■	■	□	■	■	■	15	9	4	2	0.60 (1)	1.33 (1)						
40	■	□	■	■	■	□	□	■	■	■	■	■	□	□	■	□	■	■	■	13	7	4	2	0.60 (1)				9.09 (1)			
Number of KIR Genotypes																								40	28	14	9	11	10	9	6

Figure 8: Frequency of KIR genotypes in populations in Subset A. Forty distinct KIR types were seen in these 168 individuals that differ from each other by the presence of (shaded box) or absence (white box) of 19 KIR genes (KIR2DL5 broken down into 2DL5A, 2DL5B, and 2DL5All; KIR2DS4 broken down into 1D and 2DS4). Frequency (%F) of each genotype is expressed as a percentage and is defined as the number of individuals having the genotype (N+) divided by the number of individuals (n) in the population or tuberculosis status group.

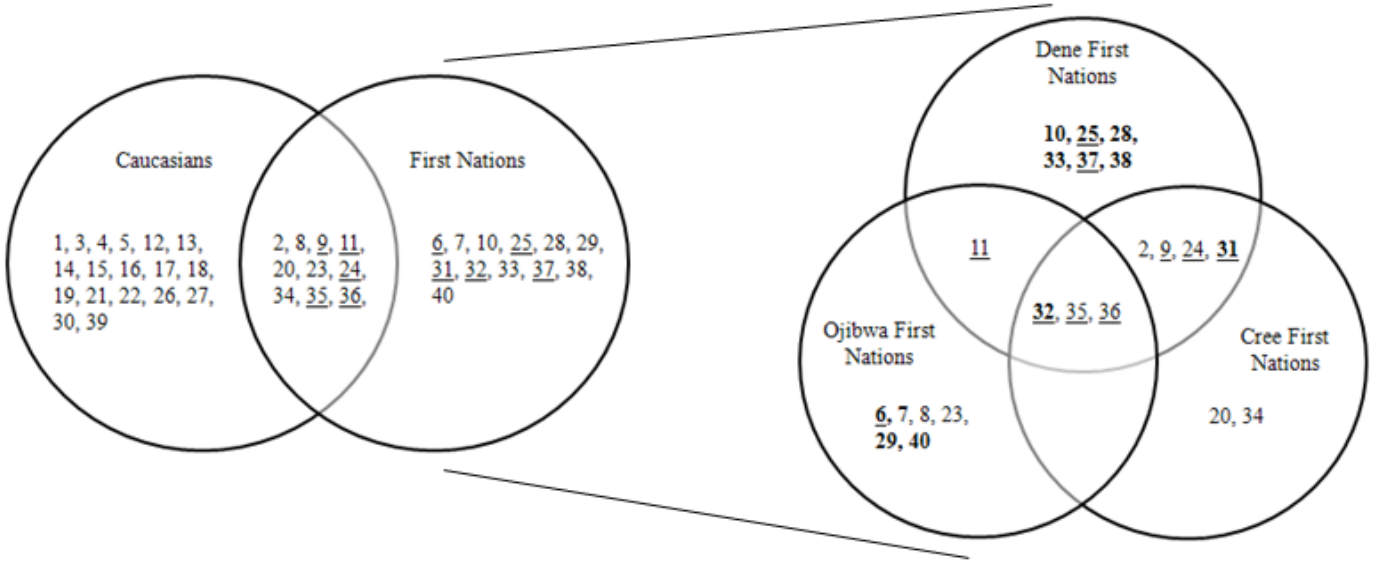


Figure 9: Distribution of KIR genotypes among Dene First Nations, Cree First Nations, and Ojibwa First Nations in Subset A. Genotypes containing at least one active/latent tuberculosis case are underlined. Bolded genotypes are seen in First Nations only, and not represented in the Caucasian population.

5.1.3. Haplotype Analysis

In order to determine differences in KIR haplotypes between population groups and disease status groups, individuals were categorized into haplotypes using the scheme described in Section 4.4 (Specific Objective iii.). Centromeric and telomeric haplotypes were determined as AA, AB, or BB, with A contributing only one activating gene (KIR2DS4 and KIR1D considered the same loci) to the profile, and B contributing 2 or more activating genes (KIR2DS2, KIR2DS3, KIR3DS1, KIR2DS5, and/or KIR2DS1) to the profile.

KIR haplotypes can be broken down into the centromeric cluster/portion and the telomeric cluster/portion to be analyzed separately. When looking exclusively at the centromeric portion of the KIR cluster, Caucasian individuals have more centromeric (cen)-AB (49.33%) than AA (40.00%) or BB (10.67%) compared to First Nations. The majority of First Nations individuals have an AA (81.72%) centromeric KIR cluster rather than AB (13.98%) or BB (4.30%). Likewise, 85.71% of individuals with latent TB fall in the cen-AA group, with the remaining 14.29% in the cen-AB group. Active TB cases also are found only in cen-AA (90.00%) and cen-AB (10.00%). Uninfected individuals are within all three centromeric groups (77.97% AA, 15.25% AB, and 6.68% BB).

When looking exclusively at the telomeric portion of the KIR cluster, the majority of Caucasians have an AA (62.67%) telomeric KIR cluster rather than AB (33.33%) or BB (2.67%). First Nations individuals have more telomeric (tel)-AA (46.24%) than AB

(36.56%) or BB (17.20%). Unlike the centromeric cluster, individuals with latent TB are seen not only in AA (35.71%) and AB (42.86%), but also in the tel-BB cluster (21.43%). The same can be said for active TB (50.00% AA, 35.00% AB, and 15.00% BB). Uninfected individuals also fell into all three telomeric groups (47.46% AA, 35.60% AB, and 16.95% BB) (Table 5).

Table 5: Frequency of centromeric and telomeric KIR clusters in different ethnic groups and tuberculosis status groups (Subset A)

KIR Cluster		Ethnicity		Tuberculosis Status in First Nations		
Centromeric		Caucasian	First Nations	Latent	Active	Uninfected
	AA	40.00%	81.72%	85.71%	90.00%	77.97%
	AB	49.33%	13.98%	14.29%	10.00%	15.25%
	BB	10.67%	4.30%	0.00%	0.00%	6.68%
Telomeric						
	AA	62.67%	46.24%	35.71%	50.00%	47.46%
	AB	33.33%	36.56%	42.86%	35.00%	35.60%
	BB	2.67%	17.20%	21.43%	15.00%	16.95%
	-/-	1.33%				

When the centromeric and telomeric KIR clusters are combined, haplotypes can be compared (Table 6). Centromeric and telomeric clusters are separated by a hyphen when describing haplotype. First Nations individuals were significantly more likely to have AA-AB (26.9% vs. 6.7%, P=0.0006) and AA-BB (14.0% vs. 0.0%, P=0.0006) haplotypes, and less likely to have AB-AA (2.2% vs. 28.0%, P<0.0001) and AB-AB (8.6% vs. 20.0%, P=0.0421) haplotypes than Caucasians.

The majority of individuals within both the population groups as well as the disease status groups had a centromeric-Ax haplotype (Caucasian – 89.3%, First Nations – 95.7%; latent TB – 100.0%, active TB – 100.0%). In addition, significantly more First Nations individuals with TB status (30/34, 88.2%) were found to have a centromeric-AA haplotype than those with cen-AB or –BB (4/34, 11.8%, P<0.0001). Half of the active cases of tuberculosis (10/20, 50.00%) were of the AA-AA haplotype, which contains the fewest number of activating genes of all haplotypes. Only 12 individuals had centromeric BB-haplotypes (8/75 Caucasians, 4/93 First Nations). Within this group there was one Caucasian individual (genotype #16, Figure 8 in Section 4.1.3) lacking a telomeric haplotype by definition of the haplotype determination scheme.

Table 6: Frequency of centromeric and telomeric haplotypes in different ethnic and tuberculosis status groups (Subset A)

Haplotype		Ethnicity			Tuberculosis Status in First Nations		
		Caucasian	First Nations		Latent	Active	Uninfected
Centromeric	Telomeric	(n = 75)	(n = 93)	P-value	(n = 14)	(n = 20)	(n = 59)
AA	AA	25 (33.3)	38 (40.9)	0.3399	5 (35.7)	10 (50.0)	23 (39.0)
AA	AB	5 (6.7)	25 (26.9)	**0.0006**	4 (28.6)	6 (30.0)	15 (25.4)
AB	AA	21 (28.0)	2 (2.2)	**<0.0001**			2 (3.4)
AB	AB	15 (20.0)	8 (8.6)	**0.0421**	2 (14.3)	1 (5.0)	5 (8.5)
AA	BB	0 (0.0)	13 (14.0)	**0.0006**	3 (21.4)	2 (10.0)	8 (13.6)
AB	BB	1 (1.3)	3 (3.2)	0.6296		1 (5.0)	2 (3.4)
BB	AA	1 (1.3)	3 (3.2)	0.6296			3 (5.1)
BB	AB	5 (6.7)	1 (1.1)	0.0900			1 (1.7)
BB	BB	1 (1.3)	0 (0.0)	0.4464			
BB	-/-	1 (1.3)	0 (0.0)	0.4464			

Significant P-values (≤ 0.05) are bolded

5.2. Subset B: Ethnically Diverse Manitoban Cohort

5.2.1. KIR Gene Frequencies

In order to determine the differences in KIR gene frequencies between different ethnic groups (Canadian-born Aboriginals, Canadian-born non-Aboriginals, and foreign born individuals) and disease status groups (active, latent, and uninfected TB status), the KIR gene frequency data obtained was analyzed and compared (Specific Objective i.). The data showed consistent presence of framework genes KIR2DL4, KIR3DL2, KIR3DL3, and the pseudogenes KIR2DP1 and KIR3DP1 in all groups. There were many significant differences (P-value $\leq$0.05) in the frequencies of KIR genes between ethnic groups (Table 7). The following KIR genes were lower in frequency in Aboriginals compared to non-Aboriginals: KIR2DL2 (29.63% vs. 49.35%, *P*=0.0306), KIR2DS2 (29.63% vs. 49.35%, *P*=0.0306), and KIR2DS3 (5.56% vs. 29.87%, *P*=0.0006). KIR2DS5 (53.70% vs. 25.97%, *P*=0.0017) was higher in frequency in Aboriginals compared to non-Aboriginals. The same four genes differed significantly between Aboriginals and foreign born individuals: KIR2DL2 (29.63% vs. 50.00%, *P*=0.0211), KIR2DS2 (29.63% vs. 50.00%, *P*=0.0211), and KIR2DS3 (5.56% vs. 38.46%, *P*<0.0001) were lower in frequency and KIR2DS5 (53.70% vs. 30.77%, *P*=0.0113) was seen in higher frequency. Overall, significant differences were seen in KIR gene frequencies between Aboriginals and non-Aboriginals, Aboriginals and foreign born, but not between non-Aboriginals and foreign born individuals.

Of the 209 samples, five KIR genes (KIR2DL2, 2DL5all, 2DL5B, 2DS2, and

2DS3) differed significantly ($P \leq 0.05$) in frequency between disease status groups (Table 8). Only two genes, KIR2DL2 (33.33% vs. 55.77%, $P=0.0014$) and KIR2DS2 (34.29% vs. 54.81%, $P=0.0035$) differed between individuals with any TB status and uninfected individuals. However, the underlying differences can be exposed when analyzing latent and active TB separately. KIR2DL5all and KIR2DL5B (both 73.91% vs. 51.92%, $P=0.0125$) were present in higher frequency in latently infected individuals compared to uninfected individuals. KIR2DL2 (27.12% vs. 55.77%, $P=0.0005$), KIR2DS2 (27.12% vs. 54.82%, $P=0.0010$), and KIR2DS3 (8.47% vs. 30.77%, $P=0.0009$) were present in a lower frequency in actively infected individuals compared to uninfected individuals. Lastly, significant differences were seen between gene frequencies in latently and actively infected individuals: KIR2DL5all (73.91% vs. 49.15%, $P=0.0156$), KIR2DL5B (73.91% vs. 49.15%, $P=0.0156$), and KIR2DS3 (39.13% vs. 8.47%, $P=0.0002$). Overall, not only were differences in KIR gene frequencies seen between infected and uninfected TB status, but also between those with active and latent TB disease.

Table 7: Comparison of killer immunoglobulin-like receptor (KIR) gene frequencies in Canadian born non-Aboriginal, Canadian born Aboriginal, and foreign born individuals (Subset B)

Frequency (%)	KIRs																		
	2DL1	2DL2	2DL3	2DL4	2DL5all	2DL5A	2DL5B	2DS1	2DS2	2DS3	1D	2DS4	2DS5	3DL1	3DL2	3DL3	3DS1	2DP1	3DP1
Canadian born non-aboriginal (n = 77)	75 (97.40)	38 (49.35)	70 (90.91)	77 (100.00)	38 (49.35)	29 (37.66)	38 (49.35)	30 (38.96)	38 (49.35)	23 (29.87)	60 (77.92)	76 (98.70)	20 (25.97)	75 (97.40)	77 (100.00)	77 (100.00)	31 (40.26)	77 (100.00)	77 (100.00)
Canadian born aboriginal (n=54)	53 (98.15)	16 (29.63)	53 (98.15)	54 (100.00)	32 (59.26)	30 (55.56)	32 (59.26)	31 (57.41)	16 (29.63)	3 (5.56)	40 (74.07)	54 (100.00)	29 (53.70)	48 (88.89)	54 (100.00)	54 (100.00)	31 (57.41)	54 (100.00)	54 (100.00)
Foreign born (n=78)	77 (98.72)	39 (50.00)	72 (92.31)	78 (100.00)	47 (60.26)	32 (41.03)	47 (60.26)	33 (42.31)	39 (50.00)	30 (38.46)	52 (66.67)	78 (100.00)	24 (30.77)	73 (93.59)	78 (100.00)	78 (100.00)	36 (46.15)	78 (100.00)	78 (100.00)
P-value																			
CBnA vs CBA	1.0000	**0.0306**	0.1406	1.0000	0.2896	0.0508	0.2896	0.0502	**0.0306**	**0.0006**	0.6781	1.0000	**0.0017**	0.0643	1.0000	1.0000	0.0751	1.0000	1.0000
CBnA vs FB	0.6201	1.0000	0.7805	1.0000	0.1982	0.7430	0.1982	0.7443	1.0000	0.3105	0.1513	0.4968	0.5938	0.4423	1.0000	1.0000	0.5178	1.0000	1.0000
CBA vs FB	1.0000	**0.0211**	0.2393	1.0000	1.0000	0.1130	1.0000	0.1113	**0.0211**	**<0.0001**	0.4422	1.0000	**0.0113**	0.3559	1.0000	1.0000	0.2199	1.0000	1.0000

Significant P-values (≤0.05) are bolded; CBnA - Canadian-born non-Aboriginal, CBA - Canadian-born Aboriginal, FB - foreign born

Table 8: Comparison of killer immunoglobulin-like receptor (KIR) gene frequencies by tuberculosis status (Subset B)

Frequency (%)	KIRs																	
	2DL1	2DL2	2DL3	2DL4	2DL5all	2DL5A	2DL5B	2DS1	2DS2	2DS3	1D	2DS4	2DS5	3DL1	3DL2	3DL3	3DS1	2DP1	3DP1
Tuberculosis (TB)																			
All (n = 105)	103 (98.10)	35 (33.33)	100 (95.24)	105 (100.00)	63 (60.00)	48 (45.71)	63 (60.00)	50 (47.62)	36 (34.29)	23 (21.90)	73 (69.52)	105 (100.00)	41 (39.05)	99 (94.29)	105 (100.00)	105 (100.00)	55 (52.38)	105 (100.00)	105 (100.00)
Latent (n = 46)	45 (97.82)	19 (41.30)	42 (91.30)	46 (100.00)	34 (73.91)	24 (52.17)	34 (73.91)	25 (54.35)	20 (43.48)	18 (39.13)	36 (78.26)	46 (100.00)	16 (34.78)	45 (97.82)	46 (100.00)	46 (100.00)	26 (56.52)	46 (100.00)	46 (100.00)
Active (n = 59)	58 (98.31)	16 (27.12)	58 (98.31)	59 (100.00)	29 (49.15)	24 (40.68)	29 (49.15)	25 (42.37)	16 (27.12)	5 (8.47)	37 (62.71)	59 (100.00)	25 (42.37)	54 (91.53)	59 (100.00)	59 (100.00)	29 (49.15)	59 (100.00)	59 (100.00)
No Tuberculosis (n = 104)	102 (98.08)	58 (55.77)	95 (91.35)	104 (100.00)	54 (51.92)	43 (41.35)	54 (51.92)	44 (42.31)	57 (54.81)	32 (30.77)	79 (75.96)	103 (99.04)	32 (30.77)	97 (93.27)	104 (100.00)	104 (100.00)	43 (41.35)	104 (100.00)	104 (100.00)
P-value																			
TB vs No TB	1.0000	**0.0014**	0.2837	1.0000	0.2664	0.5776	0.2664	0.4879	**0.0035**	0.1599	0.3519	0.4976	0.2463	0.7832	1.0000	1.0000	0.1279	1.0000	1.0000
Latent TB vs No TB	1.0000	0.1137	1.0000	1.0000	**0.0125**	0.2853	**0.0125**	0.2141	0.2190	0.3505	0.8363	1.0000	0.7050	0.4357	1.0000	1.0000	0.1099	1.0000	1.0000
Active TB vs No TB	1.0000	**0.0005**	0.0957	1.0000	0.7472	1.0000	0.7472	1.0000	**0.0010**	**0.0009**	0.1047	1.0000	0.1716	0.7583	1.0000	1.0000	0.4121	1.0000	1.0000
Actve TB vs Latent TB	1.0000	0.1474	1.0000	1.0000	**0.0156**	0.3237	**0.0156**	0.2432	0.0988	**0.0002**	0.0933	1.0000	0.5457	0.2272	1.0000	1.0000	0.5552	1.0000	1.0000

Significant P-values (≤0.05) are bolded

All of the same trends were noted when both ethnicity and tuberculosis status were analysed in conjunction; however, not all findings were statistically significant (Table 9). Frequencies of KIR2DL5all (62.86% vs. 25.00%, *P*=0.0424), KIR2DL5A (57.14% vs. 16.67%, *P*=0.0205), KIR2DL5B (62.86% vs. 25.00%, *P*=0.0424), KIR2DS1 (60.00% vs. 16.67%, *P*=0.0173), KIR2DS5 (60.00% vs. 16.67%, *P*=0.0173), and KIR3DS1 (60.00% vs. 25.00%, *P*=0.0490) were all significantly higher in Aboriginals with active tuberculosis compared to non-Aboriginals with active tuberculosis. Frequency of KIR2DS2 (43.48% vs. 18.33%, *P*=0.0112) and KIR2DS3 (39.13% vs. 2.5%, *P*<0.0001) were higher in foreign born individuals with tuberculosis when compared to Aboriginals. Frequency of KIR2DS5 in individuals with a TB status was lower in foreign born individuals when compared to Aboriginals (26.09% vs. 60.00%, *P*= 0.0021) and more specifically in those with active TB (16.67% vs. 60.00%, *P*=0.0173). No significant differences were seen between KIR gene frequencies between non-Aboriginals and foreign born individuals.

Within the non-Aboriginal population group, KIR2DL2 and KIR2DS2 gene frequencies in those individuals with a TB status (26.32% vs. 56.90%, *P*=0.0332) and specifically with active TB (16.67% vs. 56.90%, *P*=0.0234) were lower than uninfected non-Aboriginal individuals. Additionally, KIR2DL5A and KIR2DS1 frequencies were higher (both 71.43% vs. 16.67%, *P*=0.0449) in those with latent TB compared to those with active TB. Within the foreign born population group, KIR2DS5all and KIR2DL5B frequencies were higher (both 76.47% vs. 33.33%, *P*=0.0126) in those with latent TB

compared to those with active TB. Within the Aboriginal population group, no significant differences were seen between different disease status groups. This analysis shows that KIR gene frequencies associate with both ethnicity and TB status when analyzed in parallel.

Table 9: Comparison of KIR gene frequencies by tuberculosis status in A) Aboriginals, B) non-Aboriginals, and C) foreign born individuals (Subset B)

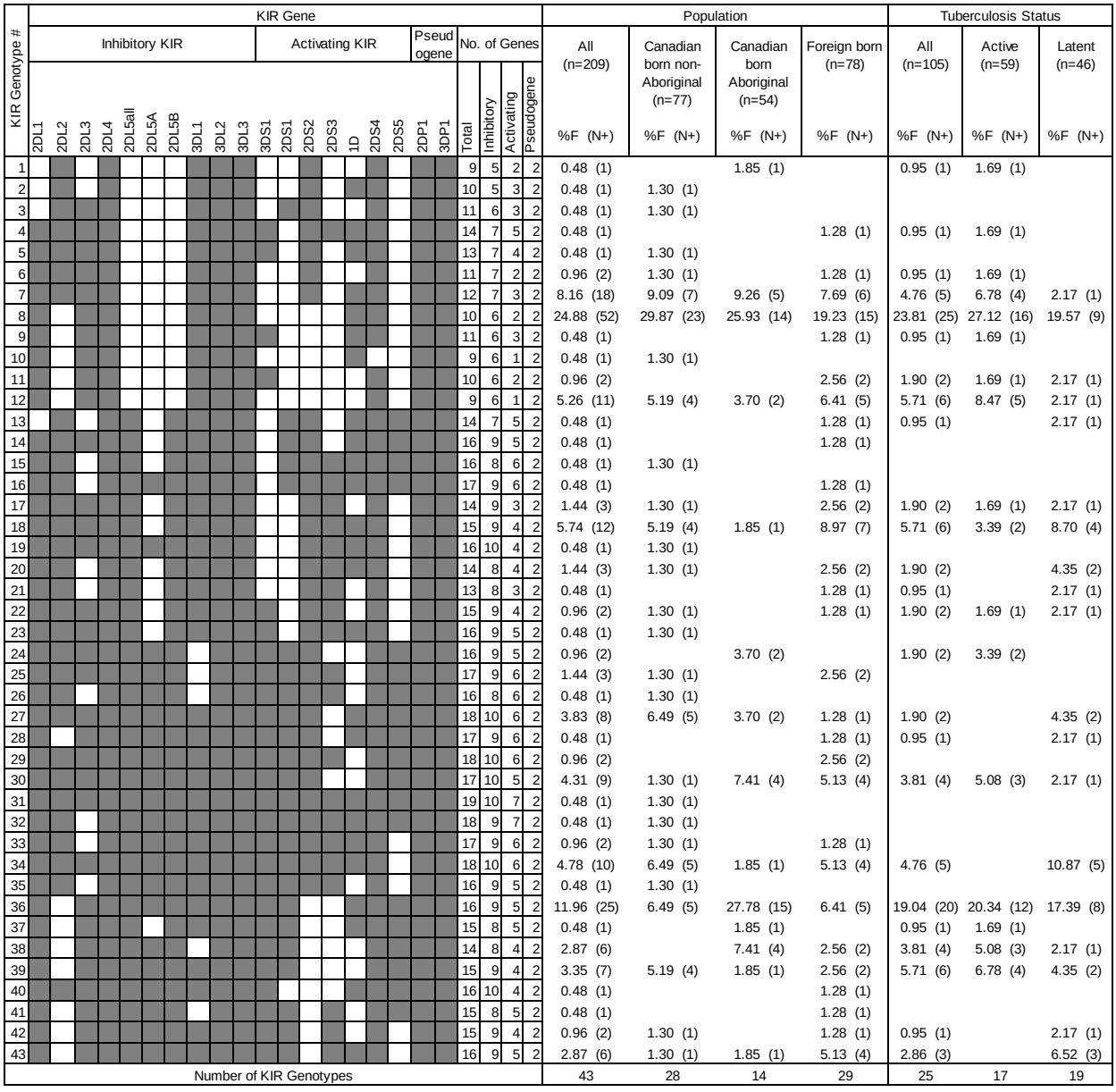
	Frequency (%)	KIRs																	
A)		2DL1	2DL2	2DL3	2DL4	2DL5all	2DL5A	2DL5B	2DS1	2DS2	2DS3	1D	2DS4	2DS5	3DL1	3DL2	3DL3	3DS1	2DP1	3DP1
	Tuberculosis (TB)																			
	All (n = 40)	97.50	27.50	97.50	100.00	62.50	57.50	62.50	60.00	18.33[e]	2.5[e]	72.50	100.00	60.00[d,e]	87.50	100.00	100.00	60.00	100.00	100.00
	Latent (n = 5)	100.00	20.00	100.00	100.00	60.00	60.00	60.00	60.00	20.00	0.00	100.00	100.00	60.00	100.00	100.00	100.00	60.00	100.00	100.00
	Active (n = 35)	97.14	28.57	97.14	100.00	62.86[d]	57.14[d,e]	62.86[d]	60.00[d,e]	28.57	2.86	68.57	100.00	60.00[d,e]	85.71	100.00	100.00	60.00[d]	100.00	100.00
	No Tuberculosis (n = 14)	100.00	35.71	100.00	100.00	50.00	50.00	50.00	50.00	35.71	14.29	78.57	100.00	25.71	92.86	100.00	100.00	50.00	100.00	100.00
B)		2DL1	2DL2	2DL3	2DL4	2DL5all	2DL5A	2DL5B	2DS1	2DS2	2DS3	1D	2DS4	2DS5	3DL1	3DL2	3DL3	3DS1	2DP1	3DP1
	Tuberculosis (TB)																			
	All (n = 19)	100.00	26.32[a]	100.00	100.00	42.11	36.84	42.11	36.84	26.32[a]	15.79	68.42	100.00	26.32[c]	100.00	100.00	100.00	42.11	100.00	100.00
	Latent (n = 7)	100.00	42.86	100.00	100.00	71.43	71.43[b]	71.43	71.43[b]	42.86	28.57	85.71	100.00	42.86	100.00	100.00	100.00	71.43	100.00	100.00
	Active (n = 12)	100.00	16.67[a]	100.00	100.00	25.00[c]	16.67[b,c]	25.00[c]	16.67[b,c]	16.67[a]	8.33	58.33	100.00	16.67[c]	100.00	100.00	100.00	25.00[c]	100.00	100.00
	No Tuberculosis (n = 58)	96.55	56.90	87.93	100.00	51.72	37.93	51.72	39.66	56.90	34.48	82.76	98.28	25.86	96.55	100.00	100.00	39.66	100.00	100.00
C)		2DL1	2DL2	2DL3	2DL4	2DL5all	2DL5A	2DL5B	2DS1	2DS2	2DS3	1D	2DS4	2DS5	3DL1	3DL2	3DL3	3DS1	2DP1	3DP1
	Tuberculosis (TB)																			
	All (n = 46)	97.83	41.30	91.30	100.00	65.22	39.13	65.22	41.30	43.48[c]	39.13[c]	67.39	100.00	26.09[c]	97.83	100.00	100.00	50.00	100.00	100.00
	Latent (n = 34)	97.06	44.12	88.24	100.00	76.47[b]	47.06	76.47[b]	50.00	47.06	47.06	73.53	100.00	29.41	97.06	100.00	100.00	52.94	100.00	100.00
	Active (n = 12)	100.00	33.33	100.00	100.00	33.33[b]	16.67[c]	33.33[b]	16.67[c]	33.33	16.67	50.00	100.00	16.67[c]	100.00	100.00	100.00	41.67	100.00	100.00
	No Tuberculosis (n = 32)	100.00	62.50	93.75	100.00	53.13	43.75	53.13	43.75	59.38	34.38	65.63	100.00	37.50	87.50	100.00	100.00	40.63	100.00	100.00

[a] significant compared to no TB; [b] significant difference between active and latent TB; [c] significant compared to Aboriginal; [d] significant compared to non-Aboriginal; [e] significant compared to foreign born; P-value <0.05 considered statistically significant

5.2.2. KIR Gene Profiles

In order to determine the differences in KIR gene frequencies between ethnic groups as well as disease status groups, gene frequencies were assembled into KIR profiles (Specific Objective ii.). Forty-three KIR profiles (genotypes) were identified in the 209 study participants (Figure 10). These profiles ranged in their distribution from as high as 24.88% (52/209) to as low as 0.48% (1/209). Twenty-two of the 43 profiles identified were unique to single individuals. Of the 43 profiles, 29 were seen in foreign born individuals, 28 were seen in Canadian born non-Aboriginals, and 14 were seen in Aboriginal individuals (overlap between these groups can be seen in Figure 11). The most prominent genotypes were #8 (52/209, 24.9%), #36 (25/209, 12.0%), #7 (18//209, 8.2%), #18 (12/209, 5.7%), and #12 (11/209, 5.3%).

Ten KIR genotypes were shared between the three population groups (genotypes #7, 8, 12, 18, 27, 30, 34, 36, 39, 43).



KIR Genotype #	KIR Gene: Inhibitory KIR 2DL1	2DL2	2DL3	2DL4	2DL5all	2DL5A	2DL5B	3DL1	3DL2	3DL3	Activating KIR 3DS1	2DS1	2DS2	2DS3	1D	2DS4	2DS5	Pseudogene 2DP1	3DP1	No. of Genes: Total	Inhibitory	Activating	Pseudogene	Population: All (n=209) %F (N+)	Canadian born non-Aboriginal (n=77) %F (N+)	Canadian born Aboriginal (n=54) %F (N+)	Foreign born (n=78) %F (N+)	Tuberculosis Status: All (n=105) %F (N+)	Active (n=59) %F (N+)	Latent (n=46) %F (N+)
1		■		■				■	■	■			■			■		■	■	9	5	2	2	0.48 (1)		1.85 (1)		0.95 (1)	1.69 (1)	
2		■		■				■	■	■			■		■	■		■	■	10	5	3	2	0.48 (1)	1.30 (1)					
3		■	■	■				■	■	■		■	■			■		■	■	11	6	3	2	0.48 (1)	1.30 (1)					
4	■	■	■	■				■	■	■	■		■	■	■	■		■	■	14	7	5	2	0.48 (1)			1.28 (1)	0.95 (1)	1.69 (1)	
5	■	■	■	■				■	■	■	■		■		■	■		■	■	13	7	4	2	0.48 (1)	1.30 (1)					
6	■	■	■	■				■	■	■			■			■		■	■	11	7	2	2	0.96 (2)	1.30 (1)		1.28 (1)	0.95 (1)	1.69 (1)	
7	■	■	■	■				■	■	■			■		■	■		■	■	12	7	3	2	8.16 (18)	9.09 (7)	9.26 (5)	7.69 (6)	4.76 (5)	6.78 (4)	2.17 (1)
8	■		■	■				■	■	■					■	■		■	■	10	6	2	2	24.88 (52)	29.87 (23)	25.93 (14)	19.23 (15)	23.81 (25)	27.12 (16)	19.57 (9)
9	■		■	■				■	■	■	■				■	■		■	■	11	6	3	2	0.48 (1)			1.28 (1)	0.95 (1)	1.69 (1)	
10	■		■	■				■	■	■					■			■	■	9	6	1	2	0.48 (1)	1.30 (1)					
11	■		■	■				■	■	■	■					■		■	■	10	6	2	2	0.96 (2)			2.56 (2)	1.90 (2)	1.69 (1)	2.17 (1)
12	■		■	■				■	■	■						■		■	■	9	6	1	2	5.26 (11)	5.19 (4)	3.70 (2)	6.41 (5)	5.71 (6)	8.47 (5)	2.17 (1)
13		■		■	■		■	■	■	■			■		■	■	■	■	■	14	7	5	2	0.48 (1)			1.28 (1)	0.95 (1)		2.17 (1)
14	■	■	■	■	■		■	■	■	■		■	■		■	■	■	■	■	16	9	5	2	0.48 (1)			1.28 (1)			
15	■	■		■	■		■	■	■	■		■	■	■	■	■	■	■	■	16	8	6	2	0.48 (1)	1.30 (1)					
16	■	■		■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	17	9	6	2	0.48 (1)			1.28 (1)			
17	■	■	■	■	■		■	■	■	■			■	■		■		■	■	14	9	3	2	1.44 (3)	1.30 (1)		2.56 (2)	1.90 (2)	1.69 (1)	2.17 (1)
18	■	■	■	■	■		■	■	■	■			■	■	■	■		■	■	15	9	4	2	5.74 (12)	5.19 (4)	1.85 (1)	8.97 (7)	5.71 (6)	3.39 (2)	8.70 (4)
19	■	■	■	■	■	■	■	■	■	■				■	■	■		■	■	16	10	4	2	0.48 (1)	1.30 (1)					
20	■	■		■	■		■	■	■	■			■	■	■	■		■	■	14	8	4	2	1.44 (3)	1.30 (1)		2.56 (2)	1.90 (2)		4.35 (2)
21	■	■		■	■		■	■	■	■			■	■		■		■	■	13	8	3	2	0.48 (1)			1.28 (1)	0.95 (1)		2.17 (1)
22	■	■	■	■	■		■	■	■	■	■		■	■		■		■	■	15	9	4	2	0.96 (2)	1.30 (1)		1.28 (1)	1.90 (2)	1.69 (1)	2.17 (1)
23	■	■	■	■	■		■	■	■	■			■	■	■	■		■	■	16	9	5	2	0.48 (1)	1.30 (1)					
24	■	■	■	■	■	■	■		■	■	■	■	■			■	■	■	■	16	9	5	2	0.96 (2)		3.70 (2)		1.90 (2)	3.39 (2)	
25	■	■	■	■	■	■	■		■	■	■	■	■	■		■	■	■	■	17	9	6	2	1.44 (3)	1.30 (1)		2.56 (2)			
26	■	■		■	■	■	■		■	■	■	■	■	■		■	■	■	■	16	8	6	2	0.48 (1)	1.30 (1)					
27	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	18	10	6	2	3.83 (8)	6.49 (5)	3.70 (2)	1.28 (1)	1.90 (2)		4.35 (2)
28	■		■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	17	9	6	2	0.48 (1)			1.28 (1)	0.95 (1)		2.17 (1)
29	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	18	10	6	2	0.96 (2)			2.56 (2)			
30	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	17	10	5	2	4.31 (9)	1.30 (1)	7.41 (4)	5.13 (4)	3.81 (4)	5.08 (3)	2.17 (1)
31	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	19	10	7	2	0.48 (1)	1.30 (1)					
32	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	18	9	7	2	0.48 (1)	1.30 (1)					
33	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	17	9	6	2	0.96 (2)	1.30 (1)		1.28 (1)			
34	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	18	10	6	2	4.78 (10)	6.49 (5)	1.85 (1)	5.13 (4)	4.76 (5)		10.87 (5)
35	■	■		■	■	■	■	■	■	■	■	■	■	■		■		■	■	16	9	5	2	0.48 (1)	1.30 (1)					
36	■		■	■	■	■	■	■	■	■	■	■			■	■	■	■	■	16	9	5	2	11.96 (25)	6.49 (5)	27.78 (15)	6.41 (5)	19.04 (20)	20.34 (12)	17.39 (8)
37	■		■	■	■		■	■	■	■	■	■			■	■	■	■	■	15	8	5	2	0.48 (1)		1.85 (1)		0.95 (1)	1.69 (1)	
38	■		■	■	■	■	■		■	■	■	■	■			■	■	■	■	14	8	4	2	2.87 (6)		7.41 (4)	2.56 (2)	3.81 (4)	5.08 (3)	2.17 (1)
39	■		■	■	■	■	■	■	■	■	■	■	■			■	■	■	■	15	9	4	2	3.35 (7)	5.19 (4)	1.85 (1)	2.56 (2)	5.71 (6)	6.78 (4)	4.35 (2)
40	■	■	■	■	■	■	■	■	■	■	■				■	■	■	■	■	16	10	4	2	0.48 (1)			1.28 (1)			
41	■		■	■	■	■	■		■	■	■	■		■		■	■	■	■	15	8	5	2	0.48 (1)			1.28 (1)			
42	■		■	■	■	■	■	■	■	■	■	■		■		■		■	■	15	9	4	2	0.96 (2)	1.30 (1)		1.28 (1)	0.95 (1)		2.17 (1)
43	■		■	■	■	■	■	■	■	■	■		■	■	■	■		■	■	16	9	5	2	2.87 (6)	1.30 (1)	1.85 (1)	5.13 (4)	2.86 (3)		6.52 (3)
Number of KIR Genotypes																								43	28	14	29	25	17	19

Figure 10: Frequency of KIR genotypes in populations in Subset B. Forty-three distinct KIR types were seen in these 209 individuals that differ from each other by the presence of (shaded box) or absence (white box) of 19 KIR genes (KIR2DL5 broken down into 2DL5A, 2DL5B, and 2DL5all; KIR2DS4 broken down into 1D and full length 2DS4). Frequency (%F) of each genotype is expressed as a percentage and is defined as the number of individuals having the genotype (N+) divided by the number of individuals (n) in the specific ethnic or specific tuberculosis status group.

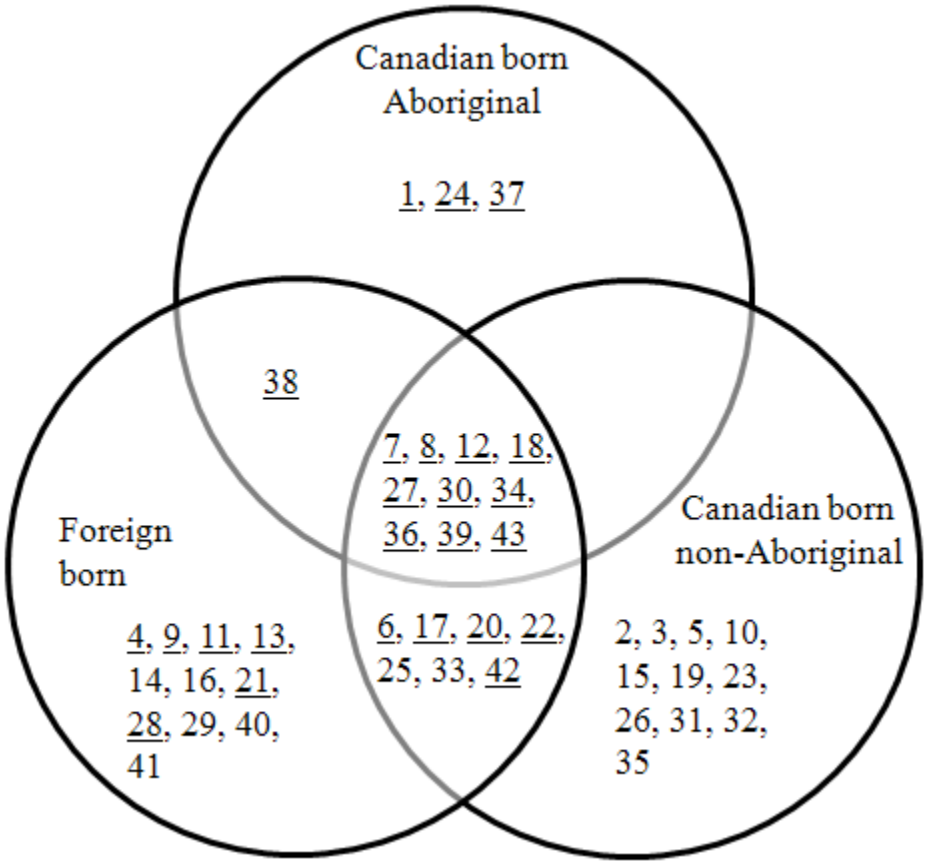


Figure 11: Distribution of KIR genotypes among Canadian born Aboriginals, Canadian born non-Aboriginals, and foreign born individuals in Subset B. Genotypes containing at least one active/latent tuberculosis case are underlined.

Each of the 10 genotypes shared between all groups included at least one case of TB (active and/or latent). Seven genotypes were shared between Canadian-born non-Aboriginals and foreign born individuals (genotypes #6, 17, 20, 22, 25, 33, 42), 5 with TB cases (genotypes #6, 17, 20, 22, 42). Only genotype #38 was shared between Canadian-born Aboriginals and foreign born individuals, and was linked to TB cases. No genotypes were shared between Canadian-born Aboriginals and non-Aboriginals. KIR genotypes were unique to individual populations as follows: foreign born – 11 (genotypes # 4, 9, 11, 13, 14, 16, 21, 28, 29, 40, 41; six containing TB cases: genotypes # 4, 9 ,11, 13, 21, 28), Canadian born Aboriginal – 3 (genotypes # 1, 24, 37; all including TB cases), and Canadian born non-Aboriginal – 11 (genotypes # 2, 3, 5, 10, 15, 19, 23, 26, 31, 32, 35; none including TB cases).

Those individuals with a TB status (active or latent) were found within 25 of the 43 genotypes, most predominantly in #8 (25/105, 23.8%) and #36 (20/105, 19.0%). Over forty-two percent of TB cases were found in these two genotypes. More specifically, in the active TB group, 17 of the 25 genotypes including TB cases were represented. Within the latent TB group, 19 of the 25 genotypes including TB cases were represented.

5.2.3. Haplotype Analysis

In order to determine differences in KIR haplotypes between ethnic groups and disease status groups, individuals were categorized into haplotypes using the scheme described in Section 4.4 (Specific Objective iii.). When looking exclusively at the

centromeric portion of the KIR cluster, non-Aboriginal and foreign born individuals have very similar distribution of cen-AA (50.65%; 50.00%, respectively), AB (40.26%; 42.31%) and BB (9.09%; 7.69%). Aboriginals however have significantly more AA (70.37%) than AB (27.68), and very little BB (1.85%) in their centromeric cluster. Like the previous cohort, latent TB and active TB cases were more likely to have cen-AA (58.70%; 72.88%) than AB (32.61%' 16.95) or BB (8.70%' 1.69%). However, unlike the previous cohort, the majority of uninfected individuals fell into the cen-AB category (47.12%) (Table 10).

When looking exclusively at the telomeric portion of the KIR cluster, again the frequencies look similar for non-Aboriginals and foreign born (59.74%; 53.85%, respectively for tel-AA, 37.66%; 39.74% tel-AB, and 2.60%; 6.41% tel-BB) compared to Aboriginals (42.59% AA, 46.30% AB, and 11.11% BB). The distribution of telomeric genes within TB status groups were as follows: latent TB (43.48% AA, 54.35% AB, and 2.17% BB); active TB (50.85% AA, 40.68% AB, and 8.47%); uninfected (58.65% AA, 34.65 AB, and 6.73%).

Table 10: Frequency of centromeric and telomeric KIR clusters in different ethnic population and tuberculosis status groups (Subset B)

KIR Cluster		Population Group			Tuberculosis Status		
Centromeric		non-Aboriginal	Aboriginal	Foreign Born	Latent	Active	Uninfected
	AA	50.65%	70.37%	50.00%	58.70%	72.88%	44.23%
	AB	40.26%	27.78%	42.31%	32.61%	16.95%	47.12%
	BB	9.09%	1.85%	7.69%	8.70%	1.69%	8.65%
Telomeric							
	AA	59.74%	42.59%	53.85%	43.48%	50.85%	58.65%
	AB	37.66%	46.30%	39.74%	54.35%	40.68%	34.62%
	BB	2.60%	11.11%	6.41%	2.17%	8.47%	6.73%

When the centromeric and telomeric KIR clusters are combined, haplotypes can be compared (Table 11). The AA-AA haplotype was the most common haplotype among non-Aboriginals (36.4%) and foreign born individuals (25.6%). Among Aboriginal individuals, the AA-AA haplotype (29.6%) was second only to the AA-AB haplotype (33.3%). Aboriginal individuals were significantly more likely to have AA-AB (33.3% vs. 14.3%, *P*=0.0176) and AA-BB (7.4% vs. 0.0%, *P*=0.0270) haplotypes compared to non-Aboriginals. No significant differences were seen between Aboriginals and foreign born individuals or non-Aboriginals and foreign born individuals. Within population groups, the centromeric-Ax haplotype predominated (non-Aboriginal – 90.0%, Aboriginal – 98.1%, foreign born – 92.3%).

Both latent (34.8%, *P*=0.0004) and active (32.3%, *P*=0.0005) TB cases were significantly more likely to have AA-AB haplotypes than uninfected individuals (9.62%). Additionally, those individuals with active TB were less likely to have an AB-AB haplotype compared to uninfected individuals (8.5% vs. 21.15%, *P*=0.0476). The majority of the tuberculosis cases are represented in the AA-AA (latent TB – 21.7%, active TB – 35.6%) and AA-AB (latent TB – 34.8%, active TB – 32.3%) haplotypes. The AA-AA haplotype contains the fewest number of activating genes of all haplotypes. Overall, 57.14% of TB cases had a centromeric-AA haplotype. More broadly, 95.24% of TB cases had a centromeric-Ax haplotype (latent TB – 91.3%, active TB – 98.3%).

Only 14 individuals had centromeric BB-haplotypes (7/77 non-Aboriginals, 1/54 Aboriginals, and 6/78 foreign born individuals). This centromeric-BB group represented

only 4.76% of TB cases (5/105). Overall we see a trend toward haplotypes with higher "A" content in population groups as well as TB disease status groups.

Table 11: Frequency of centromeric and telomeric haplotypes in different ethnic population and tuberculosis status groups (Subset B)

Haplotype		Population Group			Tuberculosis Status		
Centromeric	Telomeric	non-Aboriginal (n=77)	Aboriginal (n=54)	Foreign born (n=78)	Latent (n=46)	Active (n=59)	Uninfected (n=104)
AA	AA	28 (36.4)	16 (29.6)	20 (25.6)	10 (21.7)	21 (35.6)	33 (31.73)
AA	AB	11 (14.3)[b]	18 (33.3)[a]	16 (20.5)	16 (34.8)	19 (32.3)	10 (9.62)[c,d]
AB	AA	15 (19.5)	6 (11.1)	17 (21.8)	6 (13.0)	8 (13.6)	24 (23.08)
AB	AB	15 (19.5)	7 (13.0)	14 (17.9)	9 (19.6)	5 (8.5)	22 (21.15)[d]
AA	BB	0 (0.0)[b]	4 (7.4)[a]	3 (3.8)	1 (2.2)	3 (5.1)	3 (2.88)
AB	BB	1 (1.3)	2 (3.7)	2 (2.6)		2 (3.4)	3 (2.88)
BB	AA	3 (3.9)	1 (1.9)	5 (6.4)	4 (8.7)	1 (1.7)	4 (3.85)
BB	AB	3 (3.9)	0 (0.0)	1 (1.3)			4 (3.85)
BB	BB	1 (1.3)	0 (0.0)	0 (0.0)			1 (0.96)

[a]Significant compared to non-Aboriginal; [b]Significant compared to Aboriginal; [c]Significant compared to latent TB; [d]Significant compared to active TB; p-value ≤ 0.05 considered significant

6. Discussion

This study involved two subsets of subjects. Subset A contained individuals with active, latent, or uninfected TB status who self-identified as Dene, Cree, or Ojibwa First Nations, and who resided in one of three Northern Manitoba Reserve communities, as well as uninfected Caucasian controls from Winnipeg, Manitoba. Subset B contained individuals from three population groups (Canadian-born non-Aboriginal, Canadian-born Aboriginal, and foreign born individuals) living in Manitoba with active, latent, or uninfected TB status. Both subsets were assessed separately in three areas: KIR gene frequencies, KIR profiles, and KIR haplotype analysis for associations with ethnicity and associations with TB status.

6.1. KIR Gene Frequencies

Framework genes KIR2DL4, KIR3DL2, KIR3DL3, and the pseudogenes KIR2DP1 and KIR3DP1 were present in 100% of samples from both Subset A and Subset B, a finding that is consistent with previously published findings (58).

Comparing KIR gene frequencies to the few TB cohorts where KIR typing has been performed (74, 78, 86, 102) becomes difficult in that the genetic uniqueness of Manitoba Aboriginals has remained largely unexplored (14, 68, 69, 107) until this study. Furthermore, the foreign born group of Subset B is extremely diverse, spanning 5 continents and all World Health Organization regions (http://www.who.int/about/regions/en/index.html).

There are 4 main studies comparing KIR genes and TB disease. Mahfouz *et al*. and Mendez *et al.* both found KIR2DL3 to be the only statistically significant KIR gene frequency to differ between TB patients and controls (higher in TB patents; P=0.03 and P=0.02, respectively) (78, 86). The authors suggested that this finding indicates a greater inhibition of killer function of NK cells in a TB setting. In our study, KIR2DL3 occurred only slightly more in individuals with a TB status compared to uninfected controls: Subset A (100.0% vs. 93.22%, P=0.2927); Subset B (95.76% vs. 91.35%, P=0.2837). Pydi *et al.* found gene frequencies of KIR2DS1, KIR2DS5, KIR3DL1, and KIR2DL3 to be higher in TB patients compared to controls (P<0.0001). The authors suggest that the presence of KIR2DS1/KIR2DS5 increases the risk of developing TB and the presence of KIR2DL3/KIR3DL1 may confer susceptibility (102). Again, compared to our study, the same trends were seen (Subset B), none to the extent of statistical significance. Lastly, Lu *et al.* found gene frequencies of KIR2DS1, KIR2DS3, and KIR3DS1 to be significantly higher in TB patients compared to controls (P<0.05) (74). In our study, frequencies of KIR2DS1 and KIR3DS1 were not statistically different between those with a TB status compared to uninfected individuals (Subset B). However, when looking at KIR2DS3, our study showed a decreased frequency in TB patients (P=0.1599). This finding becomes statistically significant when comparing specifically those with active TB to uninfected individuals (P=0.0009), a comparison that more closely mirrors the Lu *et al* pulmonary TB patient group (Subset B). In each of the above mentioned studies, a different ethnic group was involved.

In 2010, an online KIR database tallied 368 different KIR genotypes in 102 populations groups (51), a number which has climbed to 418 to date. Additionally, from this study we can see that there are differences in KIR frequencies when ethnicity alone is examined. For example, KIR2DS3 is present in 38.46% of foreign born, 29.87% of non-Aboriginals, but only 5.56% of Aboriginal individuals. It is this factor, along with sample size differences, and potential association with other factors and genes affecting TB transmission that may account for differences seen compared to the literature.

Subset A and Subset B contain population groups that may be compared with the following caveat: Subset A First Nations samples are all from reserve communities and are of known ethnocultural subgroups. Subset B Aboriginal samples include First Nations (n=52), Inuit (n=1), and Metis (n=1) individuals. Furthermore, these individuals may or may not be living on reserve, and those belonging to the First Nations group are of unknown ethnocultural subgroup.

KIR2DL2, KIR2DS2, KIR2DS3, KIR1D, and KIR3DL1 were significantly lower in frequency while KIR2DL5A, KIR2DS1, KIR2DS4, KIR2DS5, and KIR3DS1 were significantly higher in frequency in Subset A First Nations compared to Caucasians (Section 5.1.1). The same trends were seen in Subset B Aboriginals when compared to non-Aboriginals, however only the relationships between KIR2DL2, KIR2DS2, KIR2DS3, and KIR2DS5 were significant.

When comparing the gene frequencies strictly between the First Nations and Aboriginal

cohorts, we see congruency in all gene frequencies except KIR1D, present in 38.71% of Subset A First Nations and 74.07% of Subset B Aboriginals ($P<0.0001$). The possible explanation for this difference in otherwise similar groups could be the genetic diversity of First Nations ethnocultural groups, as suggested by the differences seen within the Dene, Cree, and Ojibwa subpopulations (14).

When comparing tuberculosis status between the Subsets as a whole, no significant differences were seen between gene frequencies in Subset A, whereas multiple genes showed differences in Subset B, namely KIR2DL2 and KIR2DS2 were lower in frequency in TB infected individuals compared to uninfected individuals; KIR2DL5all and KIR2DL5B were higher in latently infected while KIR2DL2, KIR2DS2, and KIR2DS3 were lower in actively infected individuals compared to uninfected individuals. Lastly, frequencies of KIR2DL5all, KIR2DL5B, and KIR2DS3 were significantly different between latently and actively infected individuals. When attempting to understand why there were so many differences seen in Subset B and no differences seen in Subset A, it is important to recall that all population groups (Aboriginal, non-Aboriginal, and foreign born; n=209) of Subset B were included in this analysis, containing 105 TB cases, while only the First Nations group (n=93) of Subset A contained TB cases (n=34), and lacked Caucasian individuals with a TB status for comparison.

Again when comparing Subset A First Nations with Subset B Aboriginals, KIR1D was the only gene to differ significantly between all TB disease status groups: Any TB

(26.47% vs. 72.50%, *P*<0.0001), latent TB (28.57% vs. 100.00%, *P*=0.0108), active TB (25.00% vs. 68.57%, *P*=0.0024), uninfected (45.76% vs. 78.57%, *P*=0.0375).

The imbalance of activating and inhibitory KIR genes may affect the activation of immune cells, contributing to pathogenesis of disease (74). Each stage of the host response to *M. tuberculosis* is under genetic control (80). The differences observed in the KIR frequencies in the studied ethnic and TB disease status groups may lead to altered NK cell responses, therefore leading to different cytokine profiles and ultimately outcome to TB disease.

6.2. KIR Profiles

Certain KIR profiles differ only by heterozygosity at KIR2DL5A/KIR2DL5B (different locus), or KIR2DS4/KIR1D (same locus), which increases the number of profiles seen compared to studies that did not differentiate the KIR2DL5 and KIR2DS4 loci.

KIR2DL5 can be subtyped into KIR2DL5A and KIR2DL5B, which differ by 7 nucleotide substitutions, the latter of which is not expressed (50, 83). KIR2DL5A is located on the telomeric half and KIR2DL5B on the centromeric half of the KIR gene complex (see Figure 7, Section 4.4) (32, 132, 138). It is possible that those individuals with the presence of both KIR2DL5A and KIR2DL5B have up to four copies of the KIR2DL5 gene (32, 50).

KIR1D is an allele of KIR2DS4, with a 22 base pair deletion resulting in a truncated protein from the deletion-generated frame shift causing loss of the second transmembrane domain (2D). The loss of a transmembrane domain as well as a cytoplasmic domain in KIR1D leads to a protein that is not anchored to the membrane, therefore becoming a secreted KIR molecule (58, 88). It has been hypothesized that there may be a role for a soluble KIR to act as a ligand for an unidentified receptor, or to "mop up" soluble HLA, which could interfere with NK cell function (88, 144).

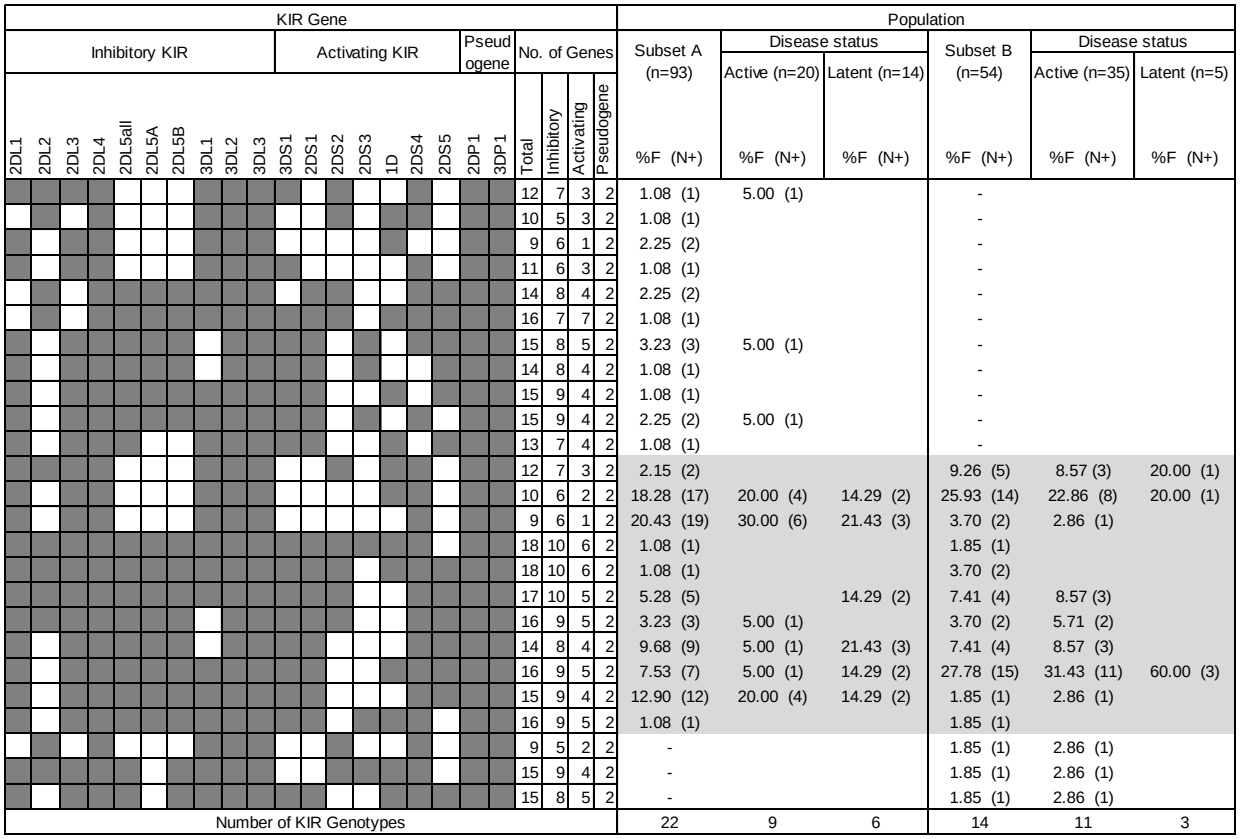
For those with KIR2DS4 as their only activating gene, the full length KIR2DS4 was more common than KIR1D, as anticipated (88). Of the forty different profiles identified in the 168 samples of subset A, only seven individuals (4.2%; genotype #8) had KIR1D as their only activating KIR compared to 20 individuals (11.9%; genotype #11) with KIR2DS4 as their only activating KIR (Figure 8 in Section 5.1.2). Those individuals with KIR1D as their only "activating" KIR ($KIR1D^{+/+}$), did not have any intact activating membrane-anchored KIR due to the loss of the transmembrane domain. Individuals from genotype #8 all (2 of 2) had an uninfected TB status, whereas 9 of the 20 individuals from genotype #11 had a tuberculosis status (3 latent, 6 active), the highest frequency within a single genotype, suggesting the need for a membrane-bound activating KIR in these individuals to activate NK cells against TB. Interestingly, those individuals who were heterozygous for KIR2DS4/KIR1D as their only activating KIR were among the most common genotype (34%; genotype #9), and represented 6 TB cases (2 latent, 4 active).

Of the 43 different profiles identified in the 209 samples of subset B, only 1 individual (0.5%; genotype #10) had KIR1D as their only activating KIR, and they were TB uninfected (Figure 7 in Section 5.2.2). Eleven individuals had KIR2DS4 as their only activating KIR (11/209, 5.3%; genotype #12), and more than half of these individuals had a TB status (1 latent, 5 active). As in subset A, the genotype containing heterozygous KIR2DS4/KIR1D as the only activating KIR in subset B was the most frequent (24.9%; genotype #8). Notably, this genotype contained the highest frequency of TB cases within a single genotype (9 latent, 16 active).

Few disease association studies (46, 143, 144) have been performed to date on KIR1D, none of them focusing on tuberculosis. The hypothesized role for a soluble KIR to act as a ligand for soluble HLA would still have an effect on NK cell function; however this specific effect is still speculative. Even though further studies are warranted, the role of KIR1D in TB disease in Aboriginals is indicative of an association between ethnicity and disease status.

Forty KIR profiles were identified in Subset A subjects (Section 5.1.2) and 43 KIR profiles were identified in Subset B subjects (Section 5.2.2). The same proportion of profiles were only seen once (i.e. twenty of the 40 profiles and 22 of the 43 profiles were unique to a single individual, respectively). In Subset A, the most predominant genotypes (#9, 11, 36, and 2) represented 43.43% of the 168 study participants and 61.76% of TB cases. In Subset B, the most predominant genotypes (#8, 36, 7, 18, and 12) represented

56.46% of the 209 study participants and 59.05% of TB cases. Three of the most common profiles from each subset overlap (Subset A genotypes #2, 9, and 11 correspond to Subset B genotypes #7, 8, and 12, respectively). As mentioned previously, the differences in population groups as well as distribution of TB cases within those groups (all Caucasians in Subset A are uninfected controls) make further comparison of all population groups from these cohorts difficult. However, a comparison can be made regarding the distribution of tuberculosis status between Subset A First Nations and Subset B Aboriginal populations (Figure 12). Twenty-two genotypes were seen in Subset A and 14 in Subset B. Eleven of these genotypes overlapped between the two Subsets, containing 82.80% (77/93) and 94.44% (51/54) of individuals from each Subset respectively. Although 73.53% of TB cases in Subset A (13/20 active, 12/14 latent) and 92.50% of TB cases Subset B (32/35 active, 5/5 latent) fell into the over lapping genotypes shared by both cohorts, the genotypes containing the most TB cases were not consistent between groups.



Total	Inhibitory	Activating	Pseudogene	Subset A (n=93) %F (N+)	Active (n=20) %F (N+)	Latent (n=14) %F (N+)	Subset B (n=54) %F (N+)	Active (n=35) %F (N+)	Latent (n=5) %F (N+)
12	7	3	2	1.08 (1)	5.00 (1)		-		
10	5	3	2	1.08 (1)			-		
9	6	1	2	2.25 (2)			-		
11	6	3	2	1.08 (1)			-		
14	8	4	2	2.25 (2)			-		
16	7	7	2	1.08 (1)			-		
15	8	5	2	3.23 (3)	5.00 (1)		-		
14	8	4	2	1.08 (1)			-		
15	9	4	2	1.08 (1)			-		
15	9	4	2	2.25 (2)	5.00 (1)		-		
13	7	4	2	1.08 (1)			-		
12	7	3	2	2.15 (2)			9.26 (5)	8.57 (3)	20.00 (1)
10	6	2	2	18.28 (17)	20.00 (4)	14.29 (2)	25.93 (14)	22.86 (8)	20.00 (1)
9	6	1	2	20.43 (19)	30.00 (6)	21.43 (3)	3.70 (2)	2.86 (1)	
18	10	6	2	1.08 (1)			1.85 (1)		
18	10	6	2	1.08 (1)			3.70 (2)		
17	10	5	2	5.28 (5)		14.29 (2)	7.41 (4)	8.57 (3)	
16	9	5	2	3.23 (3)	5.00 (1)		3.70 (2)	5.71 (2)	
14	8	4	2	9.68 (9)	5.00 (1)	21.43 (3)	7.41 (4)	8.57 (3)	
16	9	5	2	7.53 (7)	5.00 (1)	14.29 (2)	27.78 (15)	31.43 (11)	60.00 (3)
15	9	4	2	12.90 (12)	20.00 (4)	14.29 (2)	1.85 (1)	2.86 (1)	
16	9	5	2	1.08 (1)			1.85 (1)		
9	5	2	2	-			1.85 (1)	2.86 (1)	
15	9	4	2	-			1.85 (1)	2.86 (1)	
15	8	5	2	-			1.85 (1)	2.86 (1)	

Figure 12: KIR genotype distribution of active and latent TB cases in Subset A First Nations and Subset B Canadian-born Aboriginal individuals. KIR types differ from each other by the presence of (shaded box) or absence of (white box) 19 KIR genes. Frequency (%F) of each genotype is expressed as a percentage and is defined as the number of individuals having the genotype (N+) divided by the number of individuals (n) in the population or tuberculosis status group. The light grey box (right) indicates genotypes that overlap between the 2 cohorts.

Specific to Subset B, 28 of the 43 profiles were seen in Canadian-born non-Aboriginals, 14 in Canadian-born Aboriginals, and 29 in foreign born individuals. Very few profiles were uniquely found in Canadian-born Aboriginals (three) compared to non-Aboriginals (eleven) or foreign born individuals (eleven). The cultural diversity of the ancestors of Canadian-born non-Aboriginals may explain this disparity.

6.3. KIR Haplotype Analysis

Specific to Subset A subjects, we found that haplotype B centromeric genes (KIR2DL2, KIR2DS2, KIR2DS3) were reduced while haplotype B telomeric genes (KIR3DS1, KIR2DL5A, KIR2DS1, KIR2DS5) were more prominent in Manitoba First Nations when compared with Caucasians. These findings agreed with recent work by Rempel, *et al* (107). Additionally, haplotype A telomeric genes KIR2DS4 and KIR3DL1 were significantly increased, and decreased, respectively in First Nations compared to Caucasians. These additional findings along with the haplotype analysis support the view that First Nations individuals may have a stronger inhibitory genotype compared to Caucasians. This conclusion contradicts findings reported in the literature, and the difference may be attributed to different ethnocultural sample populations: Rempel, *et al* study contained 70% Oji-Cree First Nations compared to 68% Dene First Nations in our study. Our study showed that within the First Nations population, different ethnocultural groups may have different KIR frequencies, and therefore different immune outcomes to disease. The same findings were not statistically significant in the Subset B Aboriginal

population, potentially because of the mixed nature of this group.

When looking exclusively at the centromeric portion of the KIR cluster, Subset A First Nations and Subset B Aboriginals were found more frequently in the cen-AA group (81.72% and 70.37%, respectively). All other population groups had similar frequencies of AA and AB in the centromeric cluster. The cen-BB group was consistently the minority in this half of the KIR cluster. In Subset A, all active and latent TB cases were either cen-AA or cen-AB, whereas Subset B TB cases were seen in all three categories, again with the fewest in cen-BB.

When looking exclusively at the telomeric portion of the KIR cluster, differences within population groups were less distinct. One trend between the two Subsets was the increase of tel-BB in Subset A First Nations and Subset B Aboriginals compared to other population groups involved. The majority of latent TB cases were tel-AB and the majority of active TB cases were tel-AA, a trend seen in both Subsets.

When the centromeric and telomeric clusters are combined, haplotype analysis can be performed (Section 5.1.3 & 5.2.3). Haplotype analysis of Subset A revealed that the frequencies of haplotypes AA-AB, AB-AA, AB-AB, and AA-BB differed significantly between First Nations and Caucasians. The individual lacking a telomeric haplotype by definition of the haplotype determination scheme simply did not represent KIR3DL1 or KIR3DS1 in their KIR profile. They did however have 4 other activating KIR genes as part of their profile (KIR2DS1, KIR2DS3, KIR1D, and KIR2DS4).

Haplotype analysis of Subset B subjects revealed that the frequency of only 2

haplotypes (AA-AB and AA-BB) significantly differed between Aboriginals and non-Aboriginals, and that there were no differences when compared to the frequency in foreign born individuals. Haplotype BB-AB was not represented in Aboriginals, and haplotype BB-BB was not represented in either Aboriginals or foreign born individuals. A published haplotype analysis between Aboriginals and Caucasians found Aboriginals had significantly lower frequencies of AB-AA and AB-AB haplotypes, and significantly higher frequency of the AB-BB haplotype (107). These trends were observed in this study; however, they were not statistically significant, although 2 of the 3 were in Subset A. In addition, this same study did not report frequencies of the BB-AA haplotype; one that was present in all Subset B population groups of our study and contained 8.7% and 1.7% of latent and active TB cases, respectively. This further strengthens the evidence that different population groups (and further diversity within subpopulation groups) have different KIR haplotypes frequencies that may influence disease outcome.

Lastly, KIR haplotypes in Subset A First Nations and Subset B Aboriginals were compared. Half (50.00%) of the active cases of TB and 35.7% of latent cases of TB in Subset A First Nations were of the haplotype AA-AA. This is the haplotype containing the fewest number of activating genes of all haplotypes. It is speculated that the lack of activating KIR genes leads to a worsened immune response against tuberculosis infection. In Subset B Aboriginals, 25.71% of active cases were of the AA-AA haplotype. The majority of active (37.14%) and latent (60.00%) TB cases however were haplotype AA-AB, a haplotype that only 14.29% of uninfected individuals belonged to.

The increasing amount of haplotype "A" content corresponds with a decreasing presence of activating KIR genes. Again, this imbalance of inhibitory and activating genes may lead to an overall dampening of the immune response to TB infection.

In summary, there are significant differences in KIR genes, KIR profiles, and KIR haplotypes across ethnicities and tuberculosis disease status groups. When looking at tuberculosis status in Subset A, it appears that the KIR profile and centromeric haplotype are more predictive than the presence or absence of individual genes. The increased presence of all centromeric-AA haplotypes in First Nations compared to Caucasian participants, along with the overwhelming amount of TB in these same haplotypes, indicates a relationship between KIR, ethnicity, and disease. Again in Subset B, major differences can be seen in gene frequencies, profiles, and haplotypes between Canadian-born non-Aboriginal, Canadian-born Aboriginal and foreign born individuals as well as TB disease status groups. Within the province of Manitoba, each of these population groups have drastically different rates of tuberculosis.

When assessing study validity, results from the First Nations communities are not necessarily generalizable to all First Nations communities in the province of Manitoba. The small number of samples in Subset A also affects the internal validity; however, the degree of participation is not surprising in the context of research performed in Canadian Aboriginal populations in remote communities (139). It is because of this that the statistical power of the comparisons was limited. The sampling of additional individuals

is needed to confirm the generalizability of these findings to the larger provincial Aboriginal populations.

This study only assessed the variability in the frequencies of KIR genes. The diversity of these genes can be explored on a much deeper level by way analyses of KIR gene sequences. It is for this reason that the future of this project may include sequence analysis of target regions. Also, KIR-HLA disease association studies would be beneficial in determining the role that KIR play in TB disease, as HLA are the ligands upon which the receptors act. Lastly, the ultimate outcome of the KIR-HLA interaction is the release of cytokines following a cascade of molecular events. Cytokine analysis studies are needed to further clarify the relationship between infectious diseases and KIR in individuals with tuberculosis.

7. References

1. Altare F, Jouanguy E, Lamhamedi S, Döffinger R, Fischer A, Casanova JL. 1998. Mendelian susceptibility to mycobacterial infection in man. *Curr. Opin. Immunol.* 10(4):413
2. Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, et al. 1997. A vitamin d receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in japanese women. *J. Bone Miner. Res.* 12(6):915
3. Awomoyi AA, Marchant A, Howson JMM, McAdam KPWJ, Blackwell JM, Newport MJ. 2002. Interleukin-10, polymorphism in slc11a1 (formerly nramp1), and susceptibility to tuberculosis. *J. Infect. Dis.* 186(12):1808
4. Balamurugan A, Sharma SK, Mehra NK. 2004. Human leukocyte antigen class i supertypes influence susceptibility and severity of tuberculosis. *J. Infect. Dis.* 189(5):805
5. Barry CE 3rd, Boshoff HI, Dartois V, Dick T, Ehrt S, et al. 2009. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat. Rev. Microbiol.* 7(12):845
6. Bashirova AA, Martin MP, McVicar DW, Carrington M. 2006. The killer immunoglobulin-like receptor gene cluster: tuning the genome for defense. *Annu Rev Genomics Hum Genet*. 7:277
7. Bates JH, Stead WW. 1993. The history of tuberculosis as a global epidemic. *Med. Clin. North Am.* 77(6):1205
8. Bean AG, Roach DR, Briscoe H, France MP, Korner H, et al. 1999. Structural deficiencies in granuloma formation in tnf gene-targeted mice underlie the heightened susceptibility to aerosol mycobacterium tuberculosis infection, which is not compensated for by lymphotoxin. *J. Immunol.* 162(6):3504
9. Bearman JE, Kleinman H, Glyer VV, Lacroix OM. 1964. A study of variability in tuberculin test reading. *Am. Rev. Respir. Dis.* 90:913
10. Bellamy R. 2003. Susceptibility to mycobacterial infections: the importance of host genetics. *Genes Immun.* 4(1):4
11. Boom WH. 1996. The role of t-cell subsets in mycobacterium tuberculosis infection. *Infect Agents Dis*. 5(2):73
12. Boyton RJ, Altmann DM. 2007. Natural killer cells, killer immunoglobulin-like receptors and human leucocyte antigen class i in disease. *Clin. Exp. Immunol.* 149(1):1
13. Boyton RJ, Reynolds CJ, Quigley KJ, Altmann DM. 2013. Immune mechanisms and the impact of the disrupted lung microbiome in chronic bacterial lung infection and bronchiectasis. *Clin. Exp. Immunol.* 171(2):117
14. Braun K, Larcombe L, Orr P, Nickerson P, Wolfe J, Sharma M. 2013. Killer immunoglobulin-like receptor (kir) centromeric-aa haplotype is associated with ethnicity and tuberculosis disease in a canadian first nations cohort. *PLoS ONE*.

8(7):e67842
15. Brocker C, Thompson D, Matsumoto A, Nebert DW, Vasiliou V. 2010. Evolutionary divergence and functions of the human interleukin (il) gene family. *Human Genomics*. 5(1):30
16. Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, et al. 2002. A new evolutionary scenario for the mycobacterium tuberculosis complex. *Proc. Natl. Acad. Sci. U.S.A.* 99(6):3684
17. Canadian Thoracic Society. 2013. *Canadian Tuberculosis Standards*. 7ed ed.
18. Carrington M, Martin MP. 2006. The impact of variation at the kir gene cluster on human disease. *Curr. Top. Microbiol. Immunol.* 298:225
19. Carrington M, Norman P. 2003. *The KIR Gene Cluster*. Bethesda, MD: National Centre for Biotechnology Information
20. Carrington M, O'Brien SJ. 2003. The influence of hla genotype on aids. *Annu. Rev. Med.* 54:535
21. Chakravarty SD, Zhu G, Tsai MC, Mohan VP, Marino S, et al. 2008. Tumor necrosis factor blockade in chronic murine tuberculosis enhances granulomatous inflammation and disorganizes granulomas in the lungs. *Infect. Immun.* 76(3):916
22. Clifford HT. 1975. *An Introduction to Numerical Classification*. Academic Press
23. Commins SP, Borish L, Steinke JW. 2010. Immunologic messenger molecules: cytokines, interferons, and chemokines. *J. Allergy Clin. Immunol.* 125(2 Suppl 2):S53
24. Cook M, Briggs D, Craddock C, Mahendra P, Milligan D, et al. 2006. Donor kir genotype has a major influence on the rate of cytomegalovirus reactivation following t-cell replete stem cell transplantation. *Blood*. 107(3):1230
25. Cooper AM. 2009. Cell-mediated immune responses in tuberculosis. *Annu. Rev. Immunol.* 27:393
26. Danish Tuberculosis Index. 1955. The relation of tuberculin sensitivity to pulmonary calcifications as an index of tuberculosis infection. *Bull. World Health Organ.* 12(1-2):261
27. Dannenberg A. 1994. *Pathogenesis and Immunology: Basic Aspects*. New York: Springer-Verlag. 3rd ed.
28. Delgado JC, Baena A, Thim S, Goldfeld AE. 2002. Ethnic-specific genetic associations with pulmonary tuberculosis. *The Journal of Infectious Diseases*. 186(10):1463
29. Denis L, Sivula J, Gourraud P-A, Kerdudou N, Chout R, et al. 2005. Genetic diversity of kir natural killer cell markers in populations from france, guadeloupe, finland, senegal and réunion. *Tissue Antigens*. 66(4):267
30. Diel R, Loddenkemper R, Meywald-Walter K, Gottschalk R, Nienhaus A. 2009. Comparative performance of tuberculin skin test, quantiferon-tb-gold in tube assay, and t-spot.tb test in contact investigations for tuberculosis. *Chest*. 135(4):1010
31. Döhring C, Scheidegger D, Samaridis J, Cella M, Colonna M. 1996. A human

killer inhibitory receptor specific for hla-a1,2. *J. Immunol.* 156(9):3098
32. Du Z, Sharma SK, Spellman S, Reed EF, Rajalingam R. 2008. Kir2dl5 alleles mark certain combination of activating kir genes. *Genes Immun.* 9(5):470
33. Dubaniewicz A, Moszkowska G, Szczerkowska Z, Hoppe A. 2003. Analysis of dqb1 allele frequencies in pulmonary tuberculosis: preliminary report. *Thorax.* 58(10):890
34. Dutta RK, Kathania M, Raje M, Majumdar S. 2012. Il-6 inhibits ifn-γ induced autophagy in mycobacterium tuberculosis h37rv infected macrophages. *Int. J. Biochem. Cell Biol.* 44(6):942
35. Edwards LB, Acquaviva FA, Livesay VT, Cross FW, Palmer CE. 1969. An atlas of sensitivity to tuberculin, ppd-b, and histoplasmin in the united states. *Am. Rev. Respir. Dis.* 99(4):Suppl:1
36. Ellner JJ. 1997. Review: the immune response in human tuberculosis--implications for tuberculosis control. *J. Infect. Dis.* 176(5):1351
37. Esin S, Batoni G, Källenius G, Gaines H, Campa M, et al. 1996. Proliferation of distinct human t cell subsets in response to live, killed or soluble extracts of mycobacterium tuberculosis and myco. avium. *Clin. Exp. Immunol.* 104(3):419
38. Eum S-Y, Kong J-H, Hong M-S, Lee Y-J, Kim J-H, et al. 2010. Neutrophils are the predominant infected phagocytic cells in the airways of patients with active pulmonary tb. *Chest*. 137(1):122
39. Farhat M, Greenaway C, Pai M, Menzies D. 2006. False-positive tuberculin skin tests: what is the absolute effect of bcg and non-tuberculous mycobacteria? *Int. J. Tuberc. Lung Dis.* 10(11):1192
40. Flynn JL, Chan J. 2001. Immunology of tuberculosis. *Annu. Rev. Immunol.* 19:93
41. Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfeffer K, et al. 1995. Tumor necrosis factor-alpha is required in the protective immune response against mycobacterium tuberculosis in mice. *Immunity*. 2(6):561
42. Fox W, Ellard GA, Mitchison DA. 1999. Studies on the treatment of tuberculosis undertaken by the british medical research council tuberculosis units, 1946-1986, with relevant subsequent publications. *Int. J. Tuberc. Lung Dis.* 3(10 Suppl 2):S231
43. Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C. 2003. Tuberculosis. *Lancet*. 362(9387):887
44. Garcia CA, Robinson J, Madrigal JA, Marsh SGE. 2003. Natural killer cell receptors: functional roles. *Inmunologia*. 22(2):190
45. Gazit R, Garty B-Z, Monselise Y, Hoffer V, Finkelstein Y, et al. 2004. Expression of kir2dl1 on the entire nk cell population: a possible novel immunodeficiency syndrome. *Blood*. 103(5):1965
46. Giebel S, Nowak I, Wojnar J, Krawczyk-Kulis M, Holowiecki J, et al. 2008. Association of kir2ds4 and its variant kir1d with leukemia. *Leukemia*. 22(11):2129
47. Gil DP, León LG, Correa LI, Maya JR, París SC, et al. 2004. Differential induction of apoptosis and necrosis in monocytes from patients with tuberculosis and healthy

control subjects. *J. Infect. Dis.* 189(11):2120
48. Goldfeld AE, Delgado JC, Thim S, Bozon MV, Uglialoro AM, et al. 1998. Association of an hla-dq allele with clinical tuberculosis. *JAMA.* 279(3):226
49. Gomez LM, Anaya J-M, Martin J. 2005. Genetic influence of ptpn22 r620w polymorphism in tuberculosis. *Hum. Immunol.* 66(12):1242
50. Gómez-Lozano N, Gardiner CM, Parham P, Vilches C. 2002. Some human kir haplotypes contain two kir2dl5 genes: kir2dl5a and kir2dl5b. *Immunogenetics*. 54(5):314
51. Gonzalez-Galarza FF, Christmas S, Middleton D, Jones AR. 2011. Allele frequency net: a database and online repository for immune gene frequencies in worldwide populations. *Nucleic Acids Res.* 39(Database issue):D913
52. Greenwood CM, Fujiwara TM, Boothroyd LJ, Miller MA, Frappier D, et al. 2000. Linkage of tuberculosis to chromosome 2q35 loci, including nramp1, in a large aboriginal canadian family. *Am. J. Hum. Genet.* 67(2):405
53. Gumperz JE, Paterson JC, Litwin V, Valiante N, Lanier LL, et al. 1996. Specificity of two anti-class i hla monoclonal antibodies that block class i recognition by the nkb1 killer cell inhibitory receptor. *Tissue Antigens*. 48(4 Pt 1):278
54. Gutierrez MC, Brisse S, Brosch R, Fabre M, Omaïs B, et al. 2005. Ancient origin and gene mosaicism of the progenitor of mycobacterium tuberculosis. *PLoS Pathog.* 1(1):e5
55. Gutiérrez-Rodríguez ME, Sandoval-Ramírez L, Díaz-Flores M, Marsh SGE, Valladares-Salgado A, et al. 2006. Kir gene in ethnic and mestizo populations from mexico. *Hum. Immunol.* 67(1-2):85
56. Holden M, Dubin MR, Diamond PH. 1971. Frequency of negative intermediate-strength tuberculin sensitivity in patients with active tuberculosis. *N. Engl. J. Med.* 285(27):1506
57. Hosono S, Faruqi AF, Dean FB, Du Y, Sun Z, et al. 2003. Unbiased whole-genome amplification directly from clinical samples. *Genome Res.* 13(5):954
58. Hsu KC, Liu X-R, Selvakumar A, Mickelson E, O'Reilly RJ, Dupont B. 2002. Killer ig-like receptor haplotype analysis by gene content: evidence for genomic diversity with a minimum of six basic framework haplotypes, each with multiple subsets. *J. Immunol.* 169(9):5118
59. Huebner RE, Schein MF, Bass JB Jr. 1993. The tuberculin skin test. *Clin. Infect. Dis.* 17(6):968
60. Jiang K, Zhu F-M, Lv Q-F, Yan L-X. 2005. Distribution of killer cell immunoglobulin-like receptor genes in the chinese han population. *Tissue Antigens*. 65(6):556
61. Kaufmann SH. 2001. How can immunology contribute to the control of tuberculosis? *Nat. Rev. Immunol.* 1(1):20
62. Kelley J, Walter L, Trowsdale J. 2005. Comparative genomics of natural killer cell receptor gene clusters. *PLoS Genet.* 1(2):129
63. Khader SA, Cooper AM. 2008. Il-23 and il-17 in tuberculosis. *Cytokine*. 41(2):79

64. Khakoo SI, Carrington M. 2006. Kir and disease: a model system or system of models? *Immunol. Rev.* 214:186
65. Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, et al. 2004. Hla and nk cell inhibitory receptor genes in resolving hepatitis c virus infection. *Science*. 305(5685):872
66. La D, Czarnecki C, El-Gabalawy H, Kumar A, Meyers AFA, et al. 2011. Enrichment of variations in kir3dl1/s1 and kir2dl2/l3 among h1n1/09 icu patients: an exploratory study. *PLoS ONE*. 6(12):e29200
67. Langley J, Ellis E, Deeks S. 2004. Statement on bacille calmette guérin (bcg) vaccine. *Can. Commun. Dis. Rep.* 30:1
68. Larcombe L, Rempel JD, Dembinski I, Tinckam K, Rigatto C, Nickerson P. 2005. Differential cytokine genotype frequencies among canadian aboriginal and caucasian populations. *Genes Immun.* 6(2):140
69. Larcombe LA, Orr PH, Lodge AM, Brown JS, Dembinski IJ, et al. 2008. Functional gene polymorphisms in canadian aboriginal populations with high rates of tuberculosis. *J. Infect. Dis.* 198(8):1175
70. Levinson W. 2008. *Review of Medical Microbiology and Immunology*. USA: McGraw-Hill Companies. 10th Ed ed.
71. Levinson W, Jawetz E. 2000. *Medical Microbiology & Immunology: Examination & Board Review*. USA: McGraw-Hill Companies. 6th Ed ed.
72. Lorenz E, Mira JP, Cornish KL, Arbour NC, Schwartz DA. 2000. A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. *Infect. Immun.* 68(11):6398
73. Lourembam SD, Sawian CE, Baruah S. 2011. Differential association of kir gene loci to risk of malaria in ethnic groups of assam, northeast india. *Infect. Genet. Evol.* 11(8):1921
74. Lu C, Bai XL, Shen YJ, Deng YF, Wang CY, et al. 2012. Potential implication of activating killer cell immunoglobulin-like receptor and hla in onset of pulmonary tuberculosis. *Scand. J. Immunol.* 76(5):491
75. Lu C, Shen Y-J, Deng Y-F, Wang C-Y, Fan G, et al. 2012. Association of killer cell immunoglobulin-like receptors with pulmonary tuberculosis in chinese han. *Genet. Mol. Res.* 11(2):1370
76. Lurie M. 1964. *Resistance to Tuberculosis: Experimental Studies in Native and Acquired Defensive Mechanisms*. Cambridge: Harvard University Press
77. Lykouras D, Sampsonas F, Kaparianos A, Karkoulias K, Tsoukalas G, Spiropoulos K. 2008. Human genes in tb infection: their role in immune response. *Monaldi Arch Chest Dis*. 69(1):24
78. Mahfouz R, Halas H, Hoteit R, Saadeh M, Shamseddeen W, et al. 2011. Study of kir genes in lebanese patients with tuberculosis. *Int. J. Tuberc. Lung Dis.* 15(12):1688
79. Makrigiannis AP, Anderson SK. 2003. Regulation of natural killer cell function. *Cancer Biol. Ther.* 2(6):610

80. Malik S, Schurr E. 2002. Genetic susceptibility to tuberculosis. *Clin. Chem. Lab. Med.* 40(9):863
81. Malloy PJ, Feldman D. 1999. Vitamin d resistance. *Am. J. Med.* 106(3):355
82. Martin MP, Carrington M. 2008. Kir locus polymorphisms: genotyping and disease association analysis. *Methods Mol. Biol.* 415:49
83. McErlean C, Gonzalez AA, Cunningham R, Meenagh A, Shovlin T, Middleton D. 2010. Differential rna expression of kir alleles. *Immunogenetics*. 62(7):431
84. McQueen KL, Parham P. 2002. Variable receptors controlling activation and inhibition of nk cells. *Curr. Opin. Immunol.* 14(5):615
85. Means TK, Wang S, Lien E, Yoshimura A, Golenbock DT, Fenton MJ. 1999. Human toll-like receptors mediate cellular activation by mycobacterium tuberculosis. *J. Immunol.* 163(7):3920
86. Méndez A, Granda H, Meenagh A, Contreras S, Zavaleta R, et al. 2006. Study of kir genes in tuberculosis patients. *Tissue Antigens*. 68(5):386
87. Meyer CG, May J, Stark K. 1998. Human leukocyte antigens in tuberculosis and leprosy. *Trends Microbiol.* 6(4):148
88. Middleton D, Gonzalez A, Gilmore PM. 2007. Studies on the expression of the deleted kir2ds4*003 gene product and distribution of kir2ds4 deleted and nondeleted versions in different populations. *Hum. Immunol.* 68(2):128
89. Middleton D, Gonzelez F. 2010. The extensive polymorphism of kir genes. *Immunology*. 129(1):8
90. Miller EA, Ernst JD. 2008. Illuminating the black box of tnf action in tuberculous granulomas. *Immunity*. 29(2):175
91. Moretta A, Vitale M, Bottino C, Orengo AM, Morelli L, et al. 1993. P58 molecules as putative receptors for major histocompatibility complex (mhc) class i molecules in human natural killer (nk) cells. anti-p58 antibodies reconstitute lysis of mhc class i-protected cells in nk clones displaying different specificities. *J. Exp. Med.* 178(2):597
92. Morris GAJ, Edwards DRV, Hill PC, Wejse C, Bisseye C, et al. 2011. Interleukin 12b (il12b) genetic variation and pulmonary tuberculosis: a study of cohorts from the gambia, guinea-bissau, united states and argentina. *PLoS ONE*. 6(2):e16656
93. Nandi B, Behar SM. 2011. Regulation of neutrophils by interferon-γ limits lung inflammation during tuberculosis infection. *J. Exp. Med.* 208(11):2251
94. Naper C, Shegarfi H, Inngjerdingen M, Rolstad B. 2011. The role of natural killer cells in the defense against listeria monocytogenes lessons from a rat model. *J Innate Immun*. 3(3):289
95. Nikitina-Zake L, Rajalingham R, Rumba I, Sanjeevi CB. 2004. Killer cell immunoglobulin-like receptor genes in latvian patients with type 1 diabetes mellitus and healthy controls. *Ann. N. Y. Acad. Sci.* 1037:161
96. Norman PJ, Carrington CVF, Byng M, Maxwell LD, Curran MD, et al. 2002. Natural killer cell immunoglobulin-like receptor (kir) locus profiles in african and south asian populations. *Genes Immun.* 3(2):86

97. Ogus AC, Yoldas B, Ozdemir T, Uguz A, Olcen S, et al. 2004. The arg753gln polymorphism of the human toll-like receptor 2 gene in tuberculosis disease. *Eur. Respir. J.* 23(2):219
98. Parham P. 2005. Mhc class i molecules and kirs in human history, health and survival. *Nat. Rev. Immunol.* 5(3):201
99. Peters W, Ernst JD. 2003. Mechanisms of cell recruitment in the immune response to mycobacterium tuberculosis. *Microbes Infect.* 5(2):151
100. Petrelli D, Kaushal Sharma M, Wolfe J, Al-Azem A, Hershfield E, Kabani A. 2004. Strain-related virulence of the dominant mycobacterium tuberculosis strain in the canadian province of manitoba. *Tuberculosis (Edinb).* 84(5):317
101. Public Health Agency of Canada, Canadian Lung Assocation/Canadian Thoracic Society. 2007. *Canadian Tuberculosis Standards*. Canada: Minister of Health. 6th ed.
102. Pydi SS, Sunder SR, Venkatasubramanian S, Kovvali S, Jonnalagada S, Valluri VL. 2012. Killer cell immunoglobulin like receptor gene association with tuberculosis. *Hum. Immunol.*
103. Raja A. 2004. Immunology of tuberculosis. *Indian J. Med. Res.* 120(4):213
104. Rajalingam R. 2012. Overview of the killer cell immunoglobulin-like receptor system. *Methods Mol. Biol.* 882:391
105. Recommendations for use of an isoniazid-rifapentine regimen with direct observation to treat latent mycobacterium tuberculosis infection. 2011. *MMWR Morb. Mortal. Wkly. Rep.* 60(48):1650
106. Reid JK, Ward H, Marciniuk D, Hudson S, Smith P, Hoeppner V. 2007. The effect of neonatal bacille calmette-guérin vaccination on purified protein derivative skin test results in canadian aboriginal children. *Chest*. 131(6):1806
107. Rempel JD, Hawkins K, Lande E, Nickerson P. 2011. The potential influence of kir cluster profiles on disease patterns of canadian aboriginals and other indigenous peoples of the americas. *Eur. J. Hum. Genet.* 19(12):1276
108. Rooney JJ Jr, Crocco JA, Kramer S, Lyons HA. 1976. Further observations on tuberculin reactions in active tuberculosis. *Am. J. Med.* 60(4):517
109. Rothel JS, Andersen P. 2005. Diagnosis of latent mycobacterium tuberculosis infection: is the demise of the mantoux test imminent? *Expert Rev Anti Infect Ther*. 3(6):981
110. Russell DG, Cardona P-J, Kim M-J, Allain S, Altare F. 2009. Foamy macrophages and the progression of the human tuberculosis granuloma. *Nat. Immunol.* 10(9):943
111. Russell DG, VanderVen BC, Lee W, Abramovitch RB, Kim M, et al. 2010. Mycobacterium tuberculosis wears what it eats. *Cell Host Microbe*. 8(1):68
112. Saraiva M, O'Garra A. 2010. The regulation of il-10 production by immune cells. *Nat. Rev. Immunol.* 10(3):170
113. Schechter M, Zajdenverg R, Falco G, Barnes GL, Faulhaber JC, et al. 2006. Weekly rifapentine/isoniazid or daily rifampin/pyrazinamide for latent tuberculosis

in household contacts. *Am. J. Respir. Crit. Care Med.* 173(8):922
114. Schröder NWJ, Schumann RR. 2005. Single nucleotide polymorphisms of toll-like receptors and susceptibility to infectious disease. *Lancet Infect Dis*. 5(3):156
115. Shahsavar F, Mousavi T, Azargon A, Entezami K. 2012. Association of kir3ds1+hla-b bw4ile80 combination with susceptibility to tuberculosis in lur population of iran. *Iran J Immunol*. 9(1):39
116. Sharma MK, Al-Azem A, Wolfe J, Hershfield E, Kabani A. 2003. Identification of a predominant isolate of mycobacterium tuberculosis using molecular and clinical epidemiology tools and in vitro cytokine responses. *BMC Infect. Dis.* 3:3
117. Simpson T, Fox J, Crouse K, Field K. 2012. Screening for mycobacterium tuberculosis using an interferon-gamma release assay. *J Public Health Manag Pract.* 18(4):E19
118. Smith K, Starke J. 2004. *Bacille Calmette-Guerin Vaccine, in Vaccines*. Saunders, Philadelphia, London. 4th ed (eds S.A. Plotkin and W.A. Orenstein) ed.
119. Stead W. 1969. The new face of tuberculosis. *Hospital Practice*. 10(4):62
120. Sterling TR, Villarino ME, Borisov AS, Shang N, Gordin F, et al. 2011. Three months of rifapentine and isoniazid for latent tuberculosis infection. *New England Journal of Medicine*. 365(23):2155
121. SULTAN L, NYKA W, MILLS C, O'GRADY F, WELLS W, RILEY RL. 1960. Tuberculosis disseminators. a study of the variability of aerial infectivity of tuberculous patients. *Am. Rev. Respir. Dis.* 82:358
122. Thoma-Uszynski S, Stenger S, Takeuchi O, Ochoa MT, Engele M, et al. 2001. Induction of direct antimicrobial activity through mammalian toll-like receptors. *Science*. 291(5508):1544
123. Trajman A, Steffen RE, Menzies D. 2013. Interferon-gamma release assays versus tuberculin skin testing for the diagnosis of latent tuberculosis infection: an overview of the evidence. *Pulm Med*. 2013:601737
124. Treatment of tuberculosis. 2003. *MMWR Recomm Rep*. 52(RR-11):1
125. Tuberculosis in canada 2009 - pre-release
126. *Tuberculosis in Canada 2010, Pre-Release - Public Health Agency of Canada*. http://www.phac-aspc.gc.ca/tbpc-latb/pubs/tbcan10pre/index-eng.php
127. *Tuberculosis: Clinical Diagnosis and Management of Tuberculosis, and Measures for Its Prevention and Control*. 2011. London: National Institute for Health and Clinical Excellence (UK)
128. Uciechowski P, Imhoff H, Lange C, Meyer CG, Browne EN, et al. 2011. Susceptibility to tuberculosis is associated with tlr1 polymorphisms resulting in a lack of tlr1 cell surface expression. *J. Leukoc. Biol.* 90(2):377
129. Vernon A. 2013. Treatment of latent tuberculosis infection. *Semin Respir Crit Care Med*. 34(1):67
130. Versalovic J. *Manual of Clinical Microbiology*. Washington, DC: ASM Press. 10th ed.
131. Vidal S, Tremblay ML, Govoni G, Gauthier S, Sebastiani G, et al. 1995. The

ity/lsh/bcg locus: natural resistance to infection with intracellular parasites is abrogated by disruption of the nramp1 gene. *J. Exp. Med.* 182(3):655
132. Vilches C, Gardiner CM, Parham P. 2000. Gene structure and promoter variation of expressed and nonexpressed variants of the kir2dl5 gene. *J. Immunol.* 165(11):6416
133. Villarino ME, Burman W, Wang YC, Lundergan L, Catanzaro A, et al. 1999. Comparable specificity of 2 commercial tuberculin reagents in persons at low risk for tuberculous infection. *JAMA.* 281(2):169
134. Von Pirquet C. 1909. Frequency of tuberculosis in childhood. *JAMA: The Journal of the American Medical Association*. LII(9):675
135. W N Rom, Garay S. 1996. *Tuberculosis*. Little, Brown and Company Inc. 1st ed.
136. Whang DH, Park H, Yoon JA, Park MH. 2005. Haplotype analysis of killer cell immunoglobulin-like receptor genes in 77 korean families. *Hum. Immunol.* 66(2):146
137. Williams AP, Bateman AR, Khakoo SI. 2005. Hanging in the balance. kir and their role in disease. *Mol. Interv.* 5(4):226
138. Wilson MJ, Torkar M, Haude A, Milne S, Jones T, et al. 2000. Plasticity in the organization and sequences of human kir/ilt gene families. *Proc. Natl. Acad. Sci. U.S.A.* 97(9):4778
139. Wilson S. 2008. *Research is Ceremony: Indigenous Research methods*. Winnipeg: Fernwood Publishing. 144
140. Wolf AJ, Linas B, Trevejo-Nuñez GJ, Kincaid E, Tamura T, et al. 2007. Mycobacterium tuberculosis infects dendritic cells with high frequency and impairs their function in vivo. *J. Immunol.* 179(4):2509
141. World Health Organization. 2011. *Global Tuberculosis Control 2011*. Geneva, Switzerland: WHO Press
142. Yawata M, Yawata N, McQueen KL, Cheng NW, Guethlein LA, et al. 2002. Predominance of group a kir haplotypes in japanese associated with diverse nk cell repertoires of kir expression. *Immunogenetics*. 54(8):543
143. Zhang Y, Wang B, Ye S, Liu S, Liu M, et al. 2010. Killer cell immunoglobulin-like receptor gene polymorphisms in patients with leukemia: possible association with susceptibility to the disease. *Leuk. Res.* 34(1):55
144. Zhuang Y-L, Zhu C-F, Zhang Y, Song Y-H, Wang D-J, et al. 2012. Association of kir2ds4 and its variant kir1d with syphilis in a chinese han population. *Int. J. Immunogenet.* 39(2):114