A TUBERCULOSIS OUTBREAK IN A NATIVE COMMUNITY: HLA LINKAGE ANALYSIS AND EVALUATION OF DIAGNOSTIC TESTS

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ABSTRACT

An outbreak of pulmonary tuberculosis in a Canadian Native Indian family was analyzed. Of 66 evaluated subjects, 52 (79%) became newly-infected, twenty-four (46%) of which developed disease.

Chest radiography was the single best diagnostic test (sensitivity 92%; specificity 100%), after which neither demographic variables nor skin test reactivity detected additional disease in a multivariate discriminant analysis. Without radiography, tuberculin skin test reactivity and the subject's age together were significant but poor predictors of disease (model sensitivity 74% and specificity 64%). All diseased adults, but only 46% of children, produced culture-positive specimens (p=0.006). Therefore, children with suspected disease should be treated, regardless of culture results.

No linkage was found between HLA type and disease occurrence in any model. Higher lod scores were obtained by reclassifying subjects' phenotypes, but linkage was excluded up to a recombination fraction of 0.20 . Neither HLA class I loci nor a closely-linked recessive susceptibility locus is a major factor in tuberculosis disease development in this Canadian Indian family.

RÉSUMÉ

Une éclosion de tuberculose pulmonaire chez une famille canadienne de descendance indienne a été étudiée. Des 66 sujets évalués, 52 (79%) sont devenus nouvellement infectés dont vingt-quatre (46%) ont développé la maladie.

La radiographie pulmonaire était le meilleur test diagnostique (sensibilité 92%; spécificité 100%) après quoi aucune variante démographique ni de réactivité au test cutanée tuberculinique n'a décelé une maladie additionelle dans une analyse multifactorielle discriminante. Sans radiographie, la réactivité au test cutané et l'âge du sujet étaient significatifs mais s'avéraient de faibles prédictions de la maladie (sensibilité 74%; spécificité 64%). Tous les adultes atteints, seulement 46% d'enfants, ont produit des échantillons de culture positifs ($P = 0,006$). Conséquemment, les enfants soupçonnés de la maladie devraient être traités quand même.

Aucune liaison n'a été découverte entre le génotype HLA et l'occurrence de la maladie dans tout modèle. Des résultats supérieurs ont été obtenus en reclassifiant des phénotypes de sujets mais la liaison a été exclue à une fraction de recombinaison de 0,20 . Ni les loci HLA de classe I, ni un segment proche d'ADN ne représente un facteur majeur de gène récessif dans le développement de la tuberculose chez cette famille Canadienne de descendance indienne.

Acknowledgements

I would like to thank Dr. E. Anne Fanning, Alberta Health Tuberculosis Services, who allowed me to study the family whom she treated during the outbreak, and who served as liaison between the family members and myself. I would also like to thank Dr. Erwin Schurr for his prompt processing of the participants' blood samples in the early hours of the morning, Dr. Emil Skamene for financial support through his World Health Organization (WHO) grant, and Drs. Ken Morgan and Theresa Gyorkos for their guidance.

I would also like to acknowledge the participation of the members of the Native Indian family whom I studied, who agreed unhesitatingly to participate and give blood for the potential benefit of tuberculosis patients elsewhere in the world whom they will never meet.

Finally, I would like to thank my wife, Debbie, and my children, Jennifer and Mitchell, for their tolerance while I undertook to accomplish this thesis.

PREFACE

The idea for this project arose from an ongoing interest in tuberculosis by the applicant and informal discussions between the applicant and Dr. E. Skamene in 1988 about pursuing linkage analysis in Canadian families with tuberculosis. Dr. Skamene had received WHO funding for a study to look for linkage between tuberculosis disease and a putative "susceptibility" gene on human chromosome 2. At that time, despite the WHO study being Canadian-centred, no Canadian families had been enrolled. The applicant's interest in the clinical usefulness of diagnostic tests for the diagnosis of tuberculosis prompted a search for Canadian families who could serve as a study group to examine both issues. The blood given by the study subjects was used for HLA typing for this study, as well as being a source of DNA for the WHO-funded study.

Contact with Dr. E. A. Fanning (Alberta Health Tuberculosis Services) succeeded in locating a suitable family in Northern Alberta.

This thesis documents the results of such a research project which sought to answer the following two questions:

1) What is the usefulness of demographic data, Mantoux skin testing, mycobacterial cultures of respiratory secretions, and chest radiography for the detection of active pulmonary tuberculosis?

2) Is there linkage between HLA-A, and -B alleles and the presence of tuberculosis disease?

Both of these questions were investigated by studying an epidemic of tuberculosis in a Canadian Native Indian family in Alberta.

In order to analyze the demographic data and diagnostic tests (radiography, skin testing, and cultures), the medical and laboratory data of each individual in the family were systematically recorded and analyzed.

In order to address the role of the HLA loci, blood sampling of the family was performed, and linkage analysis was carried out using the medical data described above.

The project organization and coordination, medical record auditing and data recording, and project documentation were done solely by the applicant. The data analysis was also performed solely by the applicant, with the exception of the linkage analysis, which was completed with the help of Dr. Ken Morgan. In this respect, we are grateful to Drs. G. M. Lathrop and J. Ott, who kindly gave Dr. Morgan the LINKAGE programs, and for the computing facilities which were provided by the Howard Hughes Medical Institute genome resources project. Blood-taking of the study participants was done by both the applicant and Dr. E.A. Fanning (Alberta Health Tuberculosis Services, Edmonton, Alberta). The family's medical records were initially recorded by Dr. Fanning during the tuberculosis epidemic.

The HLA-A, -B, and -C typing was performed by Dr. F. Pazderka, Histocompatibility Laboratory, Blood Transfusion Service, Canadian Red Cross, Edmonton, Alberta.

Epstein-Barr transformation of patient lymphocytes, in order to maintain permanent DNA banks, was performed by Dr. Erwin Schurr, Department of Medicine, McGill University, Montreal, Quebec.

On-site patient care, surveillance of medications, and administration of tuberculin skin tests during the tuberculosis epidemic were done by the Alberta public health nurse in the community. Individuals requiring hospitalization for diagnosis and/or treatment were flown to the Alberta Health Tuberculosis Services in Edmonton, Alberta.

All specimens for mycobacterial staining and culture were transported to, and processed in, the Mycobacteriology Section, Alberta Public Health Laboratory, Edmonton, Alberta.

Due to ongoing land-claim negotiations with the federal government, the leader of the study family has asked for complete anonymity. Therefore, all family and community identifiers have been removed from this thesis in order to ensure this.

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1. INTRODUCTION

Tuberculosis remains an important cause of morbidity and mortality in the world, due to the large number of new cases and deaths each year[1]. This is especially true in developing nations, where 3 to 10 million new cases and over 2.5 million deaths occur annually[1,2]. In Quebec, high-risk endemic groups include the Inuit, Native Indians, immigrants, refugees, and the elderly[3,4]. Despite decades of research into all aspects of this area of infectious disease, questions remain about the usefulness of tuberculin skin testing, chest radiography, and mycobacterial cultures in the diagnosis of active pulmonary disease, the most common form. In addition, newer technology has changed the laboratory methods used to make the diagnosis of tuberculosis[5]. This technology has produced an increase in the sensitivity and speed for detecting M. tuberculosis from clinical specimens[6,7]. It is less apparent if it has enhanced the ability to diagnose disease during rural community outbreaks where culture sensitivity, for instance, may be compromised by suboptimal collection and transportation of specimens[8].

Major controversy also surrounds the pathophysiologic mechanisms which allow progression of infection to clinically overt disease in some individuals, and not in others. "Natural resistance" to tuberculosis has been suggested to exist[9], but the presence or mechanism of such

resistance has not been unequivocally documented. Although inoculum size of the infecting organism[10], as well as integrity of the immune system[11], are known to play important roles in the development of clinical disease, the **relative** importance of the pathogen and the host remain obscure. Because of ample data that HLA antigen expression is involved in human recognition of tuberculin antigens in vitro[12,14,101], the relative ease of HLA typing of individuals[15], and population data that HLA antigen expression may be associated with development of clinical disease[16-18], it is reasonable to look at linkage of HLA type with tuberculosis disease expression in affected families.

Analyses of tuberculosis epidemics have been used in the past in attempts to resolve several controversial issues, for example: infectivity of the organism[19], the predictive value of various tuberculin skin test interpretations[20], post-exposure prophylactic measures[21], and the "booster" effect of skin testing[22]. Even though patient numbers were sometimes small in these studies, such investigations have contributed greatly to our knowledge of tuberculosis. A review of 109 tuberculosis epidemics has detailed many of the non-genetic factors thought to be important in such outbreaks[167]. However, the genetic predisposition to disease was not explored.

In order to ascertain the usefulness of demographic data, tuberculin skin testing, chest radiography, and mycobacterial cultures for the detection of tuberculosis disease in a large Canadian Native Indian family, these parameters were compared to the "gold standard" of disease presence as defined by a combination of explicit clinical, radiologic, and microbiologic criteria as described by the American Thoracic Society[23].

In order to assess the contribution of HLA-linked genes on disease occurrence, a linkage analysis was performed on the same Canadian Native Indian family who had recently experienced an epidemic of tuberculosis.

2. REVIEW OF THE LITERATURE

i) Epidemiology of tuberculosis

The incidence of tuberculosis in the developed world, including Canada, as assessed by age-standardized incidence rates, has been declining since the early 1900's[24-26]. However, in certain areas of Canada[27], as well as in most of the developing world[1,26], it remains a significant health problem. It is estimated that between 3 and 10 million new cases, and over 2.5 million tuberculosis deaths, occur annually among the world's developing nations[1]. The overall incidence rate of tuberculosis in Canada is approximately 8 - 12 cases per 100,000 population[27], while in the developing areas of the world the rate is between 10 and 30 fold higher (estimated mean incidence of 171 per 100,000 overall with an upper limit as high as 229 per 100,000 in Sub-Saharan Africa)[1]. Among Canadian Native Indians and Inuit, the incidence rates in the 1970s were, respectively, 16 and 24 times greater than the non-Native rates[27]. Mortality from tuberculosis in Canada in 1986 averaged 0.6 deaths per 100,000 population[29], and in the developing world 61 per 100,000[1]. Among the Canadian Native Indian and Inuit populations, the mortality rates were almost 10-fold greater than the Canadian average, at 5.7 per 100,000 population[29]. In these Native populations, common-source outbreaks of tuberculosis still occur, but are rarely documented in the

medical literature[30,31]. Although incidence rates of tuberculosis have been falling in all groups in Canada since the early part of the 20th century, the rate of fall is lowest in Native Indians (4% per year), highest in the Inuit (16% per year), and approximately 10% per year in non-Native Canadians[27].

Several explanations have been proposed to clarify divergent world tuberculosis rates. It has been hypothesized that the presence of crowded living conditions, malnutrition leading to immunosuppression, limited access to health care, unavailability of antibiotic therapy, late recognition of disease (i.e. inability to find and eradicate cases early), and infection at a younger age contribute in major ways to the differences observed in incidence rates. However, it remains unclear how much of the diversity in incidence is due to genetically determined susceptibility and how much is due to environmental factors or, for instance, to variation in mycobacterial virulence. It has been proposed that the different rates of disease incidence in Canadian Native Indians from various regions in Canada can be partially explained by a "selection of the fittest" model, where earlier exposure to tuberculosis has led to a genetically hardier population and less disease[27]. In any single region, however, the risk of tuberculosis disease is dependent on a complex interplay of many other factors: age, gender, race, socioeconomic status, geography, temporal trends, and

occupation.

Age is an important determinant of disease development, and consistently demonstrates a trimodal risk. Peak periods of risk for pulmonary tuberculosis following infection occur in children 2 years or younger, those aged 14 to 25 years, and also in the elderly[32,33]. The severity of pediatric pulmonary disease also appears to be worse in the youngest children. Significant hilar pulmonary lymph node enlargement causing segmental pulmonary disease following infection in children is most frequent in infants less than 1 year old (43% incidence), decreases to an incidence of 25% in 1-10 year olds, and falls further to only 16% in those 11 to 15 years of age[32]. The rate of development of disease for children 2 years of age or less is so great - 50% or more[9,26,33,34] - regardless of other factors, that it is very likely that disease in this group is due almost exclusively to the naivete of the immune system and not much influenced by inherited susceptibility.

Gender is an important risk factor for tuberculosis disease and tuberculosis-related mortality in adults[24,36,37]. In childhood, however, incidence rates of disease as well as tuberculosis mortality rates are similar in the two sexes[24,36,37].

Adult males are more susceptible to disease development following infection, while females are more likely to die of tuberculosis, once disease manifests. The overall

male:female disease incidence ratio in adults is a minimum of 2:1, depending on the country studied[24,37] with a ratio of almost 4:1 in Asia[38]. In the age group over 65 years, the male:female disease incidence ratio is greater than in young adults[37], suggesting that elderly males are at greater risk for disease, once infected, or are at greater risk of primary infection.

The mortality rate divided by the disease incidence rate for a given group can be used as a proxy for the case-fatality ratio when such direct measures are absent, as with Canadian tuberculosis data. For tuberculosis disease in Canada, of which pulmonary disease accounts for 90%[24], this ratio is 0.026 in young adults and 0.21 in the elderly[29]. Interestingly, this ratio is smaller in males than in females in almost all age groups (table 1)[29]. This suggests that, although men are at higher risk for disease development, as stated above, they are less likely to die of manifested tuberculosis. No factors have been uncovered to explain these findings, although the higher incidence of cigarette smoking and pneumoconiosis in men may explain, in part, the discrepancy in disease incidence alone.

Race as a risk factor for development of tuberculosis disease remains controversial. The predisposition for the development of disease in non-whites in North America can be seen at all ages. However, this tendency has always been

Table 1. Tuberculosis morbidity and mortality rates (expressed per 100,000 population) in Canada for 1983, by age and sex.[36]

	Morbidity			Mortality			Mortality/morbidity		
Age	M	F	M/F	M	F	M/F	M	F	M/F
< 1	4	2	2	0	0	-	0	0	-
1-4	4	4	1	0	.30	-	0	.07	-
5-14	1	3	.33	0	0	-	0	0	-
15-19	4	5	.80	0	0	-	0	0	-
20-24	5	7	.71	0	0	-	0	0	-
25-34	7	6	1.2	.10	.16	.66	.02	.03	.57
35-44	11	7	1.6	.15	.23	.66	.01	.03	.42
45-64	24	10	2.4	1.1	.22	5.0	.05	.02	2.1
65-74	35	17	2.1	2.9	2.2	1.3	.08	.13	.65
≥ 75	53	27	2.0	9.7	5.8	1.7	.18	.21	.85

Mortality divided by morbidity is an indirect measure of the case-fatality ratio. M: male F: female

confounded by socioeconomic status[32,37]. Data in infected children and adolescents of similar socioeconomic status have not shown any difference in disease occurrence according to race[39]. Although a recent investigation has demonstrated racial differences in **infection** rates with Mycobacterium tuberculosis[40], important confounders such as socioeconomic status and occupational history were not controlled[41]. It remains unclear how much tuberculosis infection or disease in various racial groups is due to genetics, and how much is due to the contribution of coincident poverty, crowding, malnourishment, and other confounding factors. What is clear is that socioeconomic status remains strongly linked with disease, with some investigations showing striking correlations between socioeconomic status indicators and disease incidence rates[37]. Although correlations have also been found between disease incidence and other demographic factors, such as occupation[42], these analyses are confounded by the strong covariation of these influences with socioeconomic status.

The downward temporal trend in both tuberculosis infection and disease incidence rates in the developed world is impressive. As an example, the disease incidence in Quebec has dropped from 175 cases to 20 cases per 100,000 from 1945 to 1970[24]. A similar fall in Canada as a whole (from 115 cases to 20 cases per 100,000) occurred during this period[24]. In parallel, the mortality rate fell over

this 25-year period by over 90% (from 70 cases to less than 5 cases per 100,000 in Quebec; from 48 cases to less than 5 cases per 100,000 in Canada)[24]. Similar trends are seen for other developed countries in Europe[2,26]. However, rates in developing nations have remained stationary over the past 30 years, with some countries (e.g. Uganda) displaying small increases in infection rates of 1.4% per annum, and other nations (i.e. Lesotho) showing no change at all over this period[2,26]. Although the same tools for tuberculosis control (skin testing for detection of infection, treatment of active cases, and prophylaxis of infected contacts) are used throughout the world, the actual methods and resources available to implement this control vary widely and account for the lack of effectiveness in developing areas.

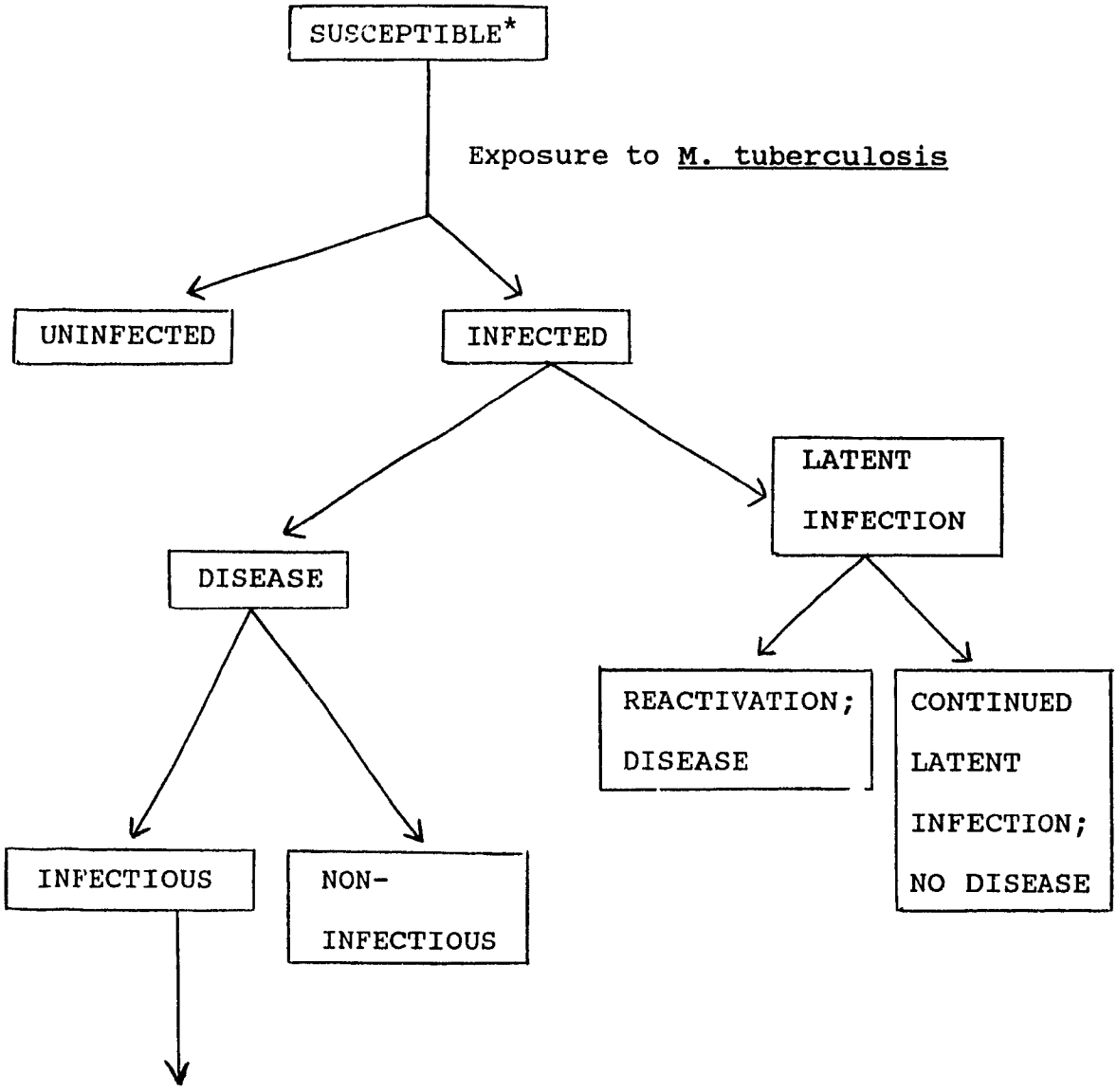
The downward trend in tuberculosis incidence rates in the United States has levelled off since 1986[43]. It is believed that this deviation from a continuing decrease is due to the large number of extra cases (14,768 cumulative excess cases since 1986) being seen because of coexistent infection with M. tuberculosis and the human immunodeficiency virus (HIV)[43-45]. Although the incidence of tuberculosis disease has not been seen to increase yet in areas of the developing world where many individuals are infected with both agents[2], most of these areas lack adequate surveillance systems and such data is only now being collected.

ii) Pathogenesis and clinical manifestations of infection and disease by Mycobacterium tuberculosis

The development of tuberculosis disease requires antecedent **infection** with the bacterium Mycobacterium tuberculosis or, much rarer, Mycobacterium bovis[46]. Infection almost always occurs via the airborne route: organisms must be contained in droplets of sufficiently small size (aerosolized by other infectious individuals) which are inhaled into the small pulmonary airways and taken up by inflammatory cells, thereby establishing infection in susceptible individuals[47]. Certain individuals, however, are able to resist infection after exposure. Of those who do become infected, either of two sequelae may develop: immediate progression to pulmonary disease, or arrest of the infection in a latent stage. Moreover, those individuals with pulmonary disease are potentially infectious for others, perpetuating the infection in the population. In addition, it is possible for previously infected individuals (regardless of development of past disease) to become re-infected with a new exposure[26,48]. Their course may be modified by the presence of cell-mediated immunity produced by the past infection. A scheme of the status of a hypothetical population exposed to M. tuberculosis is shown in figure 1.

In most of the developed world, it has been estimated that the majority of active tuberculosis cases are reactivations of old quiescent infections. For instance, in

Figure 1. Differential status of a hypothetical population exposed to M. tuberculosis, with respect to development of tuberculosis infection and disease.



*All individuals are considered susceptible to a new infection, regardless of past exposure.[48]

Quebec, they constitute 58% of endemic cases[24], most being pulmonary[24]. Therefore, such reactivations, and not new infections, are responsible for the majority of cases of endemic tuberculosis, and cause most of the spread of disease into the susceptible population. In epidemics of tuberculosis, the situation is reversed, with all disease arising from new progressive infection after exposure to an infectious individual(s).

The infectivity of the organism has been clarified in epidemic situations. Reports of outbreaks in a nursing home[20] and on a submarine[19] have served to document infectivity in closed environments. Infection rates among exposed individuals ranged from 31 - 80%, depending on physical proximity to the index case. Therefore, type and frequency of contact are primary risk factors for infection. In addition, household contacts of diseased individuals producing sputum with microscopically visualized organisms ("smear-positive") have higher infection rates (50% incidence of infection) than contacts of smear-negative individuals (5% new infection rate)[26]. The implication is that larger quantities of organisms are aerosolized, leading to both an increased probability of household contacts being infected through contact, and a larger infecting inoculum for such contacts.

For clinical and epidemiologic purposes, any population can be theoretically subdivided into those with

and those without tuberculosis infection. Infection is defined as the presence and replication of microorganisms in the host[9]. Infection with M. tuberculosis may be completely unapparent or may produce clinical signs and symptoms recognizable as "disease". As mentioned, past infection with M. tuberculosis does not prevent re-infection when exposure to a new inoculum occurs[48].

After initial replication of the organism in the lungs and pulmonary lymph nodes, a period of mycobacteremia leads to dissemination of the pathogen through the blood. This dissemination results in seeding of the organism to various body sites, most commonly the upper lungs, kidneys, bones, and brain[23]. At this point, the infection becomes quiescent in 95% of individuals, but will continue to progress in the rest[49]. Those with quiescent disease may develop, at a later time, reactivated foci of the dormant viable organisms, leading to clinical disease at that site[50,51]. As a consequence of the above sequence of events, the most frequent site of tuberculosis disease is in the lungs, either as a result of progressive primary infection or reactivation of quiescent foci. Pulmonary tuberculosis constitutes approximately 70% of tuberculosis in the developing countries, 85-90% of all disease in Canada and the United States[9,24], and approximately 65% of tuberculosis in Canadian Natives[30]. Most importantly, individuals with pulmonary tuberculosis become the source of infectious

airborne particles which can infect other susceptible persons. Individuals with the other common forms of tuberculosis disease (lymphadenitis, renal, bone, and meningeal) are not normally infectious to others.

Cell-mediated immunity (CMI), consisting mainly of T-lymphocytes and macrophages, is the major factor which keeps quiescent foci of infection from becoming active[53]. Dysfunction in CMI, however acquired, predisposes to the development of clinical tuberculosis disease in previously infected individuals. Medical conditions which adversely affect CMI and lead to an increased risk of both progression of infection and also reactivation of quiescent foci leading to disease include, most commonly, malnutrition[54], infection with the human immunodeficiency virus (HIV)[55,56], corticosteroid use[57], and renal failure[58].

The extent of clinical signs and symptoms accompanying tuberculosis **infection** are age-dependent, with increased severity in the very young. When present, the manifestations of infection are non-specific and may consist of fever, fatigue, and the immune-mediated signs of erythema nodosum and keratoconjunctivitis[9]. The clinical syndromes accompanying tuberculosis **disease** depend on the site affected. Pulmonary tuberculosis, the most common form[9,24], may be asymptomatic early on. Later in the disease progression, non-specific symptoms such as anorexia, fatigue, weight loss, chills, fever, and/or night sweats may

develop. A cough is usually also present[23]. The manifestations of extrapulmonary tuberculosis are innumerable, and are produced by the inflammatory and invasive nature of the disease at the involved site: for example, local swelling and pressure with diseased lymph nodes, pyuria and progressive renal failure with renal disease, and progressive indolent meningismis and cranial nerve palsies with meningeal involvement[9].

iii) Diagnostic tests for detection of tuberculosis infection and disease

Detection of **infection** with M. tuberculosis is currently performed by means of a delayed-type hypersensitivity (DTH) skin test. The recommended method, known as the Mantoux test, consists of an intracutaneous injection of a 5 Tine Units (TU) dose of mycobacterial purified protein derivative (PPD-S), derived from a reference strain of M. tuberculosis and commercially available[59]. The skin test has a sensitivity and specificity that is dependent on a) host factors such as age[60], immunosuppression[56], and the booster effect[61], b) previous vaccination with the mycobacterial vaccine BCG (Bacille Calmette-Guerin)[59], c) the incidence of infections with mycobacteria other than tuberculosis (MOTT) in the population[59], and d) the criterion used for determining a "positive" (i.e. size of skin induration)[59].

Some of these factors play larger roles in certain populations. Individuals previously vaccinated with BCG may have Mantoux skin test reactions of variable size, which might be differentiated from the reaction produced by natural infection with M. tuberculosis, depending on the age at vaccination[28,59]. Individuals on the African continent are much more frequently exposed to M. tuberculosis than to MOTT. Therefore, any response to the DTH skin test in these individuals is much more likely to imply tuberculosis infection, and the test provides both a sensitive and specific measure of infection when a "positive" is defined as 5 mm or more of induration. In contrast, in populations who are rarely exposed to M. tuberculosis but commonly exposed to MOTT, skin test reactivity usually implies MOTT infection. In order to preserve reasonable test operating characteristics for screening such a low-prevalence population, usual guidelines suggest a "positive" be designated as 10 mm or more of induration[59]. For individuals with a high probability (risk) of infection (i.e. individuals known to have been in contact with an infectious case), it is suggested that 5 mm of induration be used as the threshold for a "positive" test[23,59]. Similarly, the recommended threshold of skin induration to be interpreted as "positive" is 5 mm when testing individuals infected with HIV[62]. This recommendation is based on the finding of waning DTH skin reactivity with progressive immunosuppression in

this group, despite active disease[55,56]. HIV-infected patients may often present with tuberculosis disease and a non-reactive skin test[56].

The skin test is highly reliable in detecting new infection when used in the screening of non-immunosuppressed close contacts of a case of tuberculosis. In such a setting, conversion from a negative to a positive skin test (defined as progression from less than 5 mm to 5 mm or greater induration after exposure) is regarded as near-certain proof of infection, and is sufficient evidence to start prophylactic therapy[23]. Although other methods of performing tuberculin skin testing have been developed, such as the Heaf and Tine tests, they are less reliable and are not recommended for use in high-risk populations[63-65]. Although the Mantoux test is currently the best method for objectively documenting infection, it remains controversial to what extent it can: 1) signal the presence of active disease, and 2) predict the later development of disease. One survey of 625,000 American navy recruits, followed for 7 years, showed a strong association between the average annual incidence of disease and the initial skin test reaction: 25 cases per 100,000 in those with induration of 0-5 mm; 128 per 100,000 with induration of 6-11 mm; 330 per 100,000 with induration of 12 mm or more[66]. However, all reactors with 12 mm or greater induration were considered together in a single group. Thus, it remains unknown if

higher risks of disease in this group are associated in a continuous manner with increasing induration. Similar data for children showed a disease incidence rate which was higher in those with reactions of 16 mm or greater[39]. Again, this "reactor" group was considered together and no subgroup analysis was done. Another more detailed American naval study showed an increasing risk of disease only up to reactions of 15 mm. Thereafter, the risk of subsequent disease was the same, regardless of the size of induration[67]. Two surveys using the older Heaf tuberculin test in children showed contradictory results. One study showed increasing risk of eventual disease with increasing skin reactivity[68], while the other showed no association[69]. Most such surveys have indeed shown an association, while animal data has also demonstrated an increased risk of disease with large skin test reactions[35].

What remains unclear is why the presence of a larger reaction should predict a higher probability of disease occurrence. It is likely that a great part of the explanation lies in the fact that almost all individuals with large skin reactions have been infected with M. tuberculosis, whereas a significant proportion of those with smaller reactions have only been exposed to MOTT and do not carry a risk of developing tuberculosis at a later time[59]. This would also explain the threshold effect seen in the naval data, wherein those with reactions larger than 15 mm

carry the same risk of disease occurrence.

It is unlikely that the larger skin reaction indicates a heightened susceptibility to organ damage from disease, since increased severity of disease has not been shown to be associated with such larger reactions[70].

All of the above studies have addressed the prognostic ability of a tuberculin reaction to predict development of disease at a later time - usually years. However, little is known about the performance of the tuberculin test in detecting concurrent disease at the time of skin testing during an outbreak. One report of intensive screening of 1271 suspected exposed persons in Vancouver showed a median skin reaction of 20.5 mm among the 8 persons with active disease[71]. However, no data on the size of the skin tests among the non-diseased were given for comparison. Four other studies have not been able to show a difference in skin reactivity among those with and without active concurrent tuberculosis disease[72-75].

The definitive diagnosis of tuberculosis **disease** continues to rely on the ability to culture the organism <u>in vitro</u> from infected tissues or body fluids. The sensitivity of such cultures varies with the site of infection[76], the type of specimen to be cultured[77,78], and the culture method used[78,79]. In pulmonary infections, the sensitivity of sputum cultures has been shown to be approximately 40%[78]. However, most of this data was collected prior to the

technological advances in culture methods which are now in use, such as the radiometric broth system[6,7]. Moreover, the sensitivity of mycobacterial cultures of respiratory secretions is felt to be age-dependent, with poorer performance in children and infants with pulmonary tuberculosis[32]. One study in Canadians found that 15% of clinically documented cases of pulmonary tuberculosis were culture-negative in Native Indians, while 21% of non-Indians produced culture-negative respiratory specimens[27]. Another Canadian study, employing a single sputum culture as part of a mass screening campaign of inner-city adults, found that the culture of a single specimen had a sensitivity of 75%[71]. However, the number of diseased persons was very small. It must be noted that cultures for M. tuberculosis usually take several weeks before being completed. Therefore, the lack of a rapid culture result further compromises the immediate usefulness of this test. However, the immediate visualization of mycobacteria in a smear of a respiratory specimen stained with acid-fast or fluorescent staining ("acid-fast bacilli" [AFB]) is highly indicative of pulmonary tuberculosis in adults. The frequency of such a finding depends on the extent and type of disease (i.e. cavitary) present[27,31,51]. Although the proportion of smear-positive respiratory specimens depends on the extent of pulmonary disease at the time of diagnosis, two studies in Canadian Natives showed a smear-positive rate of 43 to 65

per cent[27,30], which is not much different from the usual rate seen in Canada in non-Natives[51].

In the absence of positive cultures, or when culture material is not obtainable, the diagnosis of tuberculosis must rely on the presence of compatible clinical signs and symptoms, as well as documentation of a positive tuberculin skin test[23]. However, a tuberculin skin test may be negative in the presence of active disease, if CMI is impaired for any reason. Thus, with clinical suspicion of tuberculosis disease, the DTH skin test can only be considered as an ancillary laboratory test, and not an absolute requirement proving antecedent infection.

The chest radiograph is another objective method of documenting the presence of pulmonary tuberculosis. However, except perhaps for apical cavitary disease, the radiographic appearance of tuberculosis is not specific and may be confused with malignancy[80], fungal disease[81,82], other bacterial infections[83], or any one of 55 other recognized diseases[84]. In addition, it is not a reliable way of differentiating old foci of inactive infection from active disease[23,71]. The "classical" apical or posterior involvement of the upper lobes of the lung are still seen, but lower lobe infiltrates and other patterns not characteristic of "classical" pulmonary tuberculosis may also be found[85,86]. Moreover, there have been reports of sputum culture-positive individuals having completely normal

radiographs[87]. However, these persons who were screened during an epidemic were probably detected very early in the course of their disease. In one evaluation of chest radiographs for the diagnosis of disease during a screening campaign, two of the eight diseased (proven by culture) individuals were erroneously thought to have old "inactive tuberculosis" or "other disease" on chest radiography[71].

In children, the chest radiograph is much more characteristic, with lymph node enlargement and segmental lung disease the most frequent finding. However the frequency of segmental lesions on radiography following a recent infection in children is age-dependent, reflecting the aforementioned concept that disease is less severe with increasing age in childhood. Segmental lesions can be seen radiographically after infection with M. tuberculosis in 43% of infants less than 1 year old, 25% of 1 to 10 year olds, and 16% of those aged 11 to 15 years[32].

Further diagnostic modalities, including tuberculous antigen detection in clinical specimens[88] and serologic markers of disease[89], are only in developmental phases and not available to the average clinician. Although extremely promising, these technologies are also expensive, and will likely be difficult to implement in those developing countries with the heaviest tuberculosis burden, since they also usually possess scant funding for medical diagnostics.

Therefore, the average clinician today in any area,

when faced with a patient with suspected pulmonary tuberculosis, has only the clinical presentation, the DTH skin test, radiography and mycobacterial culture at his/her disposal in order to confirm the diagnosis. Not all of these may be available to the health care worker, depending on the local economic situation. In addition, cultures are not useful for the immediate diagnosis of disease, because of the delay involved. None of the above instruments has been demonstrated to have the combined sensitivity or specificity and rapidity which would make it suitable as a single diagnostic tool.

iv) Genetic basis for host resistance to tuberculosis

Until recently, it has been thought that susceptibility to tuberculosis **infection** is dependent almost completely on factors relating to the bacterial pathogen (e.g. inoculum effect, efficiency of transmission, virulence). However, recent evidence suggests that there may be a genetic predisposition in man to the actual primary infection with M. tuberculosis, as evidenced by differential infection rates in various racial groups[40]. Confounding factors in this recent study (i.e. socioeconomic status, occupational history) prevent unanimous acceptance of this theory[41].

The role of genetic resistance in the development of human tuberculosis **disease** has been based on epidemiologic

data[26,90] and twin studies[91-93], suggesting that genetic factors contribute significantly to the host-pathogen interaction. Epidemiologic studies have suggested that there exist biologic differences in the ability to control mycobacterial replication and the development of disease among infected individuals[90]. This was demonstrated by finding that similarly exposed/infected untreated persons can vary markedly in their disease progression and in the number of detectable bacilli after similar time periods following infection.

The early studies of monozygotic and dizygotic twins, wherein siblings were assumed to be equally exposed to M. tuberculosis and one sibling developed disease, showed a greater concordance of disease in the monozygotic than in the dizygotic pairs[91,92]. For instance, Kallman and Reisner's data suggested that a monozygotic co-twin of a diseased sibling was 16 times more likely to develop disease than a dizygotic co-twin[92]. A large study of 202 twin pairs in 1952, the Prophit study[94], was conducted to re-evaluate this question. The original interpretation of the results suggested that non-genetic factors such as exposure and gender could completely explain the greater concordance of tuberculosis in monozygotic twins[94]. However, a re-analysis of the data, using multivariate regression with adjustment for potentially confounding variables, showed a relative risk of disease in monozygotic twins which was only twice

that of dizygotic pairs, but of statistical significance[93].

The genetically-directed biologic mechanisms which affect the expression of disease seem to depend to a large extent on T-lymphocyte function and macrophage-mediated mycobactericidal activity[95,97]. Anti-tuberculous CMI, as evidenced by a positive DTH skin test, seems to play a role in preventing disease after exogenous re-infection[20]. In addition, evidence exists supporting the importance of "self" major histocompatibility complex (MHC)[4] Class I and Class II molecules in the interaction between lymphocytes, macrophages, and the antigens of infectious agents[98], including tuberculin antigens[12,100-103]. The human MHC, which is termed the human leukocyte antigen (HLA) complex, consists of seven recognized highly polymorphic components on chromosome 6, which code for antigens associated with the mediation of interactions between cells of the immune system[104]. HLA-A, -B, and -C, known as class I antigens, are glycoproteins found on most nucleated cells. HLA-DR, -DQ, and -DP, the class II antigens, are primarily expressed in B lymphocytes, monocytes, macrophages, activated T lymphocytes, and endothelial cells. HLA-D expression is recognized by means of the cellular response in mixed lymphocyte cultures.[104] Although the humoral response (i.e. specific antibody production) to M. tuberculosis seems to be, in part, affected by HLA type[105,106], the humoral contribution to the prevention of tuberculosis disease is

much less important[107]. It is clear that the presence of anti-mycobacterial immunoglobulins does not prevent the development of disease with M. tuberculosis[108,109].

Genetic determinants, specifically those controlling HLA antigen expression, are almost certainly associated with human susceptibility to leprosy[110,111], another human mycobacterial disease. Regarding tuberculosis, however, the results of analyses of association between HLA type and disease have produced widely divergent results (table 2). From table 2, it can be seen that only expression of some HLA-B antigens in certain populations is associated with the development of disease[16-18], although associations with HLA-A, -B, -C, and -DR were investigated. However, it should be noted that most of the studies to date have examined the association of HLA type with disease in unrelated individuals, known as "association" studies. Very little information has yet to be collated by analyzing families with multiple affected members (i.e. "linkage" studies).

The rationale behind investigating linkage between HLA antigen expression and susceptibility to tuberculosis disease is based on evidence that T-lymphocytes, which help defend the host against M. tuberculosis[95,97], recognize mycobacterial antigens in association with products of MHC-encoded molecules on the surface of antigen-bearing cells[12-14,112]. As well, there have been demonstrated associations

Table 2. Studies investigating the association or linkage between HLA and tuberculosis.

A: association L: linkage Ref.: reference

Study type	Study subjects / TB type	Authors' Conclusions	Comments	Ref.
A	Mexican-Americans (51 with TB; 54 without TB) / pulmonary TB	HLA-DR3 less common in those with TB	If multiple tests considered (Bonferroni inequality[117]), no significant association.	113
A	Canadian caucasians from Newfoundland (46 with TB; 543 without TB) / all TB types	HLA-B8 associated with TB (EOR=5.1)	Statistically significant result.	16
A	Black Americans from Washington (60 with TB; 100 without TB) / all TB types	HLA-Bw15 associated with TB (EOR=8.1)	Statistically significant result.	17
A	Black Americans from New Jersey (72 with TB; 54 without TB but with + Mantoux) / pulmonary TB	HLA-B5, -DR5 associated with TB, and HLA-DR6y less common in TB patients	If multiple tests considered, no significant association.	114
A	Egyptians (42 with TB; 156 without TB) / pulmonary TB	HLA-A2, -B5 associated with TB.	If multiple tests considered, no significant association.	115
A	Chinese (no details available) / pulmonary and meningeal TB	HLA-Bw35 associated with TB.	Statistically significant result.	18
A	Greeks (57 with TB; 400 "controls") / pulmonary TB	HLA-B27 associated with TB.	Statistically significant result.	116

Table 2 (cont'd). Studies investigating the association or linkage between HLA and tuberculosis.

A:association L: linkage Ref.: reference

Study type	Study subjects / TB type	Authors' Conclusions	Comments	Ref.
A	Mexican Americans (100 with TB; 100 without TB pulmonary TB	No association found.		118; 119
A	European caucasians (119 with TB; unknown no. without TB) / all types of TB	No association found.		120
A	East Indians (124 with TB; 109 without TB) / pulmonary TB	No association found.		121
A, L	Russians (643 with TB; 884 without TB) / pulmonary TB	(1) HLA-DR2 associated with disease and HLA-DR3 less common in TB patients. (2) Diseased child more likely to inherit HLA haplotype of diseased parent.	(1) Associations statistically significant. (2) Increased **severity** of disease associated with HLA-DR2. (3) Increased levels of anti-PPD immunoglobulins in diseased persons with HLA-DR2.	106
L	East Indian Hindus (25 multiple-case families) / pulmonary TB	(1) HLA-DR2 linked with TB (2) Unclear whether recessive or dominant model appropriate (3) HLA-linked susceptibility to TB not highly penetrant.	HLA appears to modulate the susceptibility to TB, with other unknown factors (genetic or environmental)	122; 123

between HLA phenotypes and the host response to other intracellular infectious diseases in man, such as leishmaniasis[124], anti-measles antibody production[125], the response to influenza A vaccination[126], and recurrent herpes labialis[127], although the latter has been refuted[128]. It is unclear for most infectious diseases in man whether disease expression is causally related to genetic variation in an HLA molecule, or whether disease is due to a gene(s) closely linked to the HLA markers.

It must be noted that HLA antigen expression is, in strict terms, an expression of an individual's phenotype detected by means of a laboratory assay. However, since HLA expression is solely under genetic control, it is synonymous with "genotype" in this instance. Therefore, class I HLA antigen polymorphism results from co-dominant expression of the respective HLA alleles, and can be typed as long as suitable typing antisera are available.

In experimental animal models of mycobacterial disease, genetic factors seem to play an extremely important function in maintaining the host free of disease[129-131]. An animal model of mycobacterial infection in mice has been developed in which macrophage function plays a significant role in the modulation of clinical disease after infection by M. bovis[129,130], a bacterium closely related to M. tuberculosis. Genetic analysis of phenotypically "susceptible" and "resistant" mice has demonstrated the

existence of a gene (Bcg) which is crucial to this immunity to disease after infection[129]. The exact mechanism by which the presence of the "resistance" allele (Bcg^r) determines the resistant phenotype remains unknown, but is probably related to the superior mycobactericidal activity of the Bcg^r macrophage[132].

Analysis of the proximal region of murine chromosome 1, on which the Bcg gene is situated, has shown strong homology with a region on chromosome 2q (region 32-37) in humans[133,134]. By inference, it is assumed that there is a human gene on chromosome 2q, homologous to the mouse Bcg gene, which might be important in determining susceptibility to tuberculosis disease in humans. If this were true, then there should be close linkage between the susceptibility locus and the development of clinical tuberculosis disease. Similar linkage studies in humans have investigated the genetic susceptibility to leprosy[110,111].

v) Linkage analysis

Segregation of a disease phenotype in families with multiple affected members may appear to follow a Mendelian pattern. In such cases, phenotypic segregation studies may demonstrate the genetic model which accounts for the manifestation of disease. These studies are more difficult to perform for a disease with complex etiologies, such as tuberculosis. In the case of tuberculosis, there may be

numerous loci with a minor effect, or only a few loci with major effects. In addition, non-genetic factors have been described as playing important roles in modifying the genetic susceptibility to tuberculosis disease.

Segregation analysis may elucidate the genetic model in a given family, but it does not reveal the genetic mechanism or pinpoint the exact locus responsible for the expression of disease. Identification of the locus may be achieved by detecting co-segregation of a well-defined genetic marker with the disease phenotype. Such co-segregation, termed linkage, can serve to focus attention either on the genetic marker itself or on nearby DNA sequences as the genetic basis for the expression of, or modulation of, disease. Analysis of linkage is based on the estimation of the frequency of homologous recombination between genetic loci[135]. In the usual two-locus analysis, one locus is the genetic segment which can be typed by some means in the study subjects, while the other locus represents the "disease susceptibility" locus, as measured by the manifestation of the disease phenotype. In the present study, the typable genetic locus is the HLA-A, -B, and -C segment, while the "tuberculosis susceptibility" locus is assigned by assessing the presence of disease and assigning such a phenotype to the study subjects. Statistical testing of the recombination between these loci can be done via the maximum likelihood estimation (MLE) method, in which case a

"lod" (i.e. log, base 10, of the odds ratio) score is obtained[135]. The lod score reflects the significance of the linkage detected between a polymorphic locus and a disease locus. It is calculated by dividing the probability of observing coinheritance of two loci assumed to be genetically linked by the probability of observing coinheritance of the two loci even though they are not genetically linked. The log odds of the likelihood of the data for a specified value of the recombination fraction is expressed relative to the likelihood for unlinked loci (i.e. recombination fraction of 0.5). It is customary to accept that there exists evidence for linkage (i.e. reject the null hypothesis that no linkage exists) when the lod score is +3 or larger[135]. When the lod score is -2 or less, it is customary to say that there is evidence against linkage and that the loci are unlinked.

Three important requirements for conducting a linkage analysis are 1) a genetic model, 2) information regarding the number of phenocopies of disease, and 3) knowledge of the penetrance of the susceptibility gene. The genetic model takes into account the dominance relationships of alleles at the disease susceptibility locus. Using an inappropriate model in a linkage analysis may lead to erroneous results, especially when mistaking recessive and intermediate models of dominance[136].

A phenocopy refers to the manifestation of the

"disease" phenotype in a given individual due to environmental factors, without the presence of the susceptibility gene ("sporadic case"). It is, essentially, imitation of a presumably genetic condition by an environmental cause. With respect to tuberculosis, sporadic cases might occur secondary to factors such as a large inoculum of M. tuberculosis , increased virulence of a particular strain, or immunosuppression of the host. Such sporadic cases in a linkage analysis cause errors in phenotyping, essentially a misclassification bias. If there was no differential misclassification of phenotype (i.e. individuals with and without the proposed "susceptibility" locus may develop disease due to environmental causes), then the linkage analysis would be biased towards the null, and it would be more difficult to show linkage. If, however, sporadic cases occurred with differential rates in genetically "susceptible" and "resistant" individuals, the analysis could be biased either way, depending on the pattern of bias[137]. Although the role of some non-genetic factors (i.e. inoculum size, immunosuppression) in the development of tuberculosis disease has been studied, quantification of their prevalence in a given population is extremely difficult. Studies of leprosy have indicated that low rates of sporadic cases would not seriously affect linkage detection[111].

Penetrance of the susceptibility gene is defined as the

probability, or overall frequency, of the disease phenotype expression, given the presence of the "susceptibility" genotype. Penetrance is measured by the detection of disease among individuals of known "susceptible" genotype.

The actual phenocopy rate, as well as the penetrance of the "susceptible" gene, for the development of tuberculosis or any other disease, can be related according to the following formula, adapted from Vogel and Motulsky[138]:

$$Prev(D) = [Prev(S\ gene) \times Penet] + [Prev(NGF) \times Pheno\ rate]$$

where: Prev(D) = the prevalence of disease in the population

Prev(S gene) = the prevalence of the "susceptible" genotype in the population

Penet = penetrance of the "susceptible" genotype for causing disease

Prev (NGF) = the prevalence of non-genetic factors which predispose to disease in the population

Pheno rate = the phenocopy rate in the population

Therefore, if the other variables in the equation are known, the phenocopy rate or the penetrance may be derived.

In practice, estimates of penetrance for a "disease susceptibility" gene can be derived from segregation analyses of a disease with a clear-cut pattern of inheritance. Regarding tuberculosis, no data have been presented which ascertained the segregation of disease among multiple-affected families. One study, which investigated

HLA linkage with disease, tried both recessive and dominant models of inheritance for the presumptive susceptibility gene, but neither model satisfactorily explained the distribution of disease in these families[123]. The results of this study suggest, however, that HLA-linked susceptibility to disease is unlikely to be highly penetrant[122,123]. Studies of affected monozygotic twins have shown concordance rates for disease of 70 to 80%[92,93], suggesting that, if disease expression is indeed solely genetically determined in these pairs, penetrance of the gene(s) must be high. However, in these twin studies, it remains unknown what proportion of those with disease are truly genetically "susceptible" and what proportion are phenocopies. Therefore, penetrance cannot be inferred from this data. In summary, therefore, the pattern of inheritance and penetrance of any putative susceptibility gene for tuberculosis disease remains unknown. The allele frequency of such a gene remains also uninvestigated.

The phenocopy rate due to non-genetic factors can be estimated if such factors are known and their prevalence quantifiable. For example, the rate of sporadic cases can be estimated from the excess of isolated disease cases in segregation analyses. In the case of tuberculosis, segregation analyses have not been done, and the non-genetic factors are difficult to quantitate due to their number and interactive nature. As well, some parameters such as

exposure (i.e. inoculum) are impossible to quantify. An indirect measure of the phenocopy rate for tuberculosis disease can be made by examining the rate of development of disease in a relatively "resistant" population, where genetic susceptibility is expected to be very low. In such a population, the incidence of disease following infection would give an upper limit to the expected phenocopy rate of disease. An elderly North American population, which has descended from generations of mostly European and African ancestors exposed to repeated epidemics of tuberculosis, could be expected to manifest such a low prevalence of genetic susceptibility to disease. Epidemics of tuberculosis, prior to the development of specific therapy, were associated with high rates of disease[139] and case-fatality rates among all age groups of over 50%[140]. For example, in Eskimos and Native Indians of Alaska in the 1920's, 35% of all deaths were attributable to tuberculosis disease[141]. If one assumes that disease was largely due to genetic susceptibility, then the "fitness" of such susceptible individuals would have been markedly impaired, since many deaths occured in individuals prior to, and during, their reproductive years. Therefore, it can be argued that, after repeated exposures, there has been a diminution in the number of genetically susceptible individuals, with the survivors manifesting disease largely due to non-genetic reasons. This assumes that no advantage

to survival is conferred by being genetically susceptible to tuberculosis. In one study of elderly Americans, 6% of infected individuals contracted pulmonary disease over a 3-year period (i.e. annual risk of disease of 2%)[142]. Other studies have shown annual rates ranging from 1.5% to 17%[20,51,143]. Therefore, it could be argued that an estimate of the upper limit of the annual phenocopy rate following infection lies between 1.5% and 17%.

3. STUDY OBJECTIVES

There are 2 objectives to this study: 1) evaluate the diagnostic tests which are commonly used to make the diagnosis of active pulmonary tuberculosis disease, and 2) investigate the linkage between HLA-A,B type and the expression of tuberculosis disease.

In order to achieve the first objective, data gathered from a population of Canadian Native Indians experiencing an epidemic of pulmonary tuberculosis was used in order to assess the usefulness of demographics (age, gender), chest radiography, culture of respiratory secretions, and tuberculin (Mantoux) skin testing in predicting the presence of disease in infected individuals. To this end, univariate analyses and multivariate discriminant analyses are used, taking into consideration all of the above variables. The "gold standard" by which disease was measured is a combination of clinical and laboratory criteria, a concept well-described and endorsed by the American Thoracic Society[23].

While many population studies of association have been done, there is a paucity of linkage studies between HLA type and disease in affected families. In order to achieve the second objective, a 3-generation family which experienced an outbreak of tuberculosis will be analyzed to test whether linkage exists between development of disease and the haplotypes of the HLA-A, and -B genetic loci.

4. STUDY METHODOLOGY

i) Study design

This study was undertaken in a retrospective manner, as a means of addressing both of the study objectives. Because of the relative rarity of tuberculosis in Canada at this time, a prospective study of either objective was impractical.

A multiple affected family was chosen in a non-random manner, in order to fulfill the eligibility criteria as listed below. At the time of subject enrollment, assignment of both infection and disease status (i.e. phenotype) was performed by retrospectively ascertaining the results of the diagnostic tests done. This was accomplished by reviewing all clinical and laboratory data for each study subject.

Consenting study subjects donated blood for HLA typing, which was then analyzed with the clinical disease phenotype to ascertain if genetic linkage is present.

ii) Family eligibility and enrollment

The goal of studying the usefulness of diagnostic tests for tuberculosis required a study group with a high prevalence of tuberculosis, for whom accurate and systematic clinical and laboratory data had been accumulated. In addition, linkage analysis necessitated finding a family in which disease was likely to be present as a consequence of

genetic factors. In order to fulfill the criteria for these two studies, one large family which had undergone a documented epidemic of tuberculosis was sought.

The eligibility criteria for an analysis of diagnostic tests, and the reasons for choosing them, are listed below. Because the eligibility criteria were most restrictive for finding a family for the linkage analysis, no calculations were done to estimate the needed study sample size for the analysis of diagnostic tests. It was decided to use the largest eligible family(ies) for the two analyses. As well, criteria 2 and 4 made it evident that a family who suffered an extensive and localized epidemic of tuberculosis would best meet these requirements. The eligibility criteria for subjects for the analysis of diagnostic tests used for determining tuberculosis disease were as follows:

Eligibility Criterion	Reasoning
1. Give informed consent	Ethical and legal requirements.
2. Large number of infected and diseased individuals.	Assure reasonable power and narrow confidence intervals for the results of statistical tests of association between the diagnostic tests and outcomes.

Eligibility Criterion (cont'd)	Reasoning (cont'd)
3. No history of BCG vaccination in the study population.	Previous BCG vaccination in some or all of the study subjects would interfere with the interpretation of the skin test, and render it impossible to determine the infection status of each individual.
4. Uniform access to medical care and diagnostic services among the study group, via a single health-care team and laboratory.	Non-uniform access to, application of, or use of a diagnostic procedure would not allow assessment of its usefulness, because there may be ascertainment bias in the detection of infection and disease.
5. Detailed clinical and laboratory data documentation.	Necessary to accurately assess the results of tests, and to determine the infection and disease status of each study subject.

Eligibility criteria for a linkage analysis imposed further constraints. These criteria, and the reasoning for these choices, are listed below.

Criterion	Reasoning
1. Detailed clinical and laboratory data documentation.	As above
2. No history of BCG vaccination in the study population.	Any effect of BCG vaccination in preventing the development of disease would decrease the power of finding a significant linkage by decreasing the number of infected individuals who go on to develop disease. In addition, if BCG vaccination was not uniform in the family, BCG-associated prevention of disease would be variable among study subjects, leading to differential disease misclassification bias.

Eligibility Criterion (cont'd)	Reasoning (cont'd)
3. Sufficient time (i.e. greater than 3 months) between exposure to M. tuberculosis in the family and intervention with chemoprophylaxis of asymptomatic infected contacts.	If exposure was followed quickly by medical intervention, then insufficient time would have elapsed to allow development of disease in "susceptible" individuals. Rapid chemoprophylaxis of asymptomatic infected individuals would prevent disease development and lead to erroneous assignment of a "resistant" phenotype to a "susceptible" person (i.e. misclassification bias) and lead to a decrease in the number of "susceptibles" developing disease (i.e. loss of power to detect linkage).
4. At least 2 generations of family members able to contribute blood samples for HLA typing.	Needed for the power to obtain significant evidence for or against linkage.

Due to the increased incidence of tuberculosis, including occasional epidemics, in the aboriginal people of Canada, letters were sent to the agencies responsible for tuberculosis control in order to identify a community or communities with either high endemic incidence rates or a recent epidemic of new tuberculosis cases. The Alberta Health Tuberculosis Services in Edmonton indicated that they knew of a potential study group consisting of a family of Canadian Native Indians. This family fulfilled all the above criteria, lived in a single community, had demonstrated a history of cooperation with health practitioners, and had experienced an epidemic of tuberculosis 2½ years earlier. The medical records of this family were available at the office of the Alberta Health Tuberculosis Services.

A letter was sent to all family members aged 18 or older, explaining the study and inviting them to participate (Appendix A). Informed consent was obtained from each consenting adult member of the family for the chart review as well as the necessary phlebotomy and the subsequent HLA analysis and typing (Appendix D). All family members consented, except for 6 individuals who refused the phlebotomy. Informed consent was obtained from a parent or guardian for participation of a family member under the age of 18.

iii) Mycobacterial cultures

All family members submitted an appropriate upper respiratory tract secretion for mycobacterial staining and culture. In children, this was accomplished by doing a nasopharyngeal suction or gastric lavage. All specimens (sputum, gastric lavage, and nasopharyngeal suction in children) for mycobacterial culture were collected either in the community by an on-site public health nurse, or at the Alberta Health TB Services inpatient facility in Edmonton when individuals were hospitalized. All specimens were sent to the provincial public health laboratory in Edmonton. The usual delay from collection to processing was 1 to 3 days for specimens arriving from the community, and 1 day when arriving from the inpatient facility.

At the provincial laboratory, specimens were decontaminated and inoculated onto both solid media and into the BACTEC^{R} (Becton Dickinson, Towson, Maryland) radiometric broth system[144], cultures being kept for a maximum of 6 weeks and handled in the standard manner[145]. Positive mycobacterial cultures were noted by evidence of visible growth on the solid media or a growth index (GI) over 99 in the radiometric system. Mycobacteria were identified as to species by conventional methods, including detailed biochemical reactions[146]. In addition, each specimen was stained for microscopy upon receipt. Microscopic screening

was done with the auramine-O fluorescent method[147], followed by confirmatory Kinyoun staining[148].

iv) Administration of the tuberculin skin test (Mantoux)

All family members, except those with a history of old tuberculosis disease, underwent Mantoux skin testing. Administration of the test was done in the community by an on-site nurse who performed both the intracutaneous injection and the subsequent interpretation of the test. Tests were administered and read in the usual manner[59]. Briefly, 0.1 ml (5 TU) of tuberculin PPD (Tubersol^{R}, Connaught Laboratories Ltd.) was administered intracutaneously on the volar surface of either forearm. The reaction was measured after 48 or 72 hours, by measuring the size of the induration only (in mm) in the transverse plane. Erythema without induration was ignored.

v) Clinical data acquisition

All records pertaining to the outbreak were kept in the office of the Alberta Health Tuberculosis Services in Edmonton, Alberta. All family members had been seen during the outbreak by the same physician and standard forms were completed (Appendices C and D). Review of the charts and radiographs was performed using a questionnaire designed for this project (Appendix E). The variables documented

included: full name, date of birth, gender, result of tuberculin skin testing (mm of induration; date), mycobacterial culture results (date; site), history of previous tuberculosis disease and date(s), past exposure to BCG vaccination, and any anti-tuberculous drug therapy. Chest radiographs for each patient were reviewed without knowledge of the radiologist's report. Thereafter, the radiologist's report was also recorded on the questionnaire sheet. Differences between these two evaluations were resolved by conferring with a clinical tuberculosis specialist. The paternity of each member of the family was verified by consulting with one member of the family who claimed to have excellent knowledge of the family's ancestry.

vi) Clinical phenotyping of study subjects

According to the data acquired on the questionnaire, each individual was classified according to both his **infection** phenotype ("infected" or "uninfected"), and **disease** phenotype ("case", "culture-negative case", "old case", or "non-case"). These terms are consistent with accepted criteria[23], and defined for this study as follows:

Infected: Documentation of a reaction of ≥ 5 mm to a 5 TU tuberculin skin test OR current or remote tuberculosis disease (as defined below).

Uninfected: Individuals not meeting criteria for "infected" above.

Disease (case): Presence of one or more symptoms or signs associated with tuberculosis (i.e. fever, cough, weight loss, pulmonary infiltrate not responsive to conventional antibiotics, night sweats) during the period of the epidemic AND isolation of M. tuberculosis from respiratory specimen.

Disease (culture-negative case): Presence of one or more symptoms or signs associated with tuberculosis (i.e. fever, cough, weight loss, pulmonary infiltrate not responsive to conventional antibiotics, night sweats) during the period of the epidemic AND clinical response to antituberculous drugs AND a negative culture of respiratory secretions.

Disease (old case): Tuberculosis disease diagnosed by the appearance of appropriate signs or symptoms (as above), not necessarily accompanied by a positive culture for M. tuberculosis, OR disease responding to antituberculous therapy, PRIOR to the epidemic in question.

No disease: Absence of disease, as defined above.

Therefore, for the purposes of the phenotypic classification, individuals were considered to have been **infected** by M. tuberculosis if they fulfilled the "infected" criteria, and were considered to have had tuberculosis **disease** if they were classified as a "case", a "culture-negative case", or an "old case".

The period of the epidemic was defined as the time interval between the diagnosis of the first case (June 13, 1987) and the last new infection (June 22, 1989) prior to the data collection.

vii) HLA-A, -B, and -C typing

Seven to ten milliliters of heparinized blood was obtained from each consenting family member. Within 12 hours, the plasma and cellular fractions were separated by centrifugation, and then the lymphocyte fraction was isolated on Ficoll^{R} [149]. A sufficient quantity of lymphocytes was set aside for HLA typing. This portion of each study participants' lymphocytes was used for HLA-A, -B, and -C typing, using the microcytotoxicity method[15]. The rest of the lymphocytes were infected with a strain of Epstein-Barr virus to effect "immortalization"[150]. This produced a continuous lymphocyte cell line for each individual, thereby providing a continuous supply of DNA from each study subject. For logistic reasons, HLA-D typing was not performed on the blood samples of study

participants, but evidence suggests that linkage equilibrium between HLA-A,B,C and HLA-DQ,DR is rare in humans[103,151]. Therefore, if close linkage between a given HLA-D type and disease exists, it should also theoretically be detected between the associated HLA-A, -B, and -C types. For instance, the recombination frequency between HLA-A and HLA-C has been calculated at 0.8%; between HLA-C and HLA-B at 0.2%; and between HLA-B and HLA-D at 0.8%[151]. Moreover, no significant difference was found between males and females in the recombination frequency of the known loci on the short arm of chromosome 6, including the HLA loci[152].

viii) Data manipulation and analysis

All data were entered using a computer software system (DBASE III PLUS^R, version 1.1, Ashton-Tate, Torrance, California), from which they were selected or transformed for analysis. Graphics were performed using Harvard Graphics^R, version 2.1 (Software Publishing Corporation, Madison, Wisconsin) for the IBM PC^R (International Business Machines Corporation). Descriptive statistics and statistical analyses were performed using the True Epistat^R software package, third edition (Epistat Services, Richardson, Texas).

For the univariate analyses of the association between demographic data and diagnostic tests with the presence of disease, the following independent variables were

considered: age (continuous), gender (dichotomous: male or female), DTH skin test (continuous: millimeters of induration), culture of respiratory secretions for M. tuberculosis (dichotomous: positive or negative), and chest radiography (dichotomous: normal or presence of abnormalities consistent with active disease). The radiologic abnormalities judged to be consistent with disease included non-calcified hilar or paratracheal adenopathy, and/or parenchymal infiltration[23]. The dependent outcome variables, tuberculosis infection and disease, were considered as dichotomous: present or not present (as described on page 48 under "vi) clinical phenotyping of study subjects"). Sensitivity, specificity, positive and negative predictive values were calculated by means of the standard equations[117,153].

Comparison of continuous variables between groups was done using the Student's t-test of means[96], unless otherwise indicated. The rank sum test[96] was used when assumptions of normality of the variable were not made. Categorical variables were compared using a chi-square test[96], or the Fisher's exact test in the case of small numbers[154]. The multivariate analysis of the diagnostic tests was performed using discriminant analysis[155]. Stepwise regression of the model was done with inclusion of all variables significant at a P value less than 0.05 . The sensitivity and specificity of the discriminant analysis model in detecting

tuberculosis disease was calculated by choosing a discriminant score half-way between the values of the equation score for "diseased" and "non-diseased" individuals.

Comparison of receiver operating characteristics curves was done by comparing their areas under the curve[156]. Standard deviations for each of the points on the curve were calculated by means of the binomial distribution[117].

The P values given are always for two-tailed comparisons. A P value of 0.05 or less was interpreted as being statistically "significant". In analyses where P values of greater than 0.05 were obtained, results are labelled "not statistically significant".

The drawing and manipulation of the pedigree data were performed using PEDPACK^R, version 2.2[157,158], and "Software for calculating gene survival and multigene descent probabilities and for pedigree manipulatior and drawing"[159]. Pedpack^R is a package of programs for pedigree analysis which uses the UNIX operating system.

The linkage analysis was performed using the likelihood method[160]. Maximum-likelihood estimates of the lod score were computed using the LINKAGE^R package of programs, version 4.8[161-163]. Each individual was categorized according to two loci for his/her HLA-A,B haplotype. The HLA-A,B haplotype was coded as a single (i.e. "virtual") locus. Assumptions for the genetic model, phenocopy rate,

genotype-specific liability for disease, haplotype frequencies, penetrance, and allele frequency were derived from the medical literature and described for each analysis in the corresponding "results" section of the paper. For these analyses, the null hypothesis (i.e. no linkage present) was rejected if the derived lod score was +3 or greater[135]. Linkage was considered excluded up to a recombination rate (theta) if the lod score at that rate was -2 or less[135].

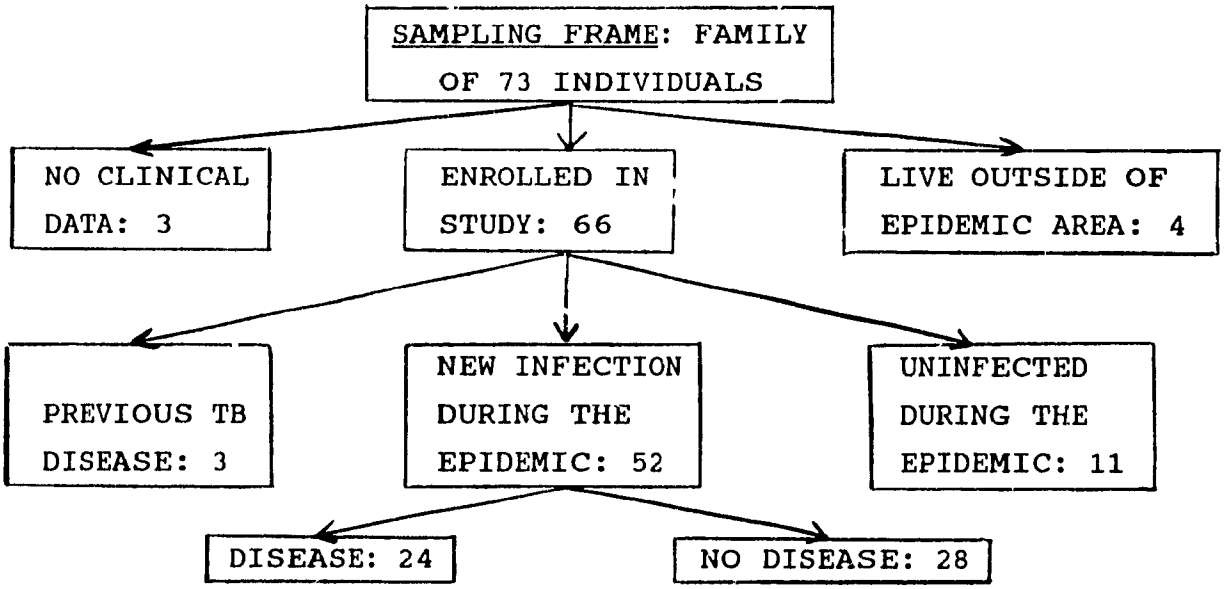
5. RESULTS

i) Descriptive epidemiology of the tuberculosis epidemic

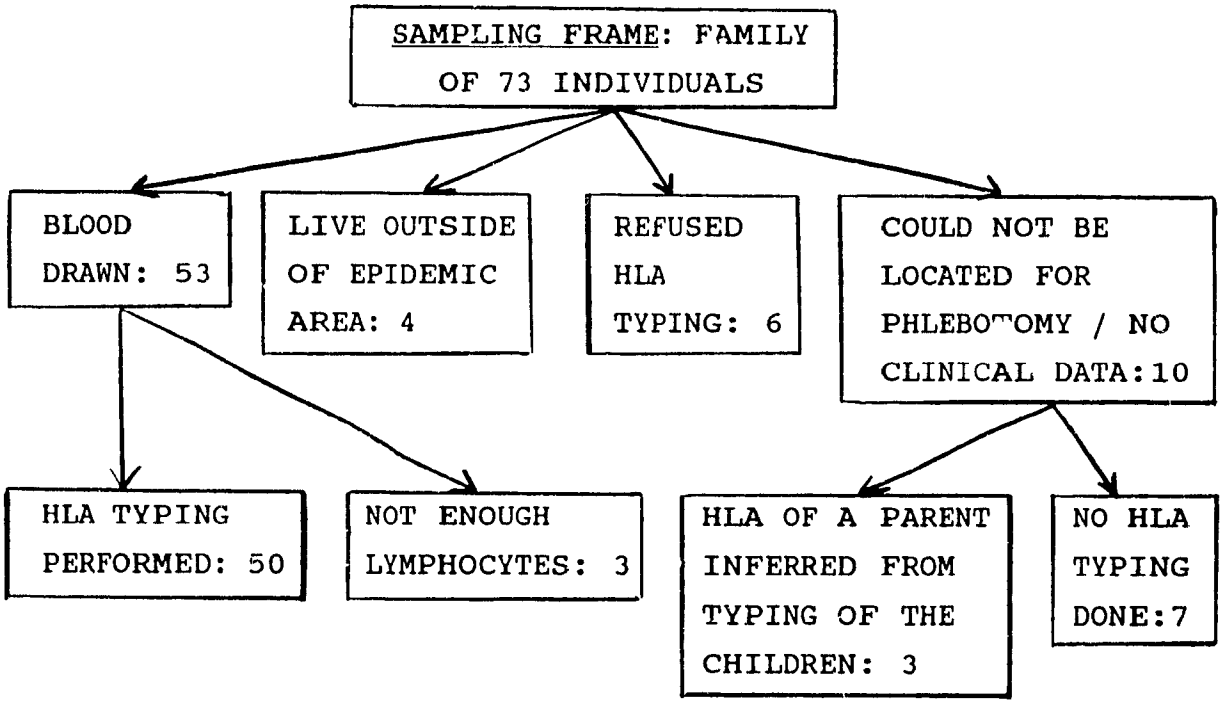
The family chosen for the study contained 73 persons (figure 2), 6 of whom did not consent to give blood samples but consented to a review of their clinical data, 4 who were living elsewhere prior to and during the tuberculosis epidemic period, and 3 for whom no clinical data could be found. Of the 73, HLA typing was not performed for the 6 individuals refusing consent, 10 individuals not available for phlebotomy, and 3 individuals from whom an insufficient number of lymphocytes was recovered. The status of study subjects after enrollment is shown in figure 2. The family lives in a rural community, situated approximately 500 km north of Edmonton, Alberta, and whose total population was 327 persons. Detailed descriptive data on the entire community are unknown, because of ongoing land claims with the government and the inability to conduct a census. The family belongs to a Native Indian band comprising 226 of the 327 individuals in the community. Routine tuberculin skin testing of school children in the band was abandoned in 1985 because of the very low numbers of new skin reactors, and skin testing of adults was stopped in 1981. There had been only one case of tuberculosis disease in this community since 1969, a non-infectious case of osteomyelitis which most likely represented reactivation of an old quiescent

Figure 2. Status of study subjects after enrollment, for each of the two objectives of the study.

a) EVALUATION OF DIAGNOSTIC TESTS



b) HLA LINKAGE ANALYSIS



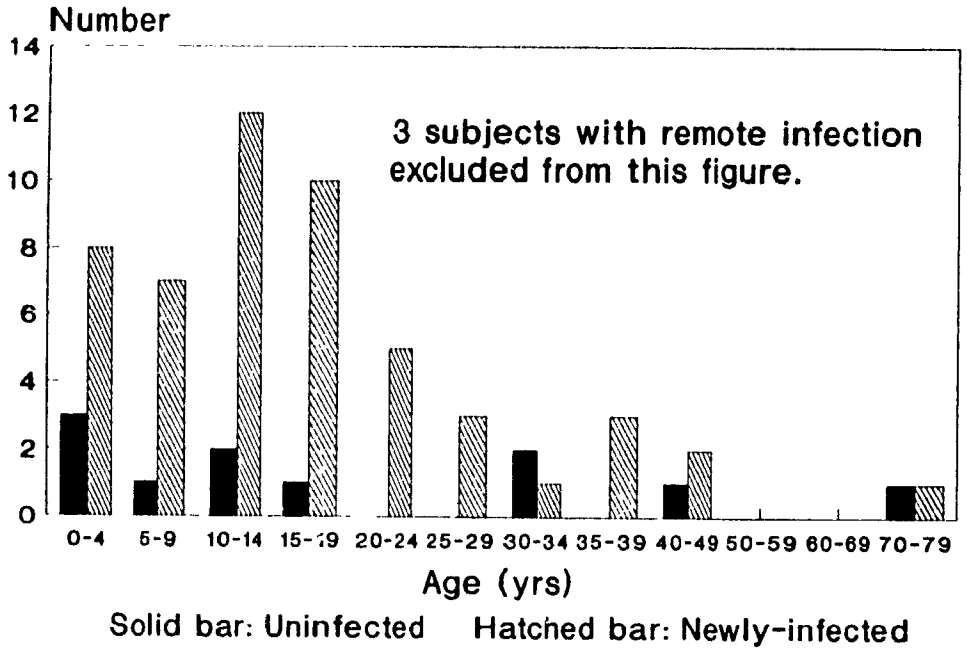
focus of infection. In addition, because of this low incidence of disease, BCG vaccination had not been performed since 1963, and had been non-uniform prior to that time.

The index case first detected at the start of the epidemic period was producing AFB-smear-positive sputum from cavitary pulmonary tuberculosis in August 1987. The 66 evaluated members of the family who were present in the area during the epidemic period (June 13, 1987 to June 22, 1989) consisted of 37 males (56%) and 29 females. Their ages at the midpoint of the epidemic period (June 18, 1988) ranged from three months to 70 years. The three-month old child was born during the epidemic period and was the last to be infected (i.e. he developed a positive tuberculin skin test on June 22, 1989). The mean age of the family members at the epidemic midpoint was 17.5 years, with an age distribution as shown in figure 3.

During the 24-month epidemic period, 52 of the 66 family members became newly infected (presence of a new positive tuberculin skin test). All infected individuals without evidence of disease received isoniazid prophylaxis. Three individuals had had tuberculosis disease in the past, and re-infection during the epidemic was impossible to assess. The remaining 11 had repeatedly negative skin tests and no clinical evidence of disease. Of the 52 newly infected individuals, 24 developed tuberculosis disease during the epidemic period (i.e. "case" or "culture-negative case").

Figure 3.

Age distribution of study subjects, by infection status.



The demographics, method of diagnosis, and phenotype for all subjects with tuberculosis disease are summarized in table 3.

There was no significant difference between the 52 infected and the 11 uninfected individuals with respect to age (mean age 16.6 years vs. 21.6 years, respectively) - figure 3. There was also no difference between the ages of the 24 diseased and 28 non-diseased individuals who had been newly-infected during the epidemic (mean age 15.0 years vs. 17.9 years, respectively) - figure 4. However, a grouped analysis (table 4) showed a significant difference in the incidence of disease among those newly-infected who were 2 years of age or younger, compared with adults 26-60 years of age ($P = 0.05$). As well, as can be seen from table 4, the incidence of disease among all age groups was significantly higher than the published risk of disease following infection (chi-square = 159.5; $P << 0.001$).

No significant difference existed between the proportion of diseased individuals among infected males when compared to infected females (15/31 versus 9/21).

ii) Analysis of diagnostic tests

Results of tuberculin skin testing done during the epidemic are shown in figure 5. The three individuals who had remote tuberculosis were removed from this analysis

Table 3. Demographics, method of diagnosis, and phenotype of study subjects with tuberculosis disease.

Patient number	Age/ sex	Chest X-ray	TB culture	Date of diagnosis	Disease phenotype
1	70/M	A	+	89/05/09	CASE
4	17/M	A	+	87/08/20	CASE
5	22/M	A	+	87/08/27	CASE
6	25/M	A	+	87/08/27	CASE
8	17/M	A	+	87/08/27	CASE
12	21/F	A	+	87/08/21	CASE
16	16/F	N	+	87/08/26	CASE
17	3/M	A	-	87/09/01	CULT-NEGATIVE CASE
20	26/F	A	+	87/08/05	CASE
21	1/M	A	-	87/06/17	CULT-NEGATIVE CASE
25	9/F	A	-	87/08/20	CULT-NEGATIVE CASE
29	11/M	A	+	87/09/02	CASE
31	9/M	A	+	87/08/26	CASE
34	8/F	A	-	87/08/20	CULT-NEGATIVE CASE
36	19/F	A	+	87/08/27	CASE
40	3/M	A	+	87/08/24	CASE
43	2/M	A	-	87/11/06	CULT-NEGATIVE CASE
44	24/M	A	+	87/08/20	CASE
48	5/F	A	-	87/08/21	CULT-NEGATIVE CASE

Table 3 (cont'd). Demographics, method of diagnosis, and phenotype of study subjects with tuberculosis disease.

Patient number	Age/ sex	Chest X-ray	TB culture	Date of diagnosis	Disease phenotype
50	40/M	N	-	1953	OLD CASE
51	39/M	N	-	1952	OLD CASE
53	18/F	N	+	87/08/27	CASE
57	11/M	A	+	87/08/20	CASE
58	8/F	A	+	87/08/26	CASE
59	2/M	A	-	87/08/25	CULT-NEGATIVE CASE
62	20/M	A	+	87/08/26	CASE
65	31/M	N	-	1958	OLD CASE

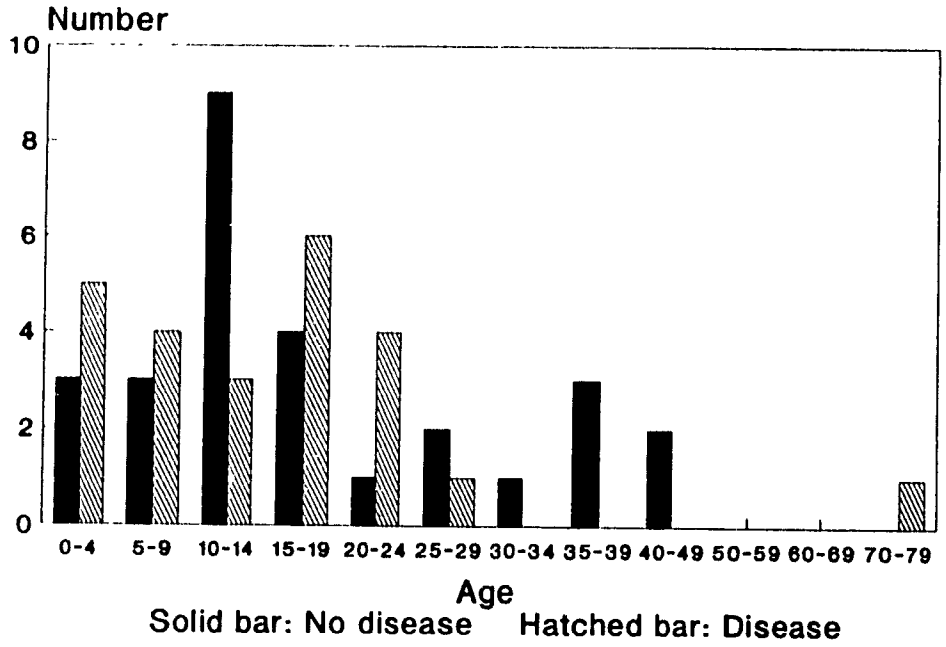
Disease: case, old case, or culture-negative case.

A: abnormal CULT: culture F: female M: male

N: normal F: female +: positive -: negative

Figure 4.

Age distribution of newly-infected subjects, by disease status.



Solid bar: No disease Hatched bar: Disease

Table 4. Incidence rate of tuberculosis disease, by age group, and the relative risk of disease among study subjects and as derived from the published data.[9,26,33,34]

	This study				Published data	
Age (yrs)	Newly-Infected (number)	Diseased (number)	Disease incidence[+]	RR	Disease incidence[+]	RR
2	4	3	75%*	6.8	50%	12.5
3-13	19	8	42%	3.8	4%	1.0
14-25	19	11	58%	5.3	9%	2.2
26-60	9	1	11%	1.0	4%	1.0
60	1	1	100%	9.0	8%	2.0

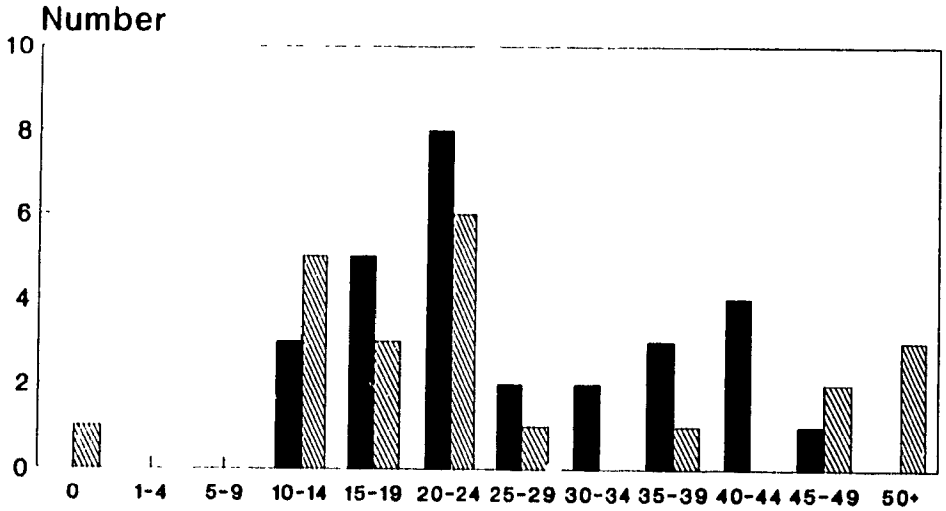
The incidence of disease among study subjects is assumed to be, on average, a one-year incidence rate. The reference group (relative risk of 1.0) is the group aged 26 to 60 years. RR: relative risk.

* Significant difference in the incidence of disease between this group and those 26-60 years of age ($P = 0.05$).

[+] Significant difference in the age group-specific incidence of disease between this study and the published data ($P << 0.001$).

Figure 5.

Distribution of tuberculin skin test results, among newly-infected study subjects, by disease status.



Solid bar: No disease Hatched bar: Disease

because performing skin tests during the epidemic was not indicated nor done in these family members due to their history of disease. Only one individual with tuberculosis disease had a negative (0 mm) tuberculin skin test: a 3 year old boy with a right upper lobe pneumonia, and a nasopharyngeal suction culture negative for M. tuberculosis. The pneumonia subsequently cleared on antituberculous therapy, and he was classified as a culture-negative case.

The size of skin induration was compared between the 28 non-diseased and the 24 diseased individuals who were infected during the epidemic. Two individuals with disease did not have the actual size of skin induration noted. Therefore, the comparison was performed by omitting these two individuals. Assignment of disease status was done according to the criteria in chapter 4.vi (page 48). There was no statistically significant difference in the average size of skin induration between those diseased and non-diseased (mean 27.6 mm vs. 25.5 mm, respectively). Using various cutoffs of the tuberculin skin test result as the threshold for predicting disease, the sensitivity, specificity, and predictive values vary as seen in table 5. For instance, using a threshold of 20 mm induration, the test has a sensitivity of 59% and a specificity of 29% in detecting disease, and positive and negative predictive values of 40% and 47%, respectively, in this population. The receiver

Table 5. Sensitivity, specificity, and predictive values of the tuberculin skin test (Mantoux) for detecting disease in study subjects. Sens:sensitivity Spec:specificity PPV:positive predictive value NPV:negative predictive value

Positive reaction (mm induration)	Sens(%)	Spec(%)	PPV*(%)	NPV*(%)
≥ 5	95	0	43	0
≥ 10	95	0	43	0
≥ 15	73	11	39	34
≥ 20	59	29	40	47
≥ 25	32	57	37	52
≥ 30	27	64	37	53
≥ 35	27	71	42	55
≥ 40	23	82	50	58
≥ 45	23	96	82	61
≥ 50	14	100	100	60
≥ 55	14	100	100	60
≥ 60	14	100	100	60
≥ 65	9	100	100	58
≥ 70	5	100	100	57
≥ 75	5	100	100	57
≥ 80	5	100	100	57
≥ 85	5	100	100	57

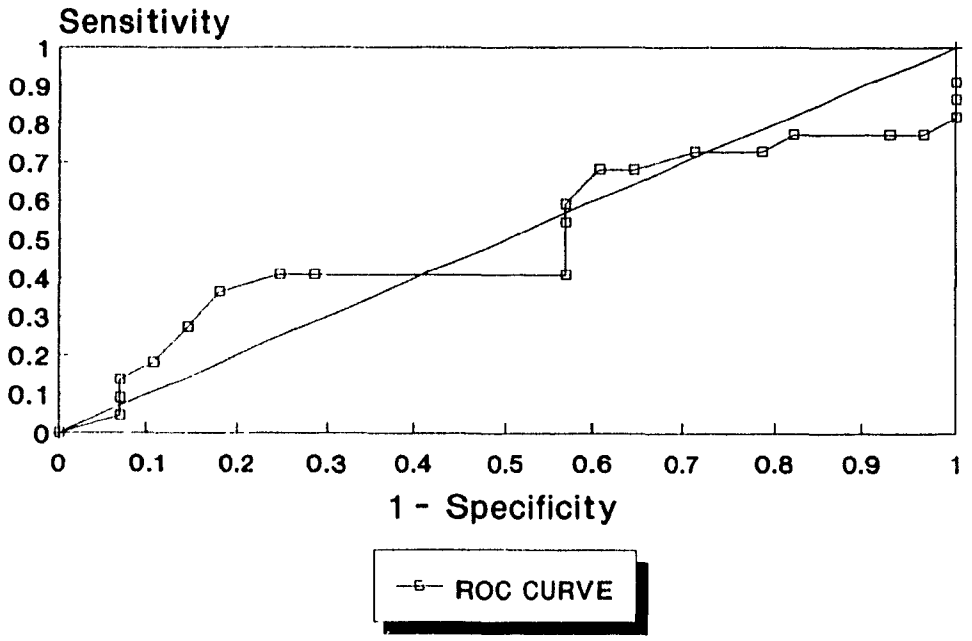
* Prevalence of disease is 44% (22 individuals of the 50 who underwent skin testing).

operating characteristics (ROC) curve of this test is depicted in figure 6. The "Y=X" line, which characterizes a test for which the post-test probability of disease equals the pre-test probability, is superimposed in figure 6. The ROC curve for tuberculin testing is not statistically different from the Y=X line. This implies that the skin test, considered alone, contributes no additional information concerning disease status beyond what was known before administering the test. Although a skin reaction of 50 mm or greater was present only in those with disease, the number of such individuals (3) is too small to allow a conclusion to be made regarding the associated risk of disease.

A univariate analysis was also carried out for the association of chest radiography and the presence of disease. An abnormal chest radiograph was highly correlated with the presence of pulmonary tuberculosis disease. Twenty-two of the 24 newly-infected individuals with disease and none of the 28 non-diseased infected individuals had an abnormal chest radiograph ($P << 0.001$). The sensitivity and specificity of the chest radiograph alone for detecting pulmonary tuberculosis disease was 92% and 100%, respectively.

In the group of 24 individuals with disease, 17 produced specimens from which M. tuberculosis was isolated. There was a highly significant difference in the ages of the culture-positive and culture-negative individuals (rank sum

Figure 6. Receiver operating characteristics curve for the tuberculin skin test (Mantoux) in diagnosing active tuberculosis disease. The "Y=X" line, which characterizes a test for which the post-test probability of disease equals the pre-test probability, is superimposed for ease of comparison.

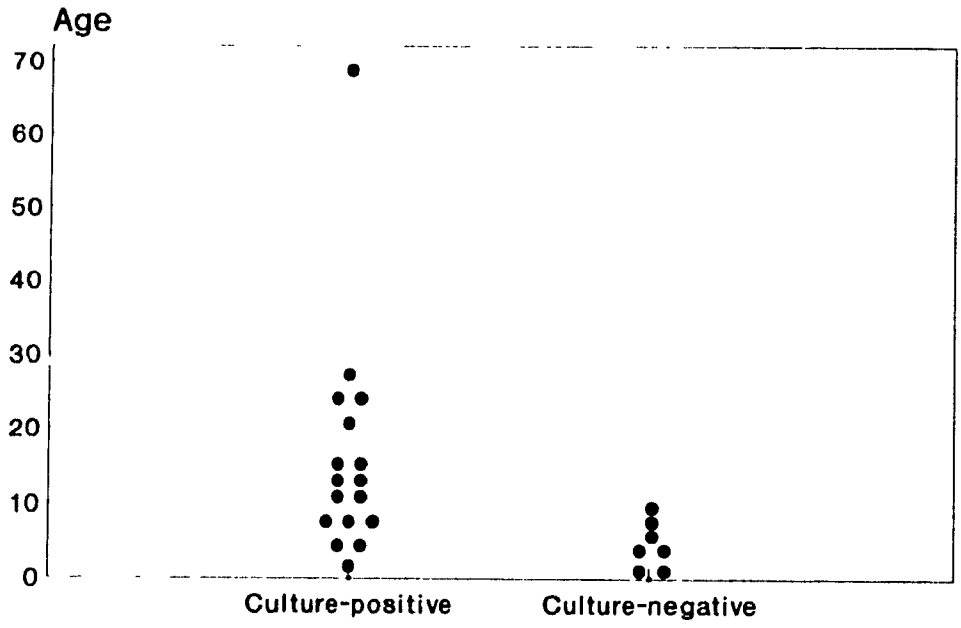


test: P = 0.001). The mean age of the 17 culture-positive individuals was 18.7 years, compared with 5.1 years for the 7 individuals in the culture-negative group (rank sum test: P = 0.001). The scattergram of ages is shown in figure 7. There was no significant difference, however, in the proportion of culture-positive individuals among the diseased males when compared to that among diseased females (11/15 versus 6/9, respectively). The probability of obtaining a positive mycobacterial culture from a study patient with disease was 100% (11/11) for those over the age of 12 years, but only 46% (6/13) in children 12 years or younger (P = 0.006). The overall sensitivity of mycobacterial cultures for detecting disease was 71%. The fluorescent smear with Kinyoun (acid-fast stain) confirmation was positive in 8 individuals. This represents 47% of all the culture-positive individuals, and 33% of those with disease.

The multivariate discriminant analysis considered the following independent variables: age, gender, DTH skin test, and chest radiography. The mycobacterial culture was not considered in the equation, since results of these tests are rarely available in less than 3 weeks' time, and therefore cannot contribute to the **initial** diagnosis of individuals with suspected disease. The dependent variable was the presence of disease. All newly-infected individuals were included in the analysis. The regression was predominantly driven by the results of the chest radiography, accounting

Figure 7.

Age distribution of individuals with new tuberculosis disease, by culture status.



for 78% of the variation in the regression ($P << 0.001$). This is consistent with the high correlation between the results of radiography and the presence of disease seen in the univariate analysis. Gender accounted for only an additional 1% variation in the regression, and was not statistically significant ($P = 0.063$ for gender). Age and PPD skin testing did not contribute significantly to the regression. Therefore, none of the additional recorded variables contributed significantly to the detection of disease, once the chest radiograph was evaluated.

A discriminant analysis was repeated without consideration of the results of chest radiography. This would be akin to the situation where demographic data are recorded and tuberculin skin testing is performed prior to, or without, chest radiography. This analysis showed that age and skin test results were significantly associated with disease, albeit accounting for only 17% of the variation in the regression (model $P = 0.008$; table 6). Age was inversely related to disease risk. Skin test results did not contribute much information ($P = 0.051$), but were kept in the model. This model has a sensitivity of 74% for detecting disease, and is capable of correctly classifying 69% of individuals in the study as to disease status.

Table 6. Results of the discriminant analysis of diagnostic tests and demographic variables (excluding chest radiography) for detecting pulmonary tuberculosis disease, among newly-infected study subjects.

Variable*	Coefficient	95% CI of coefficient	Significance
Age	-0.089	-0.149, -0.029	P = 0.015
Skin test	0.042	-0.00018, 0.085	P = 0.051
Constant	0.256		

Model P = 0.008

Model r^2 = 0.19

Model: 0.256 - 0.089 (age) + 0.042 (skin test)

With model threshold -0.256 (i.e. individual with a score $\geq$ -0.256 designated as "diseased"):

sensitivity = 74%

specificity = 64%

positive predictive value = 65%

negative predictive value = 73%

* Only independent variables having P < 0.10 are shown.

iii) HLA-A, -B, and -C typing and analysis of linkage with tuberculosis disease.

The pedigree of 56 individuals living in the epidemic area is depicted in figure 8. This pedigree includes 53 persons from whom blood samples were obtained, 3 individuals for whom HLA typing was inferred (based on typing of their children), and an additional 5 individuals (numbered 95 to 99) who were included in the pedigree for specification of biological kinship as required by the analysis programs. The latter five are unknown individuals who were not examined. Three of the 53 individuals who contributed blood could not be HLA typed, due to the paucity of lymphocytes obtained. For the 3 persons with inferred typing, the phase of the HLA-A, -B, -C alleles was assumed. Therefore, a total of fifty-three of the 66 evaluable family members were able to be typed as to HLA status. Their HLA types, phenotype and identification number are shown in table 7. The haplotype frequencies for both the entire family and just the family "founders" (i.e. unrelated parents) are shown in table 8. Paternity, as determined by HLA typing, was as indicated by the contacted family member, except for one individual. This individual was omitted from the linkage analysis.

The linkage analysis was done several different ways, with changes in many of the parameters within limits which were derived from the medical literature. In none of these models could the null hypothesis be rejected. In the

Figure 8. Pedigree of 56 subjects living in the epidemic area, for whom HLA type or clinical information is available. An additional 5 status-unknown parents (numbered 95 to 99) were included in the pedigree for specification of biological kinship as required for pedigree analysis.

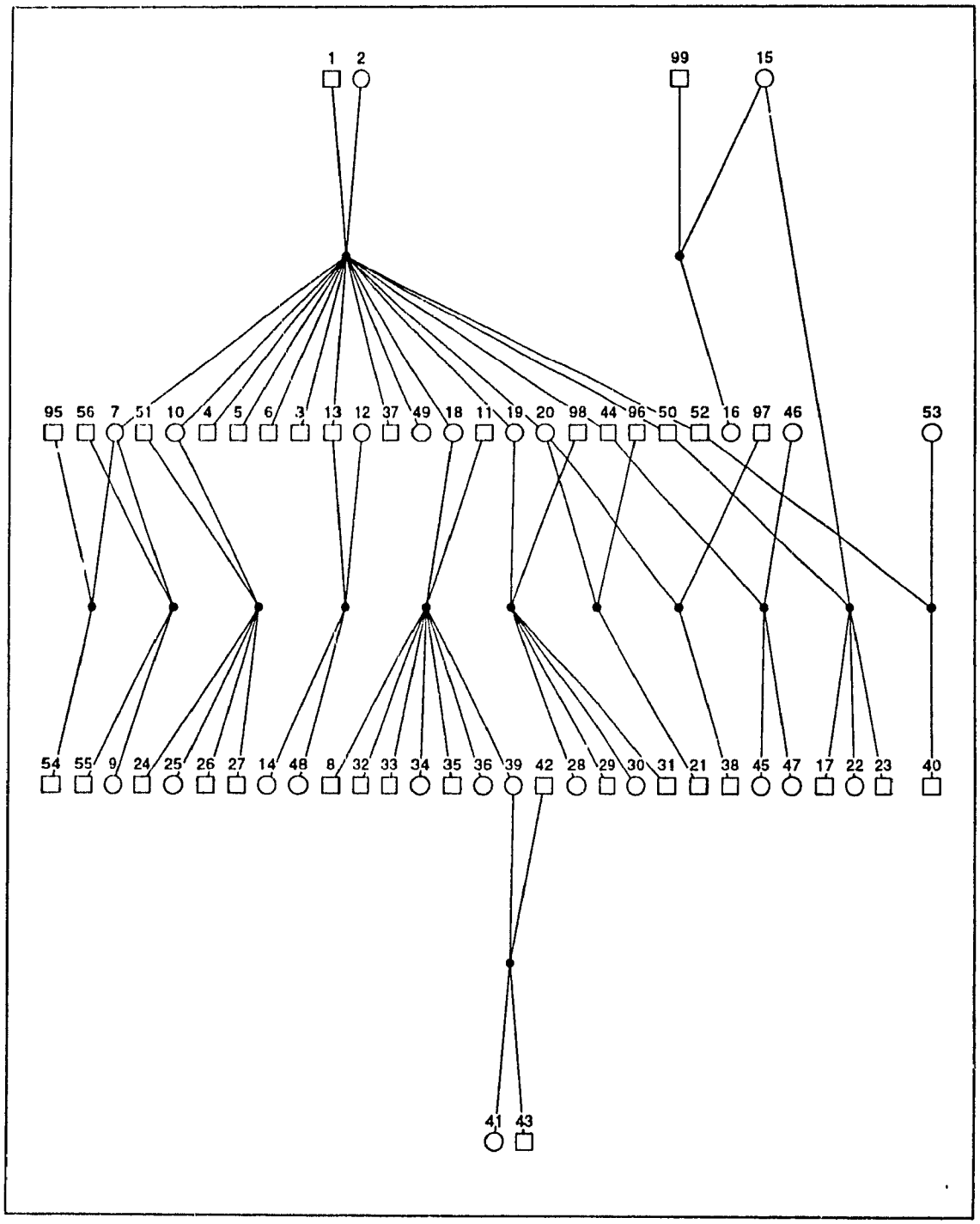


Table 7. HLA-A, -B, and -C typing and clinical phenotyping of study subjects.

Study no.	Phenotype	A1	B1	C1	A2	B2	C2
1	CASE	29	44	-	2	48	8
2	NON-CASE	2	44	5	2	62	1
3	NON-CASE	2	44	5	2	48	8
4	CASE	2	44	5	2	48	8
5	CASE	2	44	5	2	48	-
6	CASE	2	44	5	29	44	-
7	NON-CASE	2	62	1	29	44	-
8	CASE	2	48	8	2	62	1
9	NON-CASE	2	62	1	2	41	7
10	NON-CASE	2	44	5	29	44	-
11	NON-CASE	2	62	1	2	35	4
12	CASE	2	27	2	2	27	4
13	NON-CASE	2	44	5	2	48	8
14	NON-CASE	2	44	5	2	27	2
15	NON-CASE	24	62	1	68	51	4
16	CASE	2	62	1	24	62	1
17	CULTURE-NEGATIVE CASE	29	44	-	68	51	4
18	NON-CASE	2	44	5	2	48	8
19	NON-CASE	2	62	1	29	44	-
20	CASE	2	44	5	29	44	-
21	CULTURE-NEGATIVE CASE		N/A			N/A	
22	NON-CASE	29	44	-	68	51	4
23	NON-CASE	2	62	1	24	62	1
24	NON-CASE	29	44	-	2	35	4
25	CULTURE-NEGATIVE CASE	2	44	5	2	35	4
26	NON-CASE	2	44	5	2	35	4
27	NON-CASE	29	44	-	2	35	4
28	NON-CASE	29	44	-	29	44	-
29	CASE	29	44	-	29	44	-

Table 7 (cont'd). HLA-A, -B, and -C typing and phenotyping of study subjects.

Study no.	Phenotype	A1	B1	C1	A2	B2	C2
30	NON-CASE	29	44	-	2	35	4
31	CASE	2	62	1	2	35	4
32	NON-CASE	2	48	8	2	62	1
33	NON-CASE	2	48	8	2	62	1
34	CULTURE-NEGATIVE CASE	2	48	8	2	35	4
35	NON-CASE	2	48	8	2	62	1
36	CASE	2	48	8	2	62	1
37	NON-CASE	2	62	1	2	48	8
38	NON-CASE	2	44	5	2	44	5
39	NON-CASE	2	48	8	2	35	4
40	CASE		N/A			N/A	
41	NON-CASE		N/A			N/A	
42	NON-CASE	2	35	4	2	35	4
43	CULTURE-NEGATIVE CASE	2	35	4	2	48	8
44	CASE	2	44	5	29	44	-
45	NON-CASE	2	44	5	24	8	7
46	NON-CASE	24	8	7	24	62	1
47	NON-CASE	2	44	5	24	62	1
48	CULTURE-NEGATIVE CASE	2	44	5	2	27	2
49	NON-CASE	2	62	1	29	44	-
50	OLD CASE	2	62	1	29	44	0
51	OLD CASE	2	35	4	2	48	3
52	NON-CASE	2	44	5	2	48	8
53	CASE	2	44	5	3	18	7
54	NON-CASE	2	62	1	32	44	5
55	NON-CASE	2	62	1	2	58	-
56	NON-CASE	2	58	-	2	41	7
57	CASE		N/A			N/A	
58	CASE		N/A			N/A	

Table 7 (cont'd). HLA-A, -B, and -C typing and phenotyping of study subjects.

Study no.	Phenotype	A1	B1	C1	A2	B2	C2
59	CULTURE-NEGATIVE CASE		N/A			N/A	
60	NON-CASE		N/A			N/A	
61	NON-CASE		N/A			N/A	
62	CASE		N/A			N/A	
63	NON-CASE		N/A			N/A	
64	NON-CASE		N/A			N/A	
65	OLD CASE		N/A			N/A	
66	NON-CASE		N/A			N/A	

A1, B1, C1: A,B,C type on allele 1
A2, B2, C2: A,B,C type on allele 2
N/A: not available -: not typable
Disease: case, culture-negative case, or old case.

Table 8. HLA haplotype frequencies among all study subjects and among family "founders" only.

HLA-A,B,C results	Frequency in all typable subjects	HLA-A,-B results	Frequency in family "founders"
2, 27, 2	0.03	2, 27	0.08
2, 27, 4	0.01	2, 35	0.20
2, 35, 4	0.12	2, 41	0.04
2, 41, 7	0.02	2, 44	0.12
2, 44, 5	0.19	2, 48	0.08
2, 48, 3	0.01	2, 58	0.04
2, 48, 8	0.14	2, 62	0.12
2, 48, -	0.01	3, 18	0.04
2, 58, -	0.02	24, 8	0.04
2, 62, 1	0.17	24, 62	0.08
3, 18, 7	0.01	29, 44	0.08
24, 8, 7	0.02	32, 44	0.04
24, 62, 1	0.05	68, 51	0.04
29, 44, -	0.17		
32, 44, 5	0.01		
68, 51, 4	0.03		

The estimated frequencies in founders was used for the linkage analysis, and their genotypes were assumed to be in Hardy-Weinberg equilibrium.

most reasonable model (analysis II; see below), linkage could be rejected (i.e. lod score less than or equal to -2) up to a recombination frequency of approximately 0.20, suggesting that a recessive "susceptibility" gene for pulmonary tuberculosis does not reside in, or near, the HLA-A,B,C region.

The following parameters for the linkage analyses were assumed:

Classification of phenotypes: all HLA-typed individuals were included. The disease phenotype was ascertained as per the criteria in chapter 4.vi on page 49. Uninfected individuals were classified as being resistant to disease.

Genetic model: a model of recessive inheritance of disease susceptibility was assumed. The absence of data on segregation of tuberculosis disease leaves the choice of a model arbitrary, but data from families with leprosy suggest susceptibility is due to a major gene with recessive inheritance[111]. Using a dominant model, and allowing estimation of the penetrance, yielded nonsensical values for the penetrance, suggesting that such a model is not valid.

Age- and sex-specific differences: no age- or sex-specific difference was assumed to exist for penetrance, phenocopy rate, or recombination rate.

Phenocopy rate: a rate of 0% was assumed for simplification of the first three analyses. Thereafter, phenocopies were assumed to occur at a rate of 6%, which lies approximately midway between the rates derived from the literature[20,51,143].

HLA haplotype frequencies: the haplotypes were assigned by inspection of the segregation of HLA-A,B in the family. The haplotype frequencies were estimated from the founder individuals of the pedigree (table 8), whose genotypes were assumed to be in Hardy-Weinberg equilibrium.

Penetrance of the susceptibility gene: this parameter is unknown and could not be calculated from the known data on tuberculosis. Therefore, the penetrance of a homozygous susceptible was set at 100%, that of a homozygous resistant at 0%, and the penetrance of the heterozygote was estimated by the ILINK program of the LINKAGE^{R} programs, to best fit the family data.

Susceptibility allele frequency: this was assumed to be low, due to the belief that genetic susceptibility to tuberculosis is now relatively rare, as discussed in the text. An arbitrary frequency of 2.5% was asigned to the susceptibility allele (q), which translates into 0.063% (q^2) of individuals being homozygous for the trait.

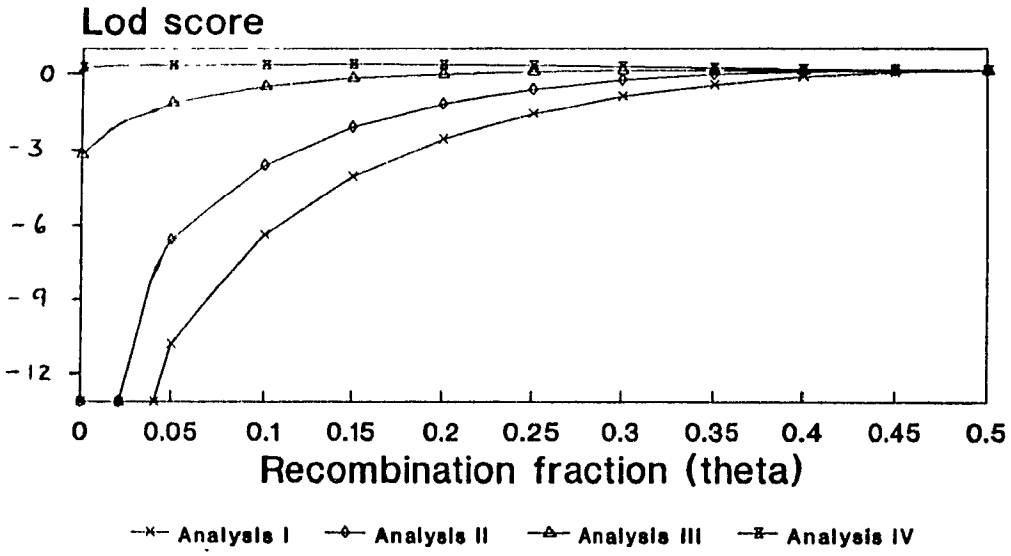
Linkage dysequilibrium: no linkage dysequilibrium was assumed to exist between the disease susceptibility locus and the HLA-A,B haplotype.

As a prelude to the first analysis (analysis I), the recombination fraction (theta) was initialized at 0.2 . Estimation of the heterozygote penetrance showed that its value tended to sit between 50% and 75%. However, the results were only mildly affected by varying the heterozygote penetrance from 0% to 75%. Therefore, subsequent analyses were run with a heterozygote penetrance of 0%, except when a phenocopy rate was introduced in the fourth analysis. The results of the lod score at various recombination fractions for analysis I are shown in figure 9. Linkage could be excluded up to a recombination fraction of 0.25 (lod score of -1.96).

A second analysis (analysis II) was performed with all of the parameters remaining as defined above. However, two groups of individuals were reclassified as to phenotype:

1) the three diseased children who were 2 years of age or less were reclassified as indeterminate phenotypes. This was based on the finding that most infected children of this age develop disease, regardless of other factors.[9,26,33,34] This argues strongly in favour of a non-genetic reason, such as immunologic naivete, which causes disease progression in this group. Because it was felt that these children added little genetic information to the linkage analysis,

Figure 9. Results of the linkage analyses.



Lod scores are plotted against recombination fractions for the four analyses (analysis I - IV). See the text for the parameter assumptions for each model.

their phenotype was made indeterminate.

2) The second group which was reclassified to an indeterminate phenotype was the "old case" group. It was reasoned that the 2 typable individuals who contracted disease in 1950, outside of the present epidemic, were infected under circumstances which might have made them phenocopies.

In this second analysis, figure 9, the null hypothesis again was not rejected, and linkage was excluded up to a recombination fraction of about 0.20 (lcd score: -1.51).

A third analysis (analysis III) examined what linkage information could be derived only from presumed genetically informative "cases". Only the diseased cases from the second analysis were retained, and all of the unaffecteds (i.e. either infected without disease or not infected at all) were classified as indeterminate phenotypes. The other parameters were kept as in the first two analyses. The results, seen again in figure 9, show lod scores which are higher than for the two previous analyses, but far from allowing rejection of the null hypothesis.

A fourth analysis (analysis IV) added a phenocopy rate of 6% to the above set of parameters. This rate was assumed to exist for both homozygous resistant and heterozygous individuals. The effect of adding the phenocopy rate was small (figure 9), but the lod score increased to a maximum of +0.28 at a recombination fraction of 0.15 . In this analysis, the curve is almost flat, due to a lack of power to either reject or accept the null hypothesis.

6. DISCUSSION

i) Evaluation of diagnostic tests for detection of tuberculosis disease

This outbreak of tuberculosis is one of the largest described in such detail among Canadian Native Indians. The ongoing frequent contact among the family members resulted in infection of 52 (79%) of the 66 evaluated individuals over a 2-year period. Because the Mantoux skin test was done in batches, the exact timing of infection is unknown, and a true epidemic curve cannot be constructed. Therefore, it is unknown whether the epidemic occurred as a result of point-source contact with the index case, or continuous person-to-person spread over time.

In this epidemic, the incidence of infection was equal among men and women. This is probably due to the preponderance of young individuals in this family (median age of 14.5 years) and the equal risk of infection described among children and young adults of either gender[24]. Subjects of all ages in this study showed similar susceptibility to **infection**, but the progression to **disease** tended to be different among the different age groups. However, only the incidence of disease among those 2 years of age or younger was statistically different from the reference group of adults 26-60 years old. The data confirm the heightened susceptibility to disease following infection among very

young children. The incidence of disease among those newly-infected in this study is strikingly higher than the age-specific figures quoted in the literature[9,26,33,34], except in the very young children. The study subjects developed disease at significantly higher rates, which were 3 to 14 times greater than would be expected from the age-specific data in previous reports. This suggests that either exposure to the pathogen was very heavy for most of the individuals, or that the study subjects were particularly susceptible to progression of infection to disease. The rapid development of disease in so many of the individuals in the study group makes it tempting to speculate that there exists a hereditary susceptibility to disease in this family.

The lack of usefulness of the Mantoux skin test for detecting the presence of disease in this population is consistent with the findings of the small number of other studies, which found no relation between tuberculin skin reactivity and the presence[72-75] or the severity[70,74] of disease. Although a positive skin test (defined as 5 mm or more induration) was found in 95% of diseased individuals, 100% of non-diseased infected persons also mounted such a reaction. The skin test was not able to reliably detect the presence of disease, nor increase the pre-test probability of disease to a significant extent. Therefore, although skin reactivity remains the only clinically useful tool for determining **infection**, it remains disappointingly inaccurate

in diagnosing active **disease.**

Cultures of respiratory secretions for M. tuberculosis were reliable for the detection of disease in individuals over the age of 12, but insensitive for those younger. Only 46% of the subjects under the age of 12 years with disease in this study yielded culture-positive specimens, while 100% of those older were positive. Overall, 8 of 24 individuals (33%) produced smear-positive specimens, which is markedly less than the proportion seen in other Native outbreaks[27,30]. This, too, may be explained by the relatively young age of the subjects in this study and the production of poorer specimens in this young population. Only 1 of the 8 smear-positive specimens was from a child 12 years of age or less. Therefore, although direct visualization of acid-fast bacilli can rapidly make the diagnosis of active pulmonary tuberculosis, it is a relatively insensitive test, with even poorer performance in children. This study confirms the generally held belief that cultures are unreliable for the documentation of active tuberculosis disease in young children[32,166]. It is believed that pediatric patients harbour smaller numbers of bacteria when diseased, and that respiratory secretions obtained from patients in this age group are of a "poorer quality" (i.e. not originating deep in the tracheo-bronchiolar tree)[32].

The overall sensitivity of culture (71%) found in this

study is similar to that obtained in another study in Canadian Natives[27]. Extrapolation of the results derived from this study means that the overall sensitivity of culture would be expected to be lower in any situation where a large number of children are involved, because of the differential yield in children versus adults. In addition, mycobacterial cultures of respiratory secretions may be theoretically less sensitive in remote populations, in contrast to hospitalized or urban populations, because of suboptimal specimen collection and delayed transport times[8]. However, this is usually not the case in practice, and one study has actually demonstrated that Native populations are more likely to be smear-positive and culture-positive than non-Natives[27]. However, the presence of large numbers of children in any epidemic would decrease the yield of these tests considerably, as demonstrated.

The multivariate discriminant analysis of age, gender, skin testing and chest radiography indicates that the radiograph is the most reliable of these indicators for the assessment of active pulmonary disease, in terms of sensitivity and specificity. However, in some instances, radiography is not immediately available, and a clinical decision as to the presence of active disease must be based on the results of the other variables noted. In this situation, only age and the results of skin testing contributed statistically significant information in a model

of disease ascertainment. Taking both into account would give the clinician a sensitivity of 74% and a specificity of 64% for detecting disease. Although these two variables significantly help determine an individual's disease status (i.e. **statistically** significant), the relatively poor sensitivity and specificity of this model make it only modestly reliable for clinically diagnosing such a contagious and progressive disease (i.e. not **clinically** significant). The poor positive and negative predictive values of this non-radiographic model (65% and 73%, respectively) would be even worse if applied to a population with a prevalence of disease less than the 46% seen in this study group. For instance, in a similar population with a prevalence of disease of 20%, the positive predictive value of this discriminant analysis model (excluding radiography) would fall to only 34%. It must be noted that the small sample size may have limited the statistical inferences which can be made from the data.

Therefore, the results indicate that the single most useful test for the detection of active pulmonary tuberculosis, while waiting for the staining and culture of respiratory secretions, is the chest radiograph. Tuberculin skin testing and knowledge of age or gender contribute little additional information. This data substantiates the long-held presumption that the size of skin test reactivity with the Mantoux test has little correlation with the

presence of active disease. In addition, the results of this analysis support the position that mycobacterial cultures from children are unreliable, and that the clinical diagnosis of pulmonary tuberculosis should not be altered on the basis of a negative culture from a child. In view of the poor performance in children of mycobacterial cultures, the less-than-perfect sensitivity of radiography, and the fact that 50%[9,26,33,34] to 75% (this study) of very young children will progress to disease once infected, a reasonable approach would treat any child aged 2 years or less with suspected disease, regardless of the outcome of the tests.

ii) Linkage analysis of HLA and tuberculosis disease

The results of the HLA typing in this study show similarities with the HLA types summarized in two other studies of North American (United States) Indians[164,165]. The most frequent HLA allele in all three studies is HLA-A2, as seen in table 9. Lower frequencies of HLA-A24 and -B62 were found in the Indian family in this study, but very similar frequencies of HLA-B35 exist in all three surveys.

The linkage analysis between tuberculosis disease expression and HLA type failed to show statistically significant linkage. There are several possible reasons for this. The most obvious explanation is that there is no disease susceptibility locus closely linked to the HLA class I region. Further linkage studies on other affected families

Table 9. Frequency of the most common HLA-A, -B alleles among "founder" Canadian Indians in this study, compared with a group of American Indians (United States).[164,165]

---: not stated.

HLA allele	Frequency (this study)	Frequency (U.S. study)[164]	Frequency (U.S. study)[165]
A2	0.68	0.45	0.65
A24	0.12	0.23*	0.46
A29	0.08	0.006	0.04
B27	0.08	0.04	0.01
B35	0.20	0.22	0.20
B44	0.24	---	0.06
B48	0.16	---	0.04
B62	0.08	---	0.41

* Figure shown is combined frequency of A23 and A24

from various populations will have to be repeated before this conclusion can be unequivocally accepted. Although data based on mouse models suggest that a single-locus model of disease susceptibility is plausible, the human situation is probably more complex. Other models, such as a 2 (or more)-locus multiplicative or additive model may be more realistic in human tuberculosis, as it is with other diseases in man[52]. As more loci linked to macrophage or T-lymphocyte function are found, such as the human homolog of the murine Bcg gene[132-134], more complex models may demonstrate linkage when HLA expression and these new loci are considered in an interactive fashion.

The lack of linkage in this study, in contrast to the demonstrated HLA/disease associations shown by several investigators in unrelated individuals (table 2), may be secondary to the severity and/or type of tuberculosis disease being studied, or the method of choosing study subjects. Studies of association between HLA type and tuberculosis disease in unrelated individuals are usually investigations of low-intensity exposure causing low endemic rates of disease. Linkage studies such as this one, done in families with multiple affected members, concern themselves with high-intensity exposure causing epidemic disease. It is conceivable that a major gene effect of HLA or a nearby region on disease expression is more important in low-exposure situations, and is overcome by other factors (i.e.

inoculum effect) in high-exposure situations. It is also possible that a disease susceptibility gene may be important in the development of reactivated disease from quiescent foci (more frequently seen in "endemic" cases) and is relatively inconsequential in modulating progressive pulmonary disease right after exposure (as is the case in this study and most "epidemic" cases). Furthermore, the presence of HLA/disease associations in studies of unrelated individuals relies heavily on the proper choice of suitably non-diseased controls. Because of this, they are more susceptible to ascertainment bias, in contrast to a linkage study.

Another reason which might explain the lack of linkage found between HLA and tuberculosis disease concerns the relatively small number of diseased subjects studied and the method of defining the "disease" phenotype. The power for detecting linkage would have been greater with the occurrence of more genetically informative diseased individuals. The phenotype of the study subjects was assessed according to a combination of explicit clinical criteria[23] which have been found useful in medical practice. However, the crude dichotomous designation of an individual as either "diseased" or "non-diseased" may not allow us to adequately study the role of an HLA-linked locus in the development of disease[99]. Perhaps, individuals with tuberculosis should be further

subclassified into a larger number of more sophisticated phenotypic categories. This could be based on specific mycobacterial antigen-directed T-lymphocyte responses as well as clinical manifestations, for instance. With such a combined cellular/clinical phenotypic classification of disease, linkage with HLA or other nearby loci might be more relevant and easier to detect. However, such subclassification would require large numbers of genetically informative persons, in order to ensure enough individuals in each category, and allow for an analysis with sufficient power.

Recombination rates between HLA-A,B,C, and HLA-DR are small, which should have made it possible to detect linkage in this analysis between disease and the HLA-DR system or a closely-linked locus. There are instances of HLA-DR/disease **associations** in studies of unrelated individuals which were not shown to be HLA-A, -B, or -C related[13,113]. In the present **linkage** analysis, an HLA-DR linkage with tuberculosis disease is unlikely to have been missed.

The linkage analysis could neither confirm nor refute a particular genetic model for the susceptibility to tuberculosis. Although a recessive model gave overall higher lod scores than a dominant model, neither reached significant levels. Variation in the penetrance and frequency of the susceptible allele did little to change the lod score. In addition, the positive changes in the lod score by altering the phenocopy rate and reclassifying the non-diseased

individuals shows that the linkage analysis is not robust with respect to the choice of genetic model, and explains little of the distribution of disease in the family. However, if the completely penetrant recessive model is correct for a major tuberculosis disease susceptibility locus, then it is not linked to HLA class I loci.

The frequency of the susceptible allele in the population was assumed, since no data is available to estimate this parameter. One could use segregation analysis to estimate the allele frequency, penetrance, and phenocopy rate, if thorough data on disease (including knowledge regarding family ascertainment) were available on multiple affected families. The parameters could then be derived from the genetic model which best explains the segregation of disease. The unavailability of estimates for these parameters led to hypothetical inferences of their magnitude, resulting in a hypothesis-generated model for the present analysis. However, in the present study family, the high disease rate following infection may imply that susceptibility is segregating in this family, in contrast to other populations.

Further studies are needed to assess the segregation of tuberculosis disease in affected families. The mode of inheritance, which may be deduced from such investigations, is necessary to construct a model with which data can be analyzed in the search for putative susceptibility loci.

7. SUMMARY / CONCLUSIONS

Data from a large extended family of Canadian Native Indians, who had experienced an epidemic of pulmonary tuberculosis, were used to: 1) assess the usefulness of diagnostic tests for the detection of disease, and 2) assess linkage between HLA-A,B type and disease expression. Seventy-nine per cent of the 66 evaluated individuals in the family became newly-infected, and 46% of the infected persons developed pulmonary disease.

The chest radiograph was the most reliable of the rapid methods for detecting disease. Knowledge of the age, gender, and Mantoux skin test result contributed little further information. In the absence of radiography, the results of tuberculin skin testing alone could not reliably indicate disease, but knowledge of both age and the skin test result were of modest help in disease ascertainment. Culture of respiratory secretions was unreliable, due to low sensitivity, in children aged 12 years and under. In view of these findings, chest radiography is strongly recommended and should be weighted heavily when assessing infected individuals for the presence of active tuberculosis disease. In addition, the results of this study suggest that children with clinically suspected disease should be treated, regardless of the result of mycobacterial cultures.

No linkage could be found between HLA-A,B haplotypes and

pulmonary tuberculosis disease. In a genetic model based on hypotheses derived from experimental animal models and epidemiologic studies of disease occurence, linkage could be excluded up to a recombination fraction of approximately 0.25. This suggests that a completely penetrant major susceptibility gene for pulmonary tuberculosis in this family, if it exists, is not located in or near the HLA complex. Therefore, although susceptibility to tuberculosis disease seems to be genetically determined, it appears that neither the HLA loci nor a nearby DNA segment is a major factor determining the development of tuberculosis disease following infection in this Native Indian family. Extrapolation of these results to other families will depend on the relative contributions of genetic and non-genetic factors, and their interaction, to the etiology of tuberculosis disease in them, compared with this Indian family.

Further research on the segregation of tuberculosis disease in multiple affected families might serve to clarify the inheritance of susceptibility to tuberculosis disease and could elucidate the proper genetic model to be used in linkage studies. Such linkage studies should probably focus on genetic loci which are involved in cell-mediated immunity.

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Appendix B.

CONSENT TO PARTICIPATE IN STUDY TO DETERMINE THE FREQUENCY OF HLA TISSUE TYPE AND PRESENCE OF BCG GENE IN INDIVIDUALS WITH A DIAGNOSIS OF ACTIVE TUBERCULOSIS AND IN THOSE WHO ARE CLOSE CONTACTS OF SMEAR POSITIVE PULMONARY CASES

INFORMATION

Tuberculosis infection does not cause disease in all those who contact it. The factors which determine who will become ill with this infection are not known and may be genetic.

A study to determine what tissue antigens are most common in those who have been diagnosed as having tuberculosis is being carried out. In addition, a search for the BCG gene which determines susceptibility to tuberculosis, will be undertaken.

If you agree to participate, 10 cc of blood will be drawn now and examined by the Histology Compatibility Laboratory at the University of Alberta Hospital and Dr. Skamene of Montreal. This study will in no way benefit you or aid in the treatment of tuberculosis. It will, however, help in the understanding of how tuberculosis infections occur.

There will be no change in your treatment or management if you do not agree to particpate.

CONSENT

I acknowledge that the research procedures described on the information sheet (above) and of which I have a copy, have been explained to me and that any questions that I have asked have been answered to my satisfaction. In addition, I know that I may contact the person designated on the form if I have further questions, either now or in the future. I have been informed of the alternatives to participation in this study. I understand the possible benefits of joining the research study as well as the possible risks and discomforts. I have been assured that personal records relating to this study will be kept confidential.

I understand that I am free to withdraw from the study at any time, without jeopardy to my continuing medical care. I further understand that if the study is not undertaken, or if it is discontinued at any time, the quality of my medical care will not be affected. I understand that if any knowledge gained from the study is forthcoming that could influence my decision to continue in this study, I will be promptly informed.

The person/s who may be contacted about the research are:

Dr. A. Fanning

Dr. F. Pazderka

Dr. M. Miller

NAME

WITNESS

DATE

SIGNATURE OF INVESTIGATOR OR DESIGNEE

Alberta

CASE DEFINITION

Appendix C.

DATE OF INTAKE YY MM DD | AHC IC | HEALTH UNIT | SUB OFFICE | DATE OF BIRTH YY MM DD | OTHER FILE NO | NAME CHECK | TB FILE NO

NAME SURNAME | FIRST | INITIAL | OTHER (MAIDEN, ALIAS) | SEX ☐M ☐F

ADDRESS | POSTAL CODE | PHONE

MARITAL ☐M ☐S ☐D ☐W ☐C/L ☐SEP | ETHNIC ORIGIN ☐METIS ☐INUIT ☐TR ☐OTHER (SPECIFY) | BAND AND NO

COUNTRY OF BIRTH | ARR IN CAN YEAR | OCCUPATION | NEXT OF KIN | PHONE NO

CORRESPONDANCE TO REFERRING DOCTOR | ADDRESS | POSTAL CODE

COPY TO PATIENT ☐YES ☐NO | COPY TO OTHER | ADDRESS | POSTAL CODE

REASON FOR REFERRAL ☐IMMIG ☐EMPLOY ☐SCHOOL ☐POST SECD ☐DOCTOR ☐SELF ☐HEALTH UNIT ☐LAB ☐POSTMORTUM ☐OTHER SPECIFY

SOURCE CASE YEAR ☐CONTACT

ASSOCIATION ☐CLOSE ☐CASUAL

TUBERCULIN LAST NEG YY MM DD | FIRST POS mm YY MM DD | CURRENT mm YY MM DD | BCG ☐U/K ☐NO ☐YES YEAR ☐SCAR

CLINICAL ☐IMMUNOSUPPRESSED ☐OTHER SPECIFY | PREVIOUS TB ☐YES ☐NO YEAR | PROV/COUNTRY | PREVIOUS MEDICATION ☐YES ☐NO ☐ACTIVE ☐PREVENT

SYMPTOMS / DURATION / DISEASE

SYMPTOMS	DURATION	DISEASE
☐FEVER		☐SILICOSIS
☐WEIGHT LOSS		☐DIABETES
☐COUGH		☐TRANSPLANT
☐SPUT		☐COLLAGEN DIS
☐OTHER		☐NIL
☐NIL		☐OTHER

TB.S DOCTOR

IF PREVIOUS TB MEDICATION DRUG / DURATION

ADMITT DATE YY MM DD | DISCHARGE DATE YY MM DD

DIAGNOSIS | DATE OF DEFINITE DIAGNOSIS YY MM DD

RESP.

☐PULM ☐MIN
☐PLEURAL ☐MA
☐PRIMARY ☐FA
☐MILIARY ☐CAVITY
☐SUSPECT ☐OTHER

NON RESP.

☐GU ☐OTHER
☐LN ☐PER
☐Abd ☐GI
☐CNS ☐MES
☐BONE OR JOINT

SPECIFY

DIAGNOSED AFTER DEATH

☐DEATH DUE TO TB (MASSIVE)
☐DEATH ACCOMPANIED BY TB
☐TB INCIDENTAL FINDING

METHOD OF DIAGNOSIS

☐CULTURE ☐PATH. ☐X-RAY ☐CLINICAL ☐OTHER

LAB RESULTS	SMEAR −	SMEAR +	SMEAR ND	CULTURE −	CULTURE +	CULTURE ND	DATE YY MM DD	(O) SENS / RES (X) I	R	S	Z	E	OTHER
SPUT													
URINE													
OTHER SPECIFY													

TREATMENT PLAN

DRUG PRESCRIBED	DOSE	DUR	MODE SELF DAILY	MODE HI DOSE OD	DATE START YY MM DD	DATE STOPPED YY MM DD	REASON

CDH 1 J6 A

SIGNATURE DATE DATE

Appendix D.

T.B. CONTROL — INTAKE/UPDATE

Date of Intake YY MM DD	AHCIC	Health Unit	Sub Office	Date of Birth YY MM DD	Other File No.	Date Of Origin Yr.	TB File No.

Name Surname	First	Initial	Other (Maiden, Alias)	Sex _ M _ F

Address	Postal Code	Phone No.

Marital Status _ M _ S _ D _ W _ C/L _ Sep.	Ethnic Origin _ Metis _ Inuit _ Tr.	Other (Specify)	Band -	Band No.

Country of Birth	Arr. in Canada	Year	Occupation -	Next of Kin	Phone No.

Correspondence to Referring Doctor	Address	Postal Code

Copy to Patient _ Yes _ No	Copy to Other	Address	Postal Code

REASON FOR REFERRAL _ Immig. _ Employ. _ School _ Post Secd. _ Doctor _ Self _ Health Unit _ Lab _ Post Mortem _ Other _____
Source Case Name _____ Year _____
_ Contact

ASSOCIATION _ Close _ Casual

Tuberculin Last Neg. YY MM DD	First Pos. YY MM DD MM	Current YY MM DD	BCG _ U/K _ No _ Yes Year Y Scar

CLINICAL _ Immunosuppressed	Other _ (Specify)	Previous T.B. _ Yes _ No Year	Prov/Country -	Previous Medication _ Yes _ No _ Active _ Prevent.

Additional Information _____

Signature of Health Nurse

Health Unit Stamp

DO NOT WRITE BELOW THIS LINE

T.B. Doctor : _____

T.B. Diagnosis
YY YY
_ Active _ Suspect Active
_ Inactive _ Presumed Inactive
Site _____ / _____ / _____
TREATMENT ADEQUATE? _ Yes _ No
Specify _____

NON-CASE ( ) _____
_ Contact _ Non-Contact
_ BCG Reactor _ Reactor
_ Non-Reactor
PROPHYLAXIS: _____
YY
Recommended _ Yes _ No
Accepted _ Yes _ No
Reason Stopped _____

ACTION	Specify Date YY MM DD
_ X-Ray	. .
_ Sputum	. .
_ CBC/Platelets	. .
_ AST	. .
_ Tuberculin	. .
_ Init/Cont Treatment	
_ Symptom Enquiry	. .
_ Visual Tests	. .
_ Report Compliance	. .
_ Other (_____)	. .

_ No further investigation required unless exposed to T.B. or symptoms of ill health appear or as required by a specific program.

CONSULTATION

X-RAY DATE: YY MM DD . . Normal _ Yes _ No

Signature _____ Date _____

_ No Copies _ File Only

Appendix E.

TUBERCULOSIS EPIDEMIOLOGY / LINKAGE ANALYSIS PROJECT

DATA FORM

Bld No.	File# &Code	Name	D.O.B. y / m / d	Sex	Mantoux mm/date	CXR report	CXR interp (MM)	Prev. TB?	BCG vaccine?	HLA type