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THE EFFECTS OF GENETIC ANCESTRY AND SOCIOCULTURAL FACTORS ON PULMONARY TUBERCULOSIS SUSCEPTIBILITY IN NORTHEASTERN MEXICO

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By

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DISSERTATION

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DEDICATION

To Norm, Shelly, Zach, and Heather, for our games of global trivia that ignited my curiosity to explore other cultures of the world.

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ABSTRACT

Genetic and environmental factors contribute to variation in tuberculosis (TB) disease risk among individuals in the Americas, although the relative contribution of each of these factors remains unclear. Genetic ancestry may serve as a proxy for underlying genetic differences in TB risk between the European, Native American, and African groups that formed many populations in the Americas, but this has never been tested. Such tests are complicated by the fact that genetic ancestry and important potential social predictors of TB are usually confounded. The urban center of Nuevo León, the Monterrey Metropolitan Area (MMA), presents a unique setting to tease apart these predictors. The MMA has excessive rates of TB disease and drug-resistant TB, and it is heterogeneous with respect to potential social predictors of disease risk and genetic ancestry.

This dissertation addressed three aims. First, we explored predictors of active TB in the MMA, including genetic ancestry, demographic, and socioeconomic characteristics. Second, we assessed the variability of genetic ancestry in the MMA to determine whether genetic ancestry could potentially capture genetic variants underlying disease risk in the parental populations. Third, we examined social and behavioral predictors of drug-resistant TB in the MMA. Data included detailed demographic and socioeconomic measures and 291,917 genetic markers from 194 individuals with latent TB infection and active pulmonary TB at the University Hospital in Monterrey.

We found that diabetes, computer ownership, and marital status predicted active TB. Substantial variation in genetic ancestry was observed, but genetic ancestry was not a risk factor for active TB after controlling for socioeconomic variables. This result indicates that: 1) genetic components of TB disease risk do not vary in the parental populations that formed the MMA, 2) effects of genetic factors are low

compared to social factors, or 3) power was too low to detect existing associations. Finally, we found that crack cocaine use predicted drug-resistant TB in this urban context. In conclusion, variation in TB rates across populations may be better understood by addressing population-specific social factors that have larger effects on active TB and drug-resistant TB susceptibility.

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"Why should tuberculosis flow such a malignant course in one person while sparing another? Chance, timing, circumstance, age at exposure, duration and severity of exposure, natural powers of resistance – all of these and more are known to play some part. But often there is no apparent reason: it is simply a mystery."

Frank Ryan, *The Forgotten Plague: How the Battle against Tuberculosis was Won – and Lost*

CHAPTER 1. INTRODUCTION

PURPOSE

Multifactorial diseases show remarkable variation across racial and ethnic groups due to a complex suite of risk factors. Host genetics, co-morbidities, and biological characteristics are contributing individual-level factors, but broader contextual issues, such as healthcare access, socioeconomic inequalities, political structures, and cultural landscapes, are major drivers of health disparities [1, 2]. Tuberculosis disease (TB) presents a unique opportunity to investigate these complex risk factors. Throughout the Americas, lower rates of TB disease are seen among people of European descent, while higher rates persist among people of Native American, Asian, and African descent. These TB disparities are largely attributed to social determinants that differentially increase exposure to environmental risk factors among those with lower proportions of European ancestry [3, 4]. TB disease disparities may further be explained by genetic factors that affect immune responses to *Mycobacterium tuberculosis*; however, it is unclear if genetic variants for susceptibility and resistance show significant differences across continental regions. Among populations of mixed descent, genetic ancestry may therefore influence TB disease risk through its association with social determinants, or possibly through its association with predisposing TB-risk alleles that differ in frequencies between parental populations. Admixed populations provide the opportunity to assess the role of genetic and environmental factors in explaining observed differences in TB disease rates between populations.

Inhabitants of Mexico are an admixed population with genetic contributions from European, Native American, and African ancestral populations. Variation in TB rates in Mexico, as well as other places throughout the Americas, has long been assumed to be partially due to genetic risk factors that differ between these parental groups. It is unknown if continental genetic ancestry predicts TB disease risk after taking into account key social determinants. Teasing apart the contributing risk factors for TB

disease is crucial for guiding public health prevention strategies. The purpose of this dissertation is to identify the sociocultural risk factors for active TB and drug-resistant TB (DRTB), and to investigate if continental genetic ancestry in admixed populations has the potential to capture the genetic differences underlying TB disease risk in the parental populations.

BACKGROUND

TB is one of the oldest known diseases in human history [5]. TB remains a major cause of morbidity in developing countries, and it is second only to HIV as a leading cause of death by infectious disease worldwide [6]. TB is spread through the air from human-to-human by aerosolized droplet nuclei containing the pathogen, *Mycobacterium tuberculosis* [7, 8]. In 2010, there were approximately 8.8 million new and relapse cases and 1.4 million deaths from TB disease [6]. The immune system of the majority of people who become infected with TB neutralizes the bacterium in the lungs, preventing its spread to other individuals, and permanently containing the infection in a state of subclinical latency [9]. Approximately 10% of infected individuals develop active disease, with 5% developing active disease during the first two years after infection, and 5% at a later point in life [9].

Rates of active TB vary substantially across racial and ethnic groups, as seen with Figure 1.1. This figure from the Centers for Disease Control and Prevention (CDC) shows clear differences in case rates in the United States from 1993 to 2009 [10]. Although rates show overall declines in all sub-groups, they are consistently lowest among Whites.

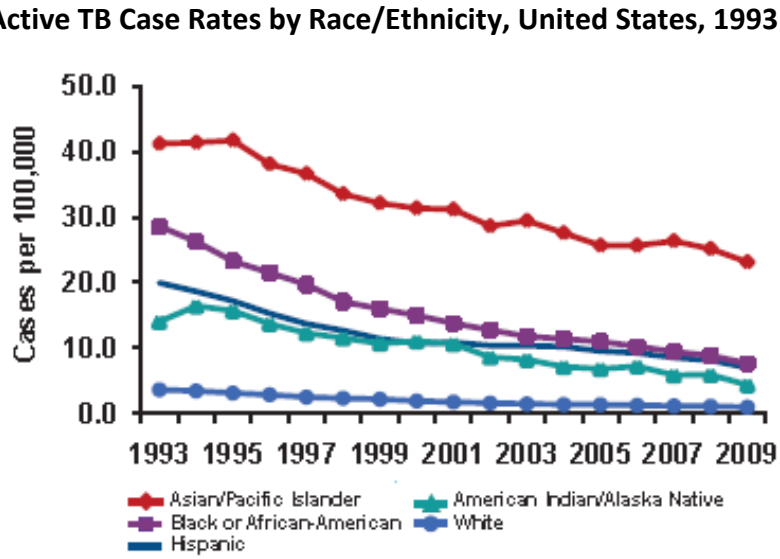


Figure 1.1. Active TB rates by race and ethnicity in the United States, 1993-2009. Taken from CDC 2011 [10].

Lower TB rates among people of western European descent is a common pattern in other areas of the world, as well [6]. This racial and ethnic variation is largely structured by environmental factors, such as sociocultural, economic, and political conditions that predispose an individual to developing active disease [3, 4, 11, 12]. Host genetics have also been shown to play a role in immunological susceptibility and resistance [13-15], although it is less clear if genetic variants differ among the continental groups that contributed to the admixed groups in the Americas [16].

Continental genetic ancestry is increasingly used in biomedical studies to investigate disease disparities. This approach assumes that the genetic component of disease risk is in fact structured by continental origins. To date, no study has assessed if genetic ancestry is independently associated with active TB. Furthermore, underlying environmental causes are mostly indicators of poverty and socioeconomic inequalities, but given the wide variation of these factors in different contexts, it is imperative to

explore population-specific environmental predictors of TB disease. This is the first study to integrate data on genetic ancestry, individual-level, and social variables.

DRTB is a unique problem for TB prevention and treatment efforts as cases with drug resistance have lower cure rates than cases with drug sensitivity [17, 18]. Treating DRTB costs 50 to 200 times more, and the duration of treatment is three to four times longer. In some areas of the world, including Mexico, rates of DRTB are increasing [19]. Pertinent risk factors for DRTB vary across populations, and it can be difficult to identify main predictors of risk for drug resistance based on patient-related factors that increase vulnerability. Among urban populations in Mexico where drug resistance is especially problematic, particularly among the U.S.-Mexico Border States, it is necessary to identify the main correlates of drug resistance in order to identify patient predictors of increased risk at clinic visits.

This dissertation combines anthropological and epidemiological perspectives to explore the effects of genetic ancestry and environmental factors on active pulmonary TB and DRTB, in an urban population of the Monterrey Metropolitan Area (MMA) in Nuevo León, Mexico. Urban areas typically have higher rates of TB compared to rural areas, partly due to greater residential crowding and higher likelihood of close contact with an active case [20]. Urbanized centers also tend to house extremes in wealth and poverty [21], and it is in pockets of severe poverty that TB thrives [12, 20]. There is the need to identify risk factors for active TB and DRTB in these contexts given increasing urbanization in developing countries.

To achieve this broad goal, we conducted research in the MMA and examined: 1) the independent contributions of population-specific sociocultural factors and genetic ancestry to active TB status; 2) the evolutionary history that produced the current pattern of genetic ancestry in the MMA; and 3) the independent predictors of DRTB.

RESEARCH DESIGN

Study population

TB disease rates have remained relatively stable in Mexico since 1990, with 16.8 new TB cases per 100,000 in 2010 [22], although increasing rates of drug-resistant TB (DRTB) greatly hinder control efforts [23]. The state of Nuevo León has almost double the rates of TB disease and mortality compared to national averages, despite its relative affluence [24]. The urban center of Nuevo León, the Monterrey Metropolitan Area (MMA), presents a unique population for this study given variation in previously reported genetic ancestry estimates [25-28], socioeconomic imbalances throughout the area [29, 30], and excessive rates of active pulmonary TB and DRTB [22, 31].

Approximately 90% of TB cases in Nuevo León occur in the MMA [32], located 140 miles southeast of Laredo, Texas (Figure 1.2). The MMA is comprised of nine municipalities totaling approximately 3.9 million inhabitants, making it the third largest population center in Mexico, after Mexico City and Guadalajara [33]. The nine municipalities include Apodaca, Escobedo, García, Guadalupe, Juárez, Monterrey, San Nicolás de la Garza, San Pedro, and Santa Catarina. Its strong industrial and business sectors make it one of the wealthiest and most developed cities in Mexico, well-known for its production of steel, cement, processed food and soda products, beer, glass, and auto parts. An industrial boom in the 1940s led to massive migrations to the MMA [25], and today, the vast majority of Nuevo León's population (90% as of 2009) lives in this urbanized center [34]. The quick expansion of the area lead to disproportionate economic development [29, 30]; today, the MMA contains one of the richest municipalities in Latin America (San Pedro) but also several severely disadvantaged sections (e.g., Colonia Independencia).

Figure 1.2. Study area in the Monterrey Metropolitan Area (red) in Nuevo León, Mexico

Brief overview of field site and recruitment

This dissertation research was conducted at the Hospital "Jose E. Gonzalez" of the Autonomous University of Nuevo León (UANL), in Monterrey, from January 2010 to February 2011. The University Hospital is a 500-bed teaching, research, and assistance facility that serves low to low-middle socioeconomic status individuals in Monterrey. The majority of patients at the hospital are residents of Monterrey or surrounding municipalities. The University Hospital treats approximately one quarter of all new TB cases in the region [35].

The overall study design was a case-control study. A total of 194 people were recruited. Cases (n=97) were comprised of individuals with bacteriologically confirmed active pulmonary TB who came to the TB clinic in the Hospital for diagnosis, treatment,

or follow-up. Controls (n=97) had latent TB infection as confirmed by a skin test (PPD); cut-off for enrollment was ≥10 millimeters [36]. Controls were recruited from the Internal Medicine clinic, hospital personnel, and people in waiting rooms. Additional details of the sample are provided in each chapter.

GUIDE TO THE DISSERTATION

The dissertation is organized by three chapters written for peer-review publications. Chapter 2 formally tests the association between genetic ancestry and active TB status, and explores individual and social variables that are important predictors of active TB in this sample. We intend to submit Chapter 2 as an original research article to the *International Journal of Tuberculosis and Lung Disease*. Chapter 3 investigates the distribution of European, Native American, and African ancestry estimates in the sample, and assesses the potential to use ancestry as an indicator of the differences in genetic risk among the parental populations that contributed to the admixed group. We plan to submit Chapter 3 as an original research article to the *American Journal of Physical Anthropology*. Chapter 4 presents an exploratory analysis of the predictors of DRTB in the sample, with clinical implications for identifying TB patients at greater risk for drug resistance. Chapter 4 for will be submitted as a brief communication to the *Pan American Journal of Public Health*. A summary of the findings and key conclusions from each chapter is presented in chapter 5.

CHAPTER 2. INDIVIDUAL AND SOCIAL CORRELATES OF ACTIVE TB: DIABETES, COMPUTER OWNERSHIP, AND MARITAL STATUS

INTRODUCTION

The main cause for transition from latent TB infection to active TB disease is immune incapacity of the host, which can be affected by genetic factors, age, sex, and medical conditions that suppress immunity, such as HIV/AIDS, malnutrition, diabetes mellitus, heavy smoking, silicosis, malignancies, and immune-suppressive treatment [9, 13, 14]. These individual risk factors are in turn influenced by a wide range of social, political, and economic conditions that vary substantially within and among regions [3, 4, 11, 12, 37]. Growing awareness of these conditions in the 20th century led to substantial progress in the fight against TB in both developed and developing nations [4], but that progress has slowed in recent years in some Latin American countries.

Mexico, for example, initiated environmental, dietetic, and hygienic programs that led to substantial declines in TB rates even before the widespread use of anti-TB medications starting in 1947, and the development of the bacille Calmette-Guérin (BCG) vaccination in 1948 [38]. As a result of these public programs, TB morbidity was halved from approximately 80 cases per 100,000 in 1920 to 41 cases per 100,00 by 1950 [38]. Anti-TB medication use and BCG vaccinations led to further declines in TB rates, to 16 cases per 100,000 in 1970 [38], but this promising trend ceased in the 1980s [19, 39, 40]. Rates have remained relatively stable since, with 16 (14-19) new TB cases per 100,000 in 2010 [22, 23, 41]. This rate contrasts with 4.1 (3.6-4.7) new cases per 100,000 in the United States in 2010 [41].

Within Mexico, there is substantial variation in TB rates across regions, but contrary to trends in the US [3, 37], the variation is not as clearly related to conventional socioeconomic measures. Nuevo León, for example, is one of the wealthiest of the 32 federal entities in Mexico as measured by living conditions, material possessions, education, employment, and infant mortality [42], yet in 2010, it ranked 9th highest in the country for TB incidence, at 24.2 new cases per 100,000 [22]. It

ranked 6th highest in the country in TB deaths, at 3.5 per 100,000, almost double the national average [42], and rates of drug-resistant TB are excessive compared to other regions [31]. This lack of association with conventional socioeconomic measures suggests that they might not capture important aspects of the social environment in developing nations, and, in particular, large urban centers [2, 3, 43].

In addition to social conditions, indigenous ancestry may also explain variation in TB rates between Mexico and other countries, as well as variation in rates throughout Mexico [44, 45]. Indigenous peoples tend to suffer disproportionately from TB disease throughout the Americas [10, 45]. This disparity may reflect underlying genetic differences between Native Americans and people of European descent [44, 46, 47], but it may also reflect persistent, wide gaps in socioeconomic status and healthcare access between these groups [4, 12]. In the Monterrey Metropolitan Area (MMA), the largest urban center in Nuevo León, estimates of indigenous ancestry range from 31-56%, and studies suggest a high European component compared to other regions in Mexico [25-27, 48, 49]. Given the relatively lower proportions of indigenous ancestry, higher proportions of European ancestry, and overall wealth of the MMA, it is paradoxical that TB rates are excessive in this region. Genomic data collected specifically from latent and active TB patients are required to assess the true levels of genetic ancestry and the association between genetic ancestry and TB disease status.

Our goal in this study is to identify the contribution of social factors and genetic ancestry to variation in TB disease status in the MMA. Our data include comprehensive, region-specific measures of social and environmental conditions, including self-reported ethnicity and indigenous language ability, and genetic ancestry estimated from 291,917 genomic markers. The results of this study have broader implications for exploring the social and genetic correlates of TB disease in urban centers in developed and developing countries.

MATERIALS AND METHODS

Study design and participants

Between January 2010 and February 2011, we conducted a case-control study at the Universidad Autónoma de Nuevo León (UANL) Hospital Jose E. Gonzalez in Monterrey. Every year, the hospital treats approximately one quarter of the new TB cases in the MMA [35]. The hospital is located in Monterrey, which is a moderate to low socioeconomic status municipality in the MMA [29, 50], and its open-door policy of treating patients independent of insurance status or income pulls in residents from all municipalities.

We recruited cases from adult patients at the hospital who currently had active pulmonary TB or had ever been diagnosed with active pulmonary TB (n = 97), as confirmed through the Mexican Ministry of Health's guidelines of bacteriological smear, culture, or histopathology [51]. Patients with extrapulmonary TB were not eligible for enrollment. Our control group was adults with latent TB infection, identified by a positive TB skin test (purified protein derivative test, PPD) of ≥10 millimeters [36, 52]. Controls had no history of transitioning to active disease, and we carefully selected them be representative of the population at risk for active disease based on known TB risk factors, such as sex, age, and low income. Our careful selection of controls that represented similar risk exposures as cases was done in an attempt to limit selection bias so that the study groups were comparable populations. Controls were recruited from the Hospital's Center of Research, Prevention, and Treatment of Respiratory Infections (CIPTIR), the Internal Medicine Clinic, and other clinic's waiting rooms within the hospital (n = 40). Individuals with diagnosed HIV were excluded from the study due to the strongly inflated risk of developing active TB and immunological anergy that could result in a false-negative PPD test [9]. The study was approved by the University of New Mexico (UNM) and UANL Institutional Review Boards, and all participants gave written consent.

Data

Data were collected through face-to-face interviews using a questionnaire that we developed from Mexican national and Latin American surveys [51, 53, 54], and established TB risk assessments used by the UANL Hospital [55, 56]. Interview questionnaires were identical for cases and controls. We partitioned these data into individual (i.e., host) and social groupings that reflected broader environmental characteristics that mediate the risk factors of the individual [1, 2]. Although certain variables, like education, can be difficult to distinguish between individual and social categories, the benefit of considering these two groupings was to recognize more comprehensive measures of socioeconomic status apart from simplistic measures at an individual-level [12, 57].

Individual variables included age, sex, self-reported indigenous ethnicity, education, employment history, personal knowledge about TB transmission and cure, alcohol and substance use, and BCG vaccination history (Appendix A, supplementary table A.1). Health characteristics included first- and second-hand smoking exposure, chronic conditions, and protein intake. Genetic ancestry was estimated from 291,917 single nucleotide polymorphisms (SNPs) assayed on DNA extracted from mouthwash samples (Illumina HumanCyotoSNP-12 v2.1 DNA Analysis BeadChip Kit). The same SNPs from 45 Native American, 54 European, and 40 African samples from the CEPH-Human Genome Diversity Panel were used for ancestral population references [58]. The chip contains a subset of 2.2 million SNPs common in Yoruban, Utah Mormon, Chinese and Japanese individuals in the International HapMap Project. We reviewed medical records to confirm demographic information and disease diagnoses, such as diabetes and history of alcohol problems.

Social variables comprised a wide-range of residential and socioeconomic factors. We ascertained data on marital status, housing structural conditions, TB contact history, and household characteristics of wealth (Appendix A, Supplementary table A.2). Socioeconomic status was estimated using a 10-question survey developed by the Mexican Association of Marketing Research and Public Opinion Agencies (AMAI) [53]. The 10 items measured computer and colored television ownership, type of floor,

number of rooms, functioning shower, exclusive bathroom, number of lights, type of stove, number of automobiles, and human capital measured by the educational achievement of the person earning the highest income in the household [59]. These variables differed from the individual-focused socioeconomic variables because they captured characteristics at a household-level. According to the AMAI protocol, points were assigned for each response for a total score ranging from zero to 366 points, and then categorized into six socioeconomic categories based on total point scores. We collapsed the six groupings into three categories representing upper-middle through highest, middle, and low-middle through lowest, due to insufficient cell counts but that still reflected the socioeconomic variation in the sample. The purpose of these categorizations allows for cross-regional comparisons of socioeconomic status throughout Mexico [60]. Each item on the 10-item AMAI survey was measured individually to assess its association with TB status, as well as the total composite survey.

Several additional social variables that estimated residential features included access to the hospital as measured by travel time to the UANL hospital in minutes, urban versus rural childhood residence, transportation by public or private vehicle, presence of a factory in the neighborhood, and municipality of residence. We also queried participants about their history of incarceration, residence in homeless shelters, and residence in nursing homes (Appendix A, Supplementary table A.2).

Statistical analysis

The SNPs were analyzed using Plink 1.05 [61] and R 2.14.2 [62]. Individual Native American, European, and African genetic ancestry proportions were estimated using maximum likelihood [63, 64]. The ancestry estimates presented are from a random subset of 10% of the loci, after filtering for minor allele frequencies of less than 5% and missing genotypes of greater than 5%, for an analyzed subset of 24,642 SNPs.

We assessed crude associations of individual and social characteristics with TB status (latent infection vs. active disease) using odds ratios (OR) with 95% confidence

intervals (CI), as well as Pearson's chi-square and Fisher's exact tests [65]. Multivariable logistic regression models were constructed from the variables in crude analyses that showed an association with active TB at a *p*-value of 0.10 or less. Variables in the logistic regression models were assessed for multicollinearity using a variance inflation factor cutoff of 2.5 [66]. The final regression model was selected based on the minimum Akaike Information Criteria (AIC), which represented the best fitted model [67]. Statistical analyses were conducted in SAS 9.2 (SAS Institute Inc., Cary, NC 2008).

RESULTS

Table 2.1 summarizes the demographic and socioeconomic characteristics of the sample. The mean age of participants was 43.8 years (± 15.9) and the majority of the sample was male (56.9%), self-reported non-indigenous ethnicity (79.6%), non-professional or never employed (67.9%), middle-level socioeconomic status (53.3%), and with a secondary educational level or less (66.4%). The average Native American, European, and African genetic ancestry proportions for the sample were 55.0% (range 25.2 – 92.6%), 37.9% (range 6.2 – 66.8%), and 7.2% (range 0.9 – 13.3%), respectively. Half of the sample lived in the municipalities of Monterrey and Guadalupe, although all nine municipalities of the MMA were represented.

Table 2.1. Demographic and socioeconomic summary of sample (N=137)

Demographic and Socioeconomic Characteristics	
	Mean ± s.d.
Age in years	43.8 ± 15.9
European genetic ancestry proportion	37.9 ± 11.3
Native American genetic ancestry proportion	55.0 ± 12.5
African genetic ancestry proportion	7.2 ± 2.3
	N (%)
Sex	
Female	59 (43.1)
Male	78 (56.9)
Indigenous ethnicity	
Indigenous heritage and/or language	28 (20.4)
Non-indigenous	109 (79.6)
Principal lifetime employment	
Professional, semi-professional, student	44 (32.1)
Non-professional or never employed	93 (67.9)
Current socioeconomic status	
Highest, Upper-Middle	39 (28.5)
Middle	73 (53.3)
Lowest, Low-Middle	25 (18.3)
Educational attainment	
Less than primary through secondary	91 (66.4)
Commercial, technical, college, specialist	46 (33.6)
Residence in Monterrey Metro Area (MMA)	
Monterrey	50 (36.5)
Guadalupe	18 (13.1)
Santa Catarina	5 (3.7)
Apodaca	15 (11.0)
San Pedro Garza García	3 (2.2)
San Nicolás de los Garza	11 (8.0)
Juárez	4 (2.9)
General Escobedo	10 (7.3)
García	4 (2.9)
Outside the MMA	17 (12.4)

In the crude analysis, 10 variables were associated with active TB ($p \leq 0.10$) (Table 2.2). Significant individual-level variables included having a secondary education or less, non-professional or unemployed history, diabetes, a history of alcohol abuse, and a lack of knowledge of TB transmission. Social conditions associated with active TB included marital status, a secondary education or less among the highest income earner in the home, and history of incarceration. While the AMAI-based composite measure of household socioeconomic status was not associated with TB status, two components showed significant associations- a lack of a functioning shower, no computers in the household. (Supplementary tables in Appendix A show all individual and social variables with their crude associations with active TB status.)

Table 2.2. Crude and adjusted logistic regression analysis (N=137)

Variable	Cases n = 97 N (%)	Controls n = 40 N (%)	Crude Odds Ratio (95% CI)	Crude *p*-value	Adjusted* Odds Ratio (95% CI)	Adjusted *p*-value*
Individual characteristics						
Educational attainment						
Less than primary through secondary	70 (72.2)	20 (50.0)	2.35 (1.1, 5.0)	0.03		
Commercial, high school, or higher	27 (27.8)	20 (50.0)	Reference	--		
Principal lifetime employment						
Professional, semi-professional, student	26 (26.8)	18 (45.0)	Reference	--		
Non-professional or unemployed	71 (73.0)	22 (55.0)	2.24 (1.04, 4.82)	0.04		
Diabetes						
No	69 (71.1)	34 (85.0)	Reference	--	Reference	--
Yes	28 (28.9)	6 (15.0)	2.42 (0.92, 6.38)	0.07	2.48 (1.0, 6.8)	0.08
History of alcohol problems						
No						
Yes	84 (86.6)	39 (97.5)	Reference	--		
	13 (13.4)	1 (2.5)	6.04 (0.76, 47.79)	0.06		
Knowledge of TB airborne transmission						
No	21 (21.7)	3 (7.5)	3.41 (0.96, 12.16)	0.06		
Yes	76 (78.4)	37 (92.5)	Reference	--		
Social characteristics						
Marital status						
Single, divorced, separated, widow	50 (51.6)	12 (30.0)	2.48 (1.13, 5.44)	0.02	2.89 (1.3, 6.6)	0.01
Married, free union	47 (48.5)	28 (70.0)	Reference	--	Reference	--
Presence of functioning shower in the house						

No	11 (11.5)	1 (2.5)	5.05 (0.63, 40.48)	0.09		
Yes	85 (88.5)	39 (97.5)	Reference	--		
Number of personal computers						
0	59 (62.1)	17 (42.5)	2.22 (1.05, 4.70)	0.04	2.28 (1.0, 5.1)	0.04
1 or more	36 (37.9)	23 (57.5)	Reference	--	Reference	--
Educational attainment of highest income earner in household						
Less than primary through secondary	65 (68.9)	20 (50.0)	2.32 (1.1, 5.0)	0.03		
Commercial, high school, or higher	28 (30.1)	20 (50.0)	Reference	--		
Ever been a resident in prison						
No						
Yes	86 (88.7)	39 (97.5)	Reference	--		
	11 (11.3)	1 (2.5)	5.00 (0.62, 40.00)	0.10		

*Odds ratios are adjusted for all variables in the table

These 10 variables were analyzed using multivariable logistic regression. The results of the best-fitted final regression model based on the minimum AIC are shown in Table 2.2. Diabetes was the only individual-level variable retained in the final model; individuals with active TB disease had two and half greater odds of having diabetes compared to individuals with latent TB infection (OR 2.48, 95% CI 1.0, 6.8). Of the social characteristics, marital status and computers in the home were independent predictors of TB disease. Cases were almost three times more likely to be single, divorced, separated, or widowed than controls (OR 2.89, 95% CI 1.3, 6.6). Cases were over two times more likely to not own a computer than controls (OR 2.28, 95% CI 1.0, 5.1).

DISCUSSION

TB presents an interesting disease phenotype because only 10% of infected individuals ever progress to active disease status [9], and non-European groups are disproportionately affected [10, 41]. Known risk factors for TB disease tend to focus on host factors, with increasing recognition of the underlying role of social structures that mediate risk [4]. Social conditions vary across populations and may help explain group differences in TB disease rates, but the MMA in Nuevo León presents a paradox since it has disproportionately high TB mortality and morbidity rates [22], despite being one of the wealthiest and most developed urban centers in Mexico [42]. Variation in genetic

ancestry has also been used as an explanation for TB discrepancies across populations [47, 68] with the assumption that ancestry is informative of group-level differences in TB-risk alleles, although this has never been formally tested using genetic markers. As a first step towards addressing these problems, we tested the hypothesis that genetic ancestry independently contributed to TB disease, while considering a wide variety individual and social characteristics. Active TB cases and latent TB controls were recruited from comparable populations, and by recruiting controls with confirmed latent TB infection, we were able to explore predictors of active disease among at-risk individuals. Overall, we found that genetic ancestry did not independently contribute to TB disease. Instead, diabetes, marital status, and computer ownership were the main correlates in this clinic-based sample in the MMA.

Diabetes is an important host risk factor for active TB due to impairment of immune defenses [69-71]. Our results are similar to a recent meta-analysis that reported a three-fold risk for active TB among diabetic patients compared to non-diabetic individuals [72]. A recent study conducted in southern Mexico concluded that diabetes may be on par with HIV co-infection in terms of co-morbidity with TB in Mexico, especially given the increasing incidence of diabetes throughout the country [73]. The prevalence of diabetes among TB patients along the border of Texas and northeastern Mexico is among the highest in the world [74], and further characterization of social and demographic factors of this co-morbid population in this region is needed. Diabetes is an important contributor to new TB cases, and it is suggested that this relationship is bidirectional since active TB may predispose people to diabetes through impaired glucose tolerance [71], although evidence of this association is inconclusive [75]. A greater emphasis on proper diabetes management and prevention may benefit TB control efforts [72, 74], especially in developing countries where TB is endemic and rates of diabetes are rapidly increasing [71].

Being married or in a lifetime partnership is increasingly recognized as a protective factor against active TB, independent of socioeconomic status [76-78]. Marital status is shown to mitigate TB disease severity and mortality [79], possibly

reflecting the influence of spousal influence on latent TB treatment completion [80]. Our finding of a significant association between active TB and being unmarried is suggestive that being married may play a protective role against progressing from latent infection to disease status. Corroborating evidence suggests the beneficial impact of "cohesive marriages" on physical and mental health [81]. Conversely, it is possible that individuals with chronic illnesses like TB may be less likely to be in a lifetime partnership due to the strain that the disease creates in the relationship [81, 82].

One unique finding of this study is the association between active TB and not having a computer in the household. A recent multilevel study in Recife, Brazil, found that living in an area where few households owned a computer was an important area-level predictor of risk of developing TB [83]. Presence of a computer likely serves as a unique proxy for socioeconomic status in the MMA, since none of our more direct measures of education, employment, and income were predictive of TB status in our final regression model. In fact, computer ownership in our sample was significantly associated with other socioeconomic variables (Appendix A, Supplementary table A.3). The measure of computers in the home might be more informative than a simpler measure of education or literacy, especially given that some studies have failed to show an association between years of schooling and TB disease [84] and latent infection treatment completion [80]. This finding may reflect the importance for access to health-related information or possibly it indicates access to resources. Internet access afforded by personal computers can directly affect health literacy [83], health-seeking behaviors, and treatment adherence [57]. The finding that not having a computer in the home is independently associated with active TB suggests a new way to identify high-risk areas in the MMA for more targeted public health interventions.

More direct measures of socioeconomic status, such as the 10-item survey (AMAI), and household crowding and income, were not predictive of TB disease status. This result could reflect the fact that we took great pains to ensure that our controls were representative of cases in terms of certain socioeconomic measures. Another possibility is that the 10-item survey used to measure socioeconomic status included

questions that were not relevant for this industrialized center, such as the use of biomass cook stoves. Variation in the impact of risk factors between and within regions emphasizes the need for more region- and community-specific studies of the determinants of health and health disparity, as well as the development of area-specific health policies.

In our sample, genetic ancestry was not associated with TB disease. This was inconsistent with the hypothesis that European genetic ancestry is protective against TB due to natural selection that conferred resistance to TB in peoples of Western European descent [46, 47, 85-87]. However, linkage between genetic ancestry and health related phenotypes is a function of the dynamics of the admixture process and organization of genetic variation in this region, which has never been assessed strictly in individuals with latent and active TB phenotypes. We plan to use the detailed genetic data collected for this study to explore the admixture process and its health-related implications.

Clear discrepancies in TB rates worldwide support the notion that people of predominately African and Native American ancestry are disproportionately susceptible to TB, whether due to underlying genetic variation and/or social disparities. In our sample in the MMA, group variation based on ethnicity and/or genetic ancestry may not be relevant for TB disease, especially since self-reported indigenous ethnicity was not associated with active TB, nor was genetic variation based on European, Native American, or African genetic ancestry. Although genetics undoubtedly play an important role in TB susceptibility [87-92], genetic ancestry may not be a useful proxy to capture the genetic basis of risk.

Several limitations of this study are important to note. First, the relatively small sample size may affect power and explain the lack of significance in certain variables that tend to be significant in other studies. The wide confidence intervals and borderline significance with several variables reflects the exploratory nature of this study and the use of proxy measures of complex social and environmental variables, and future studies should further explore these factors with larger samples. The

generalizability of study findings may be limited to industrialized, urban areas in developing countries. Lastly, social structures that predispose individuals to TB disease, such as political, economic, and cultural conditions, can be difficult to examine thoroughly, and our attempt to address some of these factors did not capture its entirety. For example, future work would benefit from including community-level measures of income inequality [93] and health service disparities [4] to dig deeper into social determinants of TB disease.

This study has several notable strengths. No previous study, to our knowledge, has incorporated such a large number of SNPs, including markers of ancestral populations, to formally assess the association of genetic ancestry and active TB. This integration of genetic ancestry, self-reported ethnicity, and social data is important for understanding the range of proximal and distal factors associated with TB disease [94]. Another strength of this study was our stringent criteria for control enrollment by a positive PPD test [95, 96]. Many case-control studies assume controls have latent TB infection if they live in a TB endemic area, but without testing for immune reactivity, there is the potential for enrolling people that have not been exposed or infected, and therefore do not actually represent an at-risk group for developing active disease. Confirmation of medical conditions and TB history through medical record reviews ensured accurate data on TB-related co-morbidities. Finally, the comparable source populations for cases and controls from representative populations based on already well-known TB risk factors allowed for further exploration of lesser-known variables, such as material possessions that were indicative of socioeconomic status specific to the MMA (i.e., computer ownership) [95].

Conclusion

This exploratory study assessed the role of genetic ancestry's contribution to TB disease susceptibility and identified potentially significant individual and social predictors of active TB among a clinic-based sample in the MMA. Genetic ancestry was not informative of TB status, while diabetes, marital status, and computer ownership

were independent predictors. The causes of TB disease disparities will be better understood by assessing population-specific social conditions that mediate individual risk. This study highlights the importance of addressing broader social conditions in TB prevention efforts.

CHAPTER 3: REJECTION OF A "ONE-TIME" ADMIXTURE HISTORY AND IMPLICATIONS FOR GENETIC STUDIES OF INFECTIOUS DISEASE

INTRODUCTION

Genetic admixture occurs when two or more previously isolated "parental" populations intermix [97, 98]. Allele frequency differences that accrue in the parental populations during isolation can be used to estimate their proportionate contributions to individuals in the admixed population. If the parental populations also differ in the frequencies of disease-causing alleles, the admixture process can create non-random associations, or linkage disequilibrium, between the disease-causing alleles and the alleles used to estimate ancestry [99]. These associations will gradually decay over time as a function of the recombination rate between the markers and the number of generations since the admixture event, unless they are maintained by continuous gene flow from one or more of the parental populations or assortative (i.e., nonrandom) mating in the admixed population [100, 101].

Genetic ancestry is increasingly used in biomedical studies of admixed populations to learn about disparities in multifactorial disease in the parental populations [97, 102, 103]. These studies assess whether genetic ancestry is predictive of disease status, independent of confounding social variables. Reiner and colleagues found, for example, that African genetic ancestry proportions predicted blood glucose levels among African Americans independent of environmental factors [104]. Other studies have identified independent associations between genetic ancestry and disease risk for breast cancer and type 2 diabetes among Hispanic American women [105, 106], and type 2 diabetes in African American women [106]. The results of these studies suggest that health disparities are partially due to differences in underlying risk alleles in the parental populations that contributed to the admixed group.

While most admixture-disease studies focus on chronic diseases, the approach is applicable to infectious diseases, like tuberculosis (TB) [9]. Pulmonary TB disease rates show clear discrepancies across populations [6], with consistently lower rates in

individuals with predominately European ancestry [6, 10]. Differential exposure to adverse social, economic, political, and cultural conditions contributes in large part to these discrepancies [1, 3, 4, 11, 37], but differences in genetic variation that affect immune response to the causative pathogen, *Mycobacterium tuberculosis* [14, 15, 107, 108] may also play an important role [16, 109-111].

A recent case-control study of TB in Monterrey, Mexico (Young et al., unpublished) did not find an association between genetic ancestry and TB disease after controlling for key sociocultural factors. They hypothesized that any association that might have existed following initial admixture between Native American, European and African individuals was gradually eliminated in the randomly mating hybrid population [28, 112]. Under this "one-time" admixture scenario, even if differences in TB-causing alleles existed in the parental populations, and even if these alleles contribute to TB disease variation in Monterrey, there would be no association between TB disease and genetic ancestry today.

Figure 3.1 diagrams this one-time admixture process with contributions from three parental populations in proportions p_1, p_2 and p_3 [98, 100]. Following this initial admixture event, there is no additional contribution from the parental groups and mating is random in the hybrid population. The dashed lines in the figure illustrate alternative scenarios involving continuous contributions from the parental populations [100].

Figure 3.2 shows a simple version of this one-time admixture process in which only Native American and European parental populations contributed in equal proportions to the admixed population 15 generations before the present ($p_{1,0} = p_{2,0} = 0.50$, $p_{3,0} = 0$) [98, 100]. The plots in the center of the figure show the probability for a random individual in the admixed population to have a given fraction of ancestry from the Native American parent after 1, 5, 10 and 15 generations. Without continuous gene flow from any of the parental populations, the distribution remains symmetric around 0.5, the initial proportionate contribution from the Native American population [100]. Due to recombination and independent assortment, the variation in this probability

decreases gradually over time, e.g., after 15 generations, the expected variance in Native American ancestry is 7.6 E-06 [100]. In this situation, individual ancestry estimates will show minimal variation after 15 generations of random mating, and therefore will not have the potential to show associations with disease phenotypes. The probability distribution takes different forms in the case of continuous contributions from one or more parental populations or assortative mating, but, importantly, in these cases, variation in ancestry proportions, and associations between genetic markers of disease and ancestry can persist indefinitely.

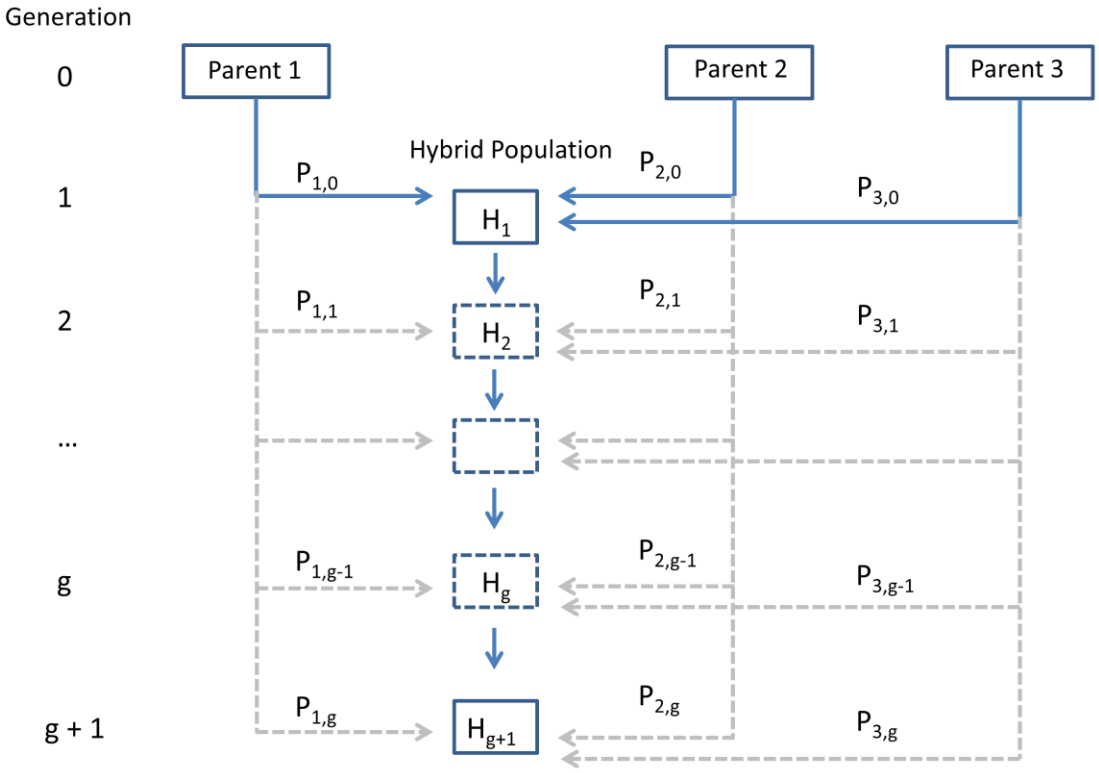


Figure 3.1. Diagram of a "one-time" admixture process (solid blue lines) with proportionate contributions from three parental populations, p_1, p_2 and p_3 at generation 1. The dashed gray lines indicate alternative scenarios in which the parental populations continue to contribute to the admixed populations. Adapted from Verdu and Rosenberg 2011, supporting material Figure S1 [100].

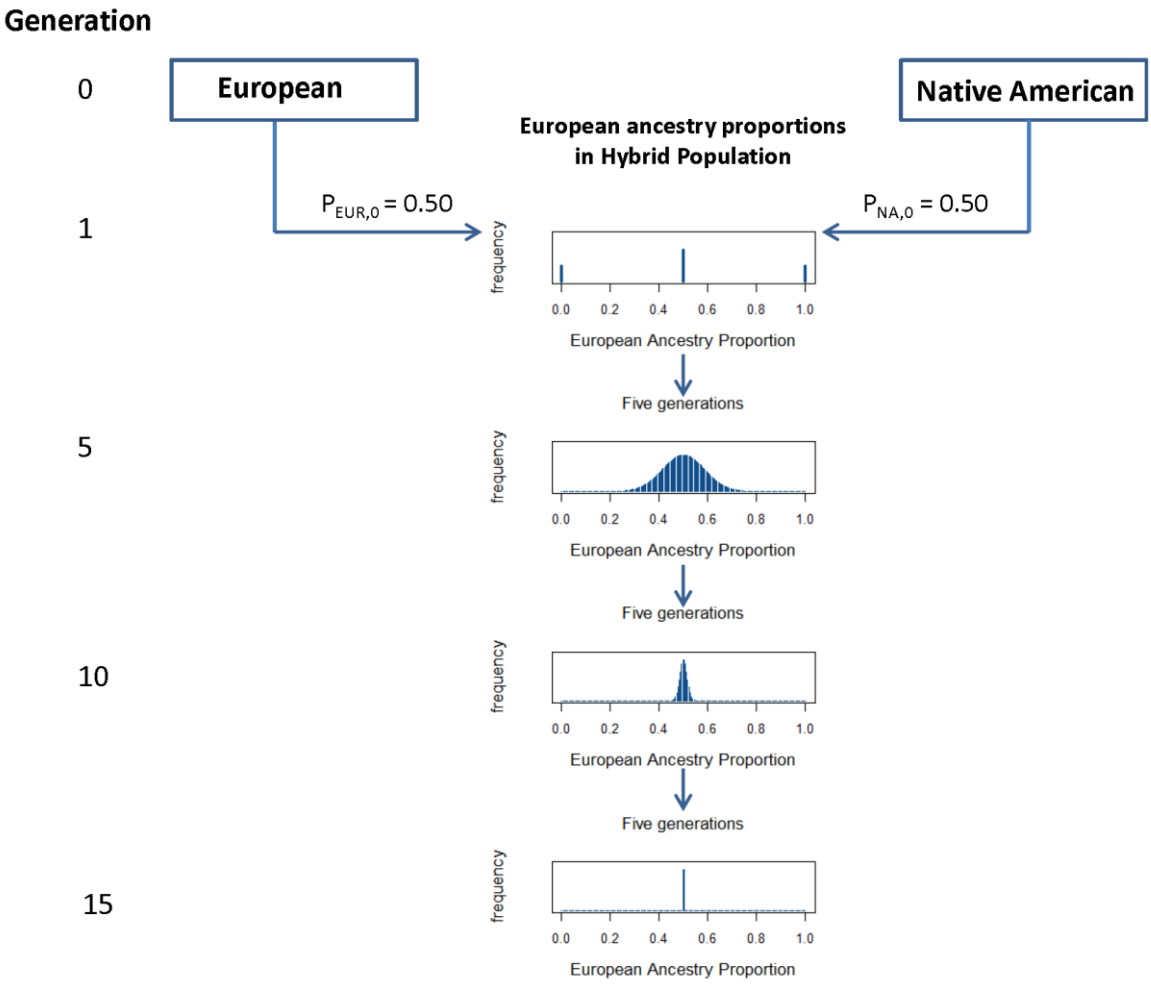


Figure 3.2. Simple version of the one-time admixture process for the case of $p_{1,0} = p_{2,0} = 0.5$, $p_{3,0} = 0$. The plots in the middle show the probability for a random individual in the admixed population to have a given fraction of ancestry from the Native American parent after 1, 5, 10 and 15 generations. Adapted from Verdu and Rosenberg 2011, Figure 2 [100].

Our goal in this study is to test the one-time admixture scenario by comparing the observed variation in genetic ancestry in the MMA today to that predicted after 15 generations, roughly equivalent to 500 years, since European and African populations first came to the Americas. If we fail to reject this scenario, then genetic ancestry cannot predict TB-disease status independent of social factors that might be associated

with genetic ancestry. If we do reject it, the finding of no association between genetic ancestry and active pulmonary TB in Monterrey suggests: 1) genetic components of TB disease risk do not vary in the parental groups that formed the MMA; 2) effects of genetic factors that differ in the parental populations are low compared to social factors; or 3) power was too low to detect existing associations. We consider the broader implications of our results for using genetic ancestry to investigate genetic contributions to disparities in multifactorial diseases in the Americas.

MATERIALS AND METHODS

Study population and sample

Monterrey is the capital of Nuevo León in northeastern Mexico. The Monterrey Metropolitan Area (MMA) is comprised of nine municipalities and is the third largest metropolitan area in Mexico, totaling over 3.5 million inhabitants [33]. Approximately 90% of Nuevo León's residents were concentrated in the MMA in 2009 [34]. Despite the fact that the MMA is one of the wealthier and more developed population centers in Mexico [42], it suffers from comparatively high rates of TB disease than other parts of Mexico [22].

The data were collected as part of a study on the effects of genetic ancestry and sociocultural variables on active TB status in the MMA. All participants signed an informed consent document, and the project was approved by the Institutional Review Boards at the University of New Mexico (UNM) and the Universidad Autónoma de Nuevo León (UANL).

Study participants included adults with confirmed active pulmonary TB (n=83) and latent TB infection (LTBI) (n=59) recruited from the UANL "Jose E. Gonzalez" Hospital in Monterrey. Participants with active TB were present at the hospital for diagnosis, treatment, or follow-up, and participants with LTBI included people in waiting rooms, hospital personnel, and patients present for other conditions. The hospital's policy of treating patients independent of insurance status or ability to pay makes it a catchment site for residents from all nine municipalities of the MMA.

We conducted face-to-face interviews to collect detailed demographic and socioeconomic data. Interview questions were developed in conjunction with local researchers and included questions from Mexican and other Latin American-based surveys [53, 54] and TB-risk assessments used at the UANL Hospital [55, 56]. Demographic variables included age, sex, and birth location. Measures of socioeconomic status included education, employment history, household income, and the Mexican AMAI 10-item survey that measured material wealth, housing conditions, and human capital [53]. The AMAI survey is scored by each question and points range from zero (lowest socioeconomic status) up to 366 (highest socioeconomic status). Indigenous ethnicity was assessed by self-report and indigenous language spoken personally or by a family member. Hospital medical records were reviewed to confirm demographic information and TB status.

Genetic data

DNA was extracted from mouthwash rinses in the molecular anthropology laboratory at UNM using a modified Puregene extraction protocol. Extracts were genotyped at the University of Michigan's DNA Sequencing Core. The genotypes consisted of 291,917 single nucleotide polymorphisms (SNPs) on the Illumina HumanCyotoSNP-12 DNA Analysis BeadChip Kit [113]. The chip contains a subset of 2.2 million SNPs common in Yoruban, Utah Mormon, Chinese and Japanese individuals in the International HapMap Project [114]. All SNP call rates exceeded 99%. To control for potential genotyping errors, we filtered the SNPs for minor allele frequencies of < 5% and missing genotypes of > 5%, resulting in a set of 246,420 SNPs for admixture analysis. Plink was used for the management of genetic data [61].

A total of 281 individuals were included in the genetic analysis. The MMA sample was comprised of 142 participants, and we additionally genotyped 54 Europeans, 45 Native Americans, and 40 Africans from the HGDP-CEPH Human Genome Diversity Cell Line Panel to serve as proxies for the parental populations [58]. The Europeans included French, Adygei, Orcadian, Russian, Sardinian, and Tuscan

individuals. The Native Americans included Mexican Pima, Maya, Colombian, Karitiana, and Surui individuals. The Africans were comprised of Yoruba, Mandenka, Bantu, and San individuals. We ran two sets of admixture analyses. In one set, all regional samples were used to represent European, Native American, and African parental populations. In the second set, we used only the French, Pima, and Yoruban individuals as proxies for the ancestral populations. Admixture estimates were essentially identical for both sets of analyses. Results presented in this paper are from the set that used regional samples as parents.

Statistical analysis

We estimated individual European, Native American, and African ancestry proportions for MMA participants with the method of maximum likelihood [63]. Estimates were obtained from random subsets of 10% of the filtered loci (24,642 SNPs) [63, 64]. We tested the one-time admixture process by comparing the observed variance in ancestry proportions in our MMA sample to the predicted variance after 15 generations, given by $V[H_{15}] = p_{x,0}(1-p_{x,0})/2^g$, for each of the three parental populations, x. The null hypothesis we tested is that our observed variance of ancestry estimates from the MMA sample are not significantly different from the expected variance of ancestry estimates under a one-time admixture history. This was assessed by testing the ratio of the observed variance/ $df_{observed}$ to the expected variance/ $df_{expected}$ equals 1.0. This ratio is an F-distributed random variable with $df_{observed} = df_{expected} = 141$ (degrees of freedom).

We used a likelihood ratio statistic, $G = -2\ [(\ln L\ (\mu_x) - \ln L\ (\hat{}\mu_i))]$ to identify individuals whose ancestry proportions deviated from expected under the one-time model [100, 115]. In the equation, μ_x is the average ancestry proportion from parental population *x*, and μ_i is the ancestry fraction that maximizes the likelihood function for the i^{th} individual. The null hypothesis is that $\mu_i = \mu_x$. G is distributed as a χ^2 random variable with degrees of freedom equal to the number of parental populations minus

one [116]. We used triangle plots of genetic ancestry to highlight the demographic characteristics of individuals for whom we rejected the null hypothesis.

RESULTS

Sample characteristics

Table 3.1 summarizes participant demographic and socioeconomic characteristics. Our sample was representative of the MMA with respect to sex and immigration status; about half of the sample was male (54.9%), and approximately half were born in the MMA. Our sample was not representative with respect to several other characteristics. First, since the sample was collected to study the correlates of active TB disease, it had a high proportion of people with active TB disease (58.5%). The median age of our sample was high compared to the median age for Nuevo León (42 vs. 26 years) [34], and the proportion of indigenous language speakers was high compared to the proportion in the MMA at large (5.6% vs. 0.8%) [117]. The sample also had slightly higher socioeconomic status than the municipality of Monterrey [60], likely due to the fact that the LTBI participants included hospital personnel with higher levels of education, employment, and income. These differences suggest that TB patients are not a random subset of the surrounding community with respect to age, ethnicity and SES.

Table 3.1. Demographic and socioeconomic summary of participants (N=142)

Participant Characteristics	
	Mean ± s.d.
Age in years	41.9 ± 15.2
European genetic ancestry proportion*	40.1 ± 12.8
Native American genetic ancestry proportion *	52.8 ±13.8
African genetic ancestry proportion *	7.1 ± 2.3
	N (%)
TB status	
Active TB disease	83 (58.5)
Latent TB infection	59 (41.5)
Sex	
Female	64 (45.1)
Male	78 (54.9)

Immigration to Monterrey	
Origin in Monterrey	72 (50.7)
Origin outside of Monterrey	70 (49.3)
Self-reported indigenous ethnicity	
Yes	24 (16.9)
No	116 (81.7)
Indigenous language spoken	
Yes	8 (5.6)
No	134 (94.4)
Principal lifetime employment	
Professional, semi-professional, student	64 (45.1)
Non-professional or never employed	78 (54.9)
Educational attainment	
Less than primary, secondary	76 (53.5)
High school, technical, college, specialist	66 (46.5)
Socioeconomic level**	
Highest (A/B)	21 (14.8)
High (C+)	38 (26.8)
Upper moderate (C)	27 (19.0)
Moderate (D+)	41 (28.9)
Low (D)	10 (7.0)
Lowest (E)	5 (3.5)

*Genetic ancestry: 24,642 random SNPs (random 10% from filtered 246,420).
**Based on the Mexican Association of Marketing Research and Public Opinion Agencies 10-item standardized survey [53].

Variation in ancestry: rejection of a one-time admixture model

Figure 3.3 summarizes individual genetic ancestry estimates for each MMA individual. Genetic ancestry varied substantially among study participants, with sample averages for Native American, European, and African genetic ancestry of 52.8% (± 13.8 s.d.), 40.1% (± 12.8), and 7.1% (± 2.3), respectively. Standard errors for individual ancestry estimates ranged from 0.004 – 0.01%. While these individual ancestry estimates fall within previously reported ranges for the MMA [26, 27, 48, 49], these studies relied on fewer markers and had higher standard errors in individual ancestry estimates.

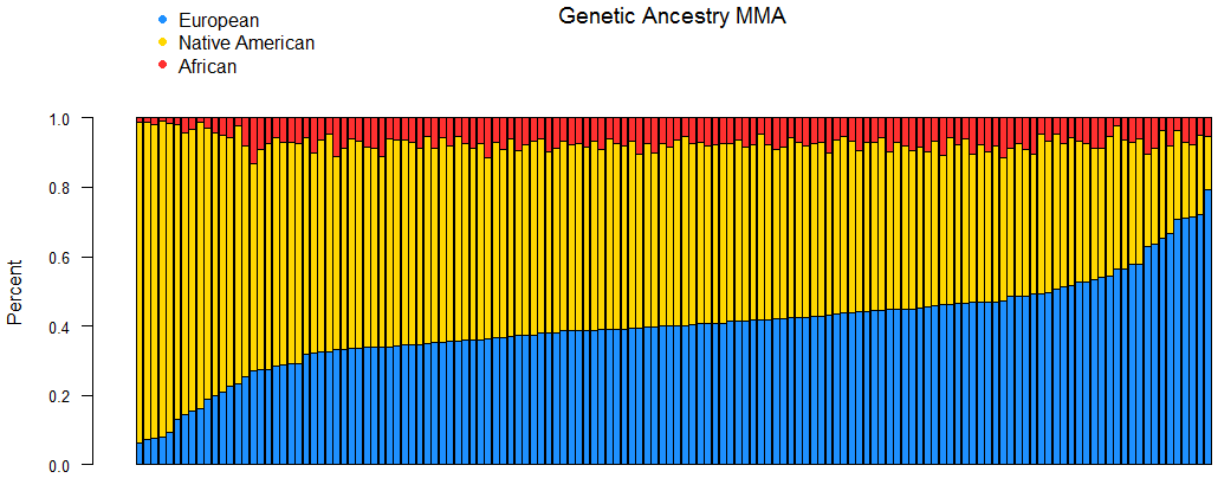


Figure 3.3. Genetic ancestry proportions in the MMA. The plot consists of 142 vertical bars representing the European, Native American and African ancestry proportions for each individual in the sample.

Figure 3.4 shows histograms of ancestry estimates for each of the three parental groups. The red vertical line shows the approximate expected range of ancestry under the hypothesis of one-time admixture if: 1) the parental populations initially contributed their respective mean amounts of observed ancestry, and 2) individuals in the admixed population mated randomly for 15 generations. The range of observed values is clearly much larger than that expected under the one-time process (F_{Eur} = 2251, F_{NA} = 2487, F_{Af} = 258, p-values < 0.0000).

Observed vs. Expected Variance of Ancestry Proportions in the MMA

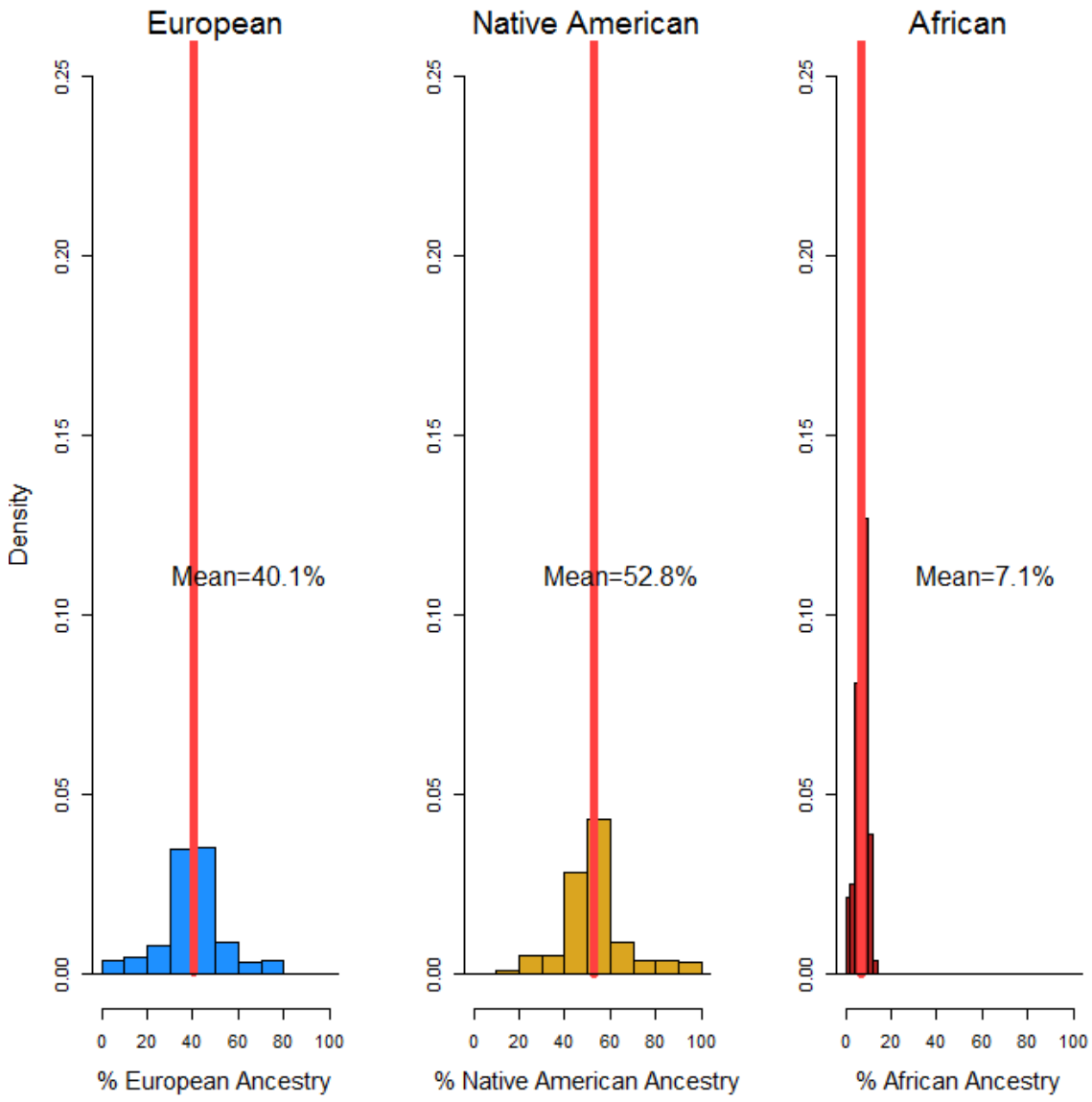


Figure 3.4. Histograms of individual genetic ancestry estimates in the MMA. The red vertical lines encompass the narrow range of variation in ancestry expected after 15 generations under the one-time admixture history.

The ancestry data are illustrated using triangle plots in Figure 3.5. The population mean is colored red. From the likelihood ratio test, the 20 individuals (14%) that were statistically indistinguishable from the population mean are colored black;

the remaining 122 individuals (86%) that significantly differed from the population mean are colored blue. Based on the F and the likelihood ratio tests, we reject the one-time admixture process.

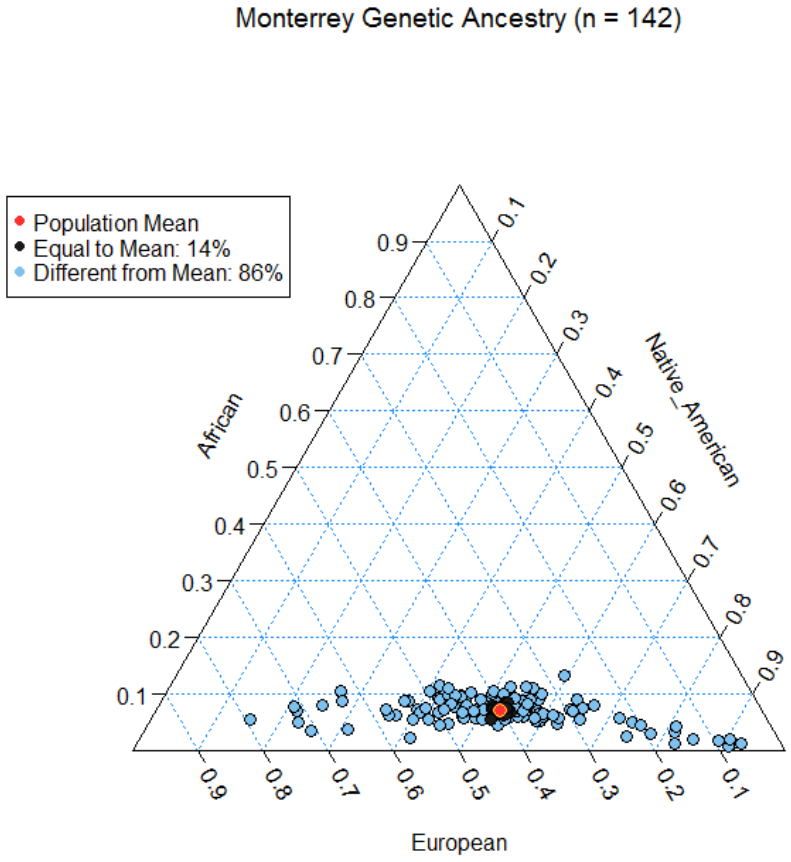


Figure 3.5. Individual ancestry estimates of the MMA. Blue dots (86% of sample) represent individuals that showed significant differences from the mean, and the black dots (14% of sample) are those that were indistinguishable from the mean.

To explore the potential processes driving our rejection of the one-time admixture process, we used the triangle plots to highlight key demographic characteristics of the sample. Figure 3.6 shows the same data as Figure 3.5, but this time individuals are color-coded by birthplace (i.e., immigration status). The plot shows that about half of the individuals in our sample were born outside of the MMA, as were the majority of individuals with high Native American ancestry. In fact, all individuals

with greater than 70% Native American ancestry were born outside of the MMA. Of these 14 immigrants with greater than 70% Native American ancestry, 36% self-reported as indigenous and 43% spoke indigenous languages or had relatives that spoke indigenous languages.

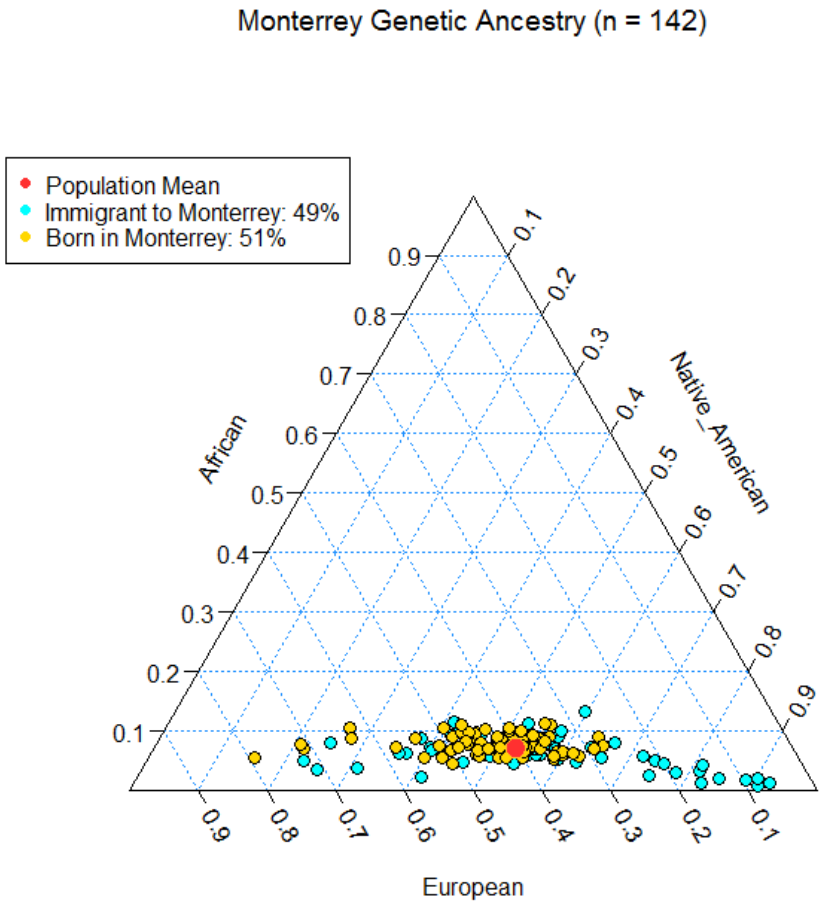


Figure 3.6. Ancestry estimates colored by immigration status to the MMA

In Figure 3.7, the individual ancestry data are color-coded to reflect recruitment status of the TB study participants. The figure shows that hospital personnel, namely nurses, secretaries, laboratory technicians, physicians, and medical students, had higher than average European ancestry in our sample. These personnel also tended to have higher socioeconomic status compared to the sample mean (238.5 AMAI points vs. 174.0 AMAI points). Approximately one-third of these individuals are also recent

immigrants to the MMA, but the majority is not. These patterns may reflect a combination of employment-related immigration and assortative mating by ancestry and socioeconomic status in the MMA.

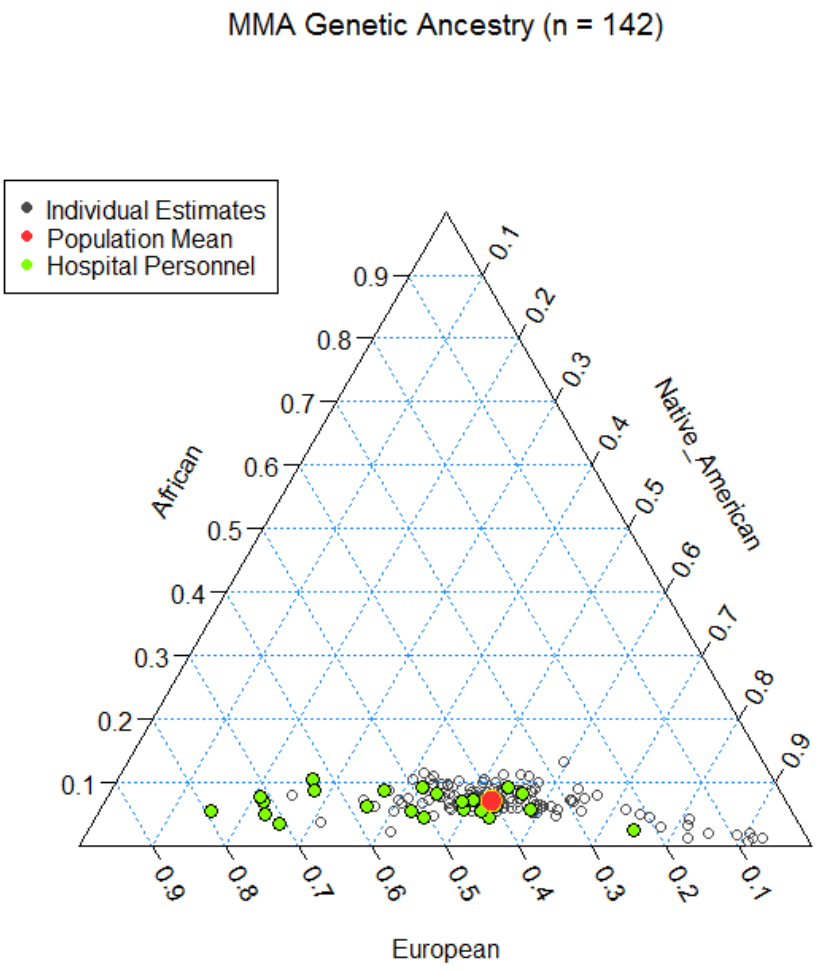


Figure 3.7. Ancestry estimates colored by recruitment status of hospital personnel

DISCUSSION

The variation in genetic ancestry in Mexico today reflects intermixing between predominately Spanish men and Native American women starting around 1519 [28, 118], and Africans starting in the early 16th century [28, 118]. Some recent African ancestry may also have entered through the European colonizers as a result of pre-Colombian gene flow between North African and Iberian populations [28, 119]. The Spanish and Portuguese continued to migrate to Mexico until the mid-19th century, and the African slave trade ceased in 1850 [28, 118].

The earliest inhabitants of Monterrey, founded in 1596 [25, 118], would have been comprised of the descendants of this intermixing. If the population in Monterrey had mated randomly following initial admixture between these parental populations beginning in the early 16th century, then we would expect the level of variation in genetic ancestry the MMA to be much lower than the observed levels identified in this study. Even if random mating only began in 1850 (approximately 7 generations), when slave traffic ceased, the observed level of variation for the European and Native American ancestry proportions would still greatly exceed the expected ($F_{Eur} = 8.8$, $F_{NA} = 9.7$, p-values < 0.0000) [100]. However, the African component is in fact consistent with the 7-generation onetime process ($F_{Af} = 1.0$, $p = 0.4816$). This result is consistent with prior evidence that Mexican spousal pairs do not exhibit assortative mating based on African ancestry [120].

The demographic characteristics of our sample suggest that continuous immigration of genetically distinctive peoples has played an important role in maintaining variation in genetic ancestry in the MMA, particularly individuals with high Native American genetic ancestry proportions. Immigration has played an important role in the massive population growth in the MMA over the past 70 years [34]. In just 20 years between 1940 and 1960, 400,000 people immigrated to Nuevo León to seek better living conditions and employment opportunities [121]. Many of these migrants came from regions with higher proportions of indigenous groups, e.g., San Luis Potosi, Tamaulipas, Mexico City, and Durango [121, 122]. In our sample, 10% of all immigrants

or their family members spoke indigenous languages, including Nauhuatl, Huasteco, Otomi, and Azteca. Six of the 14 individuals (43%) with greater than 70% Native American ancestry spoke indigenous languages. This continuous immigration of Native American peoples accounts for a large portion of the variation in genetic ancestry in our sample.

Assortative mating by ancestry or socioeconomic status may also explain deviations from the predictions of the one-time process. Many of the individuals with high European ancestry in our sample were relatively high socioeconomic status hospital staff. European ancestry is correlated with socioeconomic status in other large urban centers in Mexico (116). A previous study of spousal choice in Mexico City and the San Francisco Bay Area also showed strong correlations for assortative mating by European ancestry as well as by Native American ancestry [120]. These ancestry-based correlations for spousal pairs persisted even within socioeconomic categories and geographic subgroups [123, 124]. These results suggest that assortative mating has also played an important role in maintaining high levels of variation in genetic ancestry in the MMA.

Given its wide range of variation in the MMA, genetic ancestry has the potential to be informative about genetic differences in TB risk between the parental populations. However, in our recent study of the correlates of active pulmonary TB in the MMA, we found no association between TB-disease status and genetic ancestry (Young et al., unpublished). These findings suggests that: 1) genetic differences in TB-causing alleles do not exist between the ancestral populations that formed the Monterrey population, 2) any genetic differences that do exist contribute proportionately little to variation in TB disease compared to sociocultural factors, or 3) power was too low to detect existing associations.

With respect to the possibility that genetic differences in TB-causing alleles do not exist between ancestral populations that formed the MMA, there is weak evidence that genetic variation underlying susceptibility is geographically structured. The SLC11A1 gene, which is crucial in host immunity against infections, presents a good

example of this inconsistency. Two recent meta-analyses looked at the effects on TB susceptibility across populations from polymorphisms of the SLC11A1 gene. Li and colleagues identified differences in odds ratios between groups and differences in allele frequencies, and concluded that these allele frequency discrepancies might account for the variation in genetic risk across populations [90]. However, a more recent meta-analysis in 2011 compiled data from a larger number of studies and reported no differences in odds ratios between populations based on SLC11A1 variation [16]. Although both studies show contributions of SLC11A1 variation on TB susceptibility, there is no support for a group-specific effect of SLC11A1 polymorphisms and TB outcomes.

Genetic differences related to TB disease susceptibility that do exist often have relatively low effect sizes. Again, with SLCA11A1 polymorphisms, summed odds ratios for the 3' UTR variant was 1.35 (95% CI 1.17, 1.54), the D543N variant was 1.25 (95% CI 1.04, 1.50), the INT4 variant was 1.23 (95% CI 1.05, 1.44), and the 5' GT variant was 1.31 (95% CI 1.08, 1.59) [16]. Similarly low effect sizes and insignificant results are reported in meta-analyses for other TB-related genetic polymorphisms, such as with SP110 [110], P2X7 [125], TIRAP S180L [126], and the vitamin D receptor [109].

In contrast, numerous publications have demonstrated the role played by social factors in mediating TB disparities, most notably those associated with poverty and socioeconomic inequalities [1, 2, 4, 11, 37, 127]. For example, an analysis of 22 countries that bear 80% of the global TB burden concluded that HIV, malnutrition, smoking, diabetes, alcohol abuse, and indoor air pollution contributed substantially to population-level risk [128]. A systematic review of alcohol consumption concluded that approximately 10% of global TB cases were attributable to alcohol abuse [129]. Recent multilevel analyses in Brazil and South Africa highlighted the important effects of community-level factors on TB risk [83]. In the Brazil study, extreme poverty had a strong effect (OR 4.3, 95% CI: 2.9-6.3), and the authors concluded that 65% of all TB cases were explained by socioeconomic variables [83]. Income inequality was highlighted in the South Africa study as an independent risk factor for TB disease (OR

2.4, 95% CI: 1.6-3.5) [93]. Overall, the consistency of the findings in these studies, and the magnitude of the effects they report, suggest that variation in sociocultural factors is likely to play a more important role in TB risk than variation in genetic factors [130-132].

Conclusion

This study highlights the need for biomedical researchers to assess whether genetic ancestry in admixed groups has the potential to capture genetic differences in disease risk between the parental populations. Under a one-time admixture model, associations between genetic ancestry and disease-causing alleles, and variation in genetic ancestry in general, will quickly disappear. In admixed populations that experienced a one-time admixture event in the distant past, any current association between genetic ancestry and disease phenotypes must reflect unaccounted for sociocultural factors, not genetic differences in disease risk between the parental populations. Under more complex admixture histories, ancestry-disease marker associations may be maintained in the hybrid population, but only if disease-causing alleles show distinct differences across parental populations, and only if samples sizes of the admixed population have the power to detect effect sizes that are likely, in many cases, to be quite small. In either case, in countries throughout the Americas where healthcare resources are limited, prevention efforts may be better spent on addressing the known social conditions that so strongly affect multifactorial disease burden.

CHAPTER 4: CRACK COCAINE USE AND DRUG-RESISTANT TB

INTRODUCTION

Drug-resistant tuberculosis (DRTB) is a major public health problem worldwide that complicates TB prevention and care services in three important ways [6]. First, treatment for drug-resistant cases is 50 to 200 times more expensive than for drug-susceptible cases, and treatment duration is at least three times longer (18-24 vs. 6 months) [133]. Second, DRTB requires increased supervision from healthcare providers because more medications are required to treat the disease and because these medications are less potent and produce more serious side effects. Third and most importantly, the cure rate for drug-resistant cases averages only 70%, compared to 90-95% for drug-susceptible cases [6, 134]. In Mexico, a steady increase in DRTB was seen among new cases from 2000 to 2010 [22]. Nuevo León, a state in northeastern Mexico that shares a border with Texas, was tied with Baja California for being the second highest for drug-resistant cases in 2010 [22].

The predominate risk factors associated with selection for drug resistance have been categorized into community factors and patient conditions that increase vulnerability [17]. Community factors largely involve substandard healthcare services that influence risk [4]. Examples include high costs of treatment, inadequate healthcare access, improper dosing or duration of drugs, unavailable or poor quality drugs, and lack of directly observed treatment [4, 17, 135, 136]. Patient factors typically focus on predictors of loss to follow-up (i.e., treatment default), and therefore are indirect measures of risk for DRTB. These often include behavioral and social factors, such as illicit drug use, alcohol abuse, homelessness, incarceration, low education and income, impoverished housing conditions, and unfavorable patient-provider relations [137-141]. Patient factors that have been directly correlated with DRTB, such as age, sex, and HIV, are inconsistent across studies [133, 135]. In light of increasing rates of DRTB in Mexico, it is important to identify risk factors associated with the selection of drug resistance [142], particularly patient conditions that increase vulnerability for resistance [17].

The urban center and capital of Nuevo León, Monterrey, offers a unique setting to assess the correlates of DRTB because of routine drug-susceptibility testing (DST) at the Jose E. Gonzalez University Hospital, and evidence of extensive DRTB and recent transmission [31]. Throughout Mexico, most clinics and hospitals lack the resources for routine DST among previously treated and new TB cases. Given the lack of resources available for routine DST, a population-specific risk profile would benefit TB healthcare providers to identify patient predictors of drug resistance at clinic visits [17, 19, 136, 141]. The goal of this study was to explore links between DRTB and a variety of patient risk factors.

MATERIALS AND METHODS

Design and recruitment

This study is part of a larger project conducted between January 2010 and February 2011 as a case-control study in the TB clinic at the Jose E. Gonzalez University Hospital. The hospital is located in Monterrey, Nuevo León, which is a moderate to low socioeconomic status municipality in the MMA [29, 30]. Residents from all nine of the MMA municipalities come to this public hospital because of its policy to treat patients regardless of insurance status or ability to pay.

The data presented here represent an exploratory secondary analysis from the original study (Young et al., unpublished). Using the data from the original study, the present analysis investigates patient risk factors of DRTB among pulmonary TB patients attending the Jose E. Gonzalez Hospital. Active pulmonary TB participants (n = 95) aged 18 years or older were recruited from the hospital's TB clinic who currently had active disease or had ever been diagnosed with laboratory confirmation of active disease. All participants had disease confirmation through a positive culture, and received standard mycobacteriology DST to determine if they had drug-susceptible or DRTB [51]. We defined drug resistance according to the World Health Organization's (WHO) guidelines as a TB isolate that is not susceptible to the action of one or more anti-TB drugs [51]. Cases consisted of 25 patients with resistance to at least one TB medication, and

controls consisted of 70 patients who were sensitive to the four first-line medications. Patients with diagnosed HIV and extra-pulmonary TB were excluded from the study. This project was approved by the University of New Mexico and Autonomous University of Nuevo León Institutional Review Boards, and study participants gave informed, written consent prior to enrollment.

Data collection

To investigate patient risk factors for drug resistance, we conducted personal interviews, reviewed medical records, and assayed genetic ancestry from DNA polymorphisms. The interviews were based on questionnaires from Mexican and other Latin American surveys and hospital risk assessments [53, 54]. They assessed a wide range of patient factors known to be associated with active TB and possibly DRTB, such as socioeconomic status, demographic characteristics, health features, and social stigma [2, 143].

Socioeconomic status was measured using a nationwide survey designed for market research in Mexico that compiled 10 questions related to number of rooms in the house, housing materials and structure, possession of a colored television, computer, and automobile, type of stove used, and education of the highest income earner [53]. Personal education, employment history, household income, and additional housing measures were also collected to estimate socioeconomic status. Demographic characteristics included age, sex, marital status, and indigenous ethnicity based on self-report and indigenous language spoken. Health features included alcohol and substance abuse histories, co-morbidities, previous BCG vaccination, TB contact history, prior TB treatment, and use of alternative treatments for TB.

Additionally, in order to explore issues surrounding social stigma, discrimination, and fear towards the disease, patients were also asked during the interviews about their knowledge and attitudes towards TB [143]. Social stigma was assessed by giving the patient a hypothetical scenario of having a family member sick with TB, and then asking if they would prefer to treat that family member in secrecy [54]. Patients were

also asked if they had ever seen or felt discrimination related to TB in their community, and if they had any fears related to TB, such as not being cured, spreading it to family, or feeling isolated as a result of being sick [143].

Medical records were reviewed in the UANL Archival Department to confirm diagnoses of co-morbidities, such as diabetes. TB-related data were also confirmed through medical record reviews, including date of diagnosis and treatment history.

We measured genetic ancestry of participants because prior studies have suggested that genetic ancestry is predictive of TB susceptibility and resistance [45, 46, 85]. DNA was extracted from mouthwash samples using a modified protocol of the Puregene DNA Isolation Kit (QIAGEN, Inc., U.S. 2011). The extracts were genotyped for a panel of 291,917 single-nucleotide polymorphisms (SNPs) that vary in frequency between European, Native American, and West African parental populations (Illumina HumanCytoSNP-12 v2.1 DNA Analysis BeadChip Kit). These SNPs were also typed for 54 European, 45 Native American, and 40 African individuals from the Human Genome Diversity Panel [58]. All SNPs were filtered by minor allele frequencies of less than 5%, and missing genotypes of greater than 5%, resulting in a set of 246,240 markers. Proportions of European, Native American, and African genetic ancestry were estimated from random subsets of 10% of the filtered loci (24,642) using maximum likelihood [64].

Data analysis

We tested the association of each variable with DRTB using Pearson's chi-square tests, and Fisher's exact tests were calculated when cell counts were less than 5. Potential risk factors that differed between drug-susceptible and drug-resistant cases at an alpha level of 0.10 were introduced in the multivariable logistic regression models, and the final model was selected based on a backward elimination procedure. Variables in the logistic regression model were assessed for multicollinearity using a variance inflation factor cutoff of 2.5 [66]. Crude and adjusted odds ratios (OR) and 95%

confidence intervals (CI) were reported for the full and final models. All statistical analyses were performed in SAS 9.2 (SAS Institute Inc., Cary, NC 2008).

RESULTS

A summary of patient characteristics is provided in Table 4.1. Overall, the majority of participants were male (53.7%), of low socioeconomic status (55.8%), unemployed or non-professional lifetime employment (73.7%), possessed a secondary education or less (71.6%), and self-reported non-indigenous ethnicity (82.1%). Genetic ancestry estimated from the SNPs ranged from 6.1 – 56.5% European, 37.1 – 92.6% Native American, and 0.9 – 11.6% African. Mean sample estimates were 37.2% (s.d. 10.8), 55.7% (s.d. 12.3), and 7.1% (s.d 2.3) for European, Native American, and African proportions, respectively. Participants came from all nine municipalities in the MMA, although Monterrey had the highest representation (38.9%).

Table 4.1. Summary of study sample characteristics (N = 95)

Sample characteristics	
	Mean ± s.d.
Age in years	44.9 ± 17.1
European genetic ancestry proportion	37.2 ± 10.8
Native American genetic ancestry proportion	55.7 ± 12.3
African genetic ancestry proportion	7.1 ± 2.3
	N (%)
Resistance to at least one anti-TB drug	
No	70 (73.7)
Yes	25 (26.3)
Sex	
Female	44 (46.3)
Male	51 (53.7)
Self-reported indigenous ethnicity	
Indigenous heritage and/or language	17 (17.9)
Non-indigenous	78 (82.1)
Principal lifetime employment	
Professional, semi-professional, student	25 (26.3)
Non-professional or never employed	70 (73.7)
Current socioeconomic status	
Highest, Upper-Middle	25 (26.3)
Middle	17 (17.9)
Lowest, Low-Middle	53 (55.8)
Educational attainment	
Less than primary through secondary	68 (71.6)
Commercial, technical, college, specialist	27 (28.4)
Residence in Monterrey Metro Area (MMA)	
Monterrey	37 (38.9)
Guadalupe	13 (13.7)
Santa Catarina	3 (3.2)
Apodaca	11 (11.6)
San Pedro Garza García	2 (2.1)
San Nicolás de los Garza	6 (6.3)
Juárez	3 (3.2)
General Escobedo	5 (5.3)
García	3 (3.2)
Outside the MMA	12 (12.6)

In univariable analysis comparing patient characteristics between drug-susceptible and drug-resistant participants, seven variables were associated with drug resistance (p<0.10) (Table 4.2). BCG vaccination status was excluded from multivariable models due to insufficient data for approximation, resulting in six variables for introduction in multivariable models.

Drug-resistant patients were more likely than drug-susceptible patients to be younger, have a history of marijuana, crack cocaine, and inhalant use, report social stigma, and received prior TB treatment. All other variables failed to show significant differences between drug-susceptible and drug-resistant patients, and drug resistance status with respect to genetic ancestry was not significant (Table 4.2).

Table 4.2. Crude associations between drug-resistant TB and individual and social characteristics (N=95)

Variable	Drug-sensitive (n = 70)	Drug-resistant (n = 25)	*p*-value
	Mean ± s.d.	**Mean ± s.d.**	
Age in years	48.9 **±** 17.7	39.2 ± 13.9	**0.05**
European genetic ancestry %*	36.6 ± 10.5	40.3 ± 9.9	0.14
Native American genetic ancestry %*	56.3 ± 12.1	52.5 ± 10.9	0.17
African genetic ancestry %*	7.1 ± 2.2	7.3 ± 2.3	0.77
Mean total pack years**	7.2 ± 17.6	5.4 ± 10.2	0.63
Travel time to UANL Hospital (minutes)	65.4 ± 68.6	61.2 ± 41.4	0.77
	N (%)	**N (%)**	
Sex			
Female	35 (50.0)	9 (36.0)	0.23
Male	35 (50.0)	16 (64.0)	
Self-reported indigenous ethnicity			
Indigenous heritage	55 (78.6)	23 (92.0)	0.13
Non-indigenous	15 (21.4)	2 (8.0)	
Educational attainment			
Less than primary through secondary	52 (74.3)	16 (64.0)	0.33
Commerical, high school, or higher	18 (25.7)	9 (36.0)	
Principal lifetime employment			
Professional, semi-professional, student	17 (24.3)	8 (32.0)	0.45
Non-professional or unemployed	53 (75.7)	17 (68.0)	
Diabetes			
No	49 (70.0)	17 (68.0)	0.85
Yes	21 (30.0)	8 (32.0)	
History of alcohol problems			
No	60 (85.7)	23 (92.0)	0.51
Yes	10 (14.3)	2 (8.0)	
Asthma			
No	69 (98.6)	24 (96.0)	0.46
Yes	1 (1.4)	1 (4.0)	
Hypertension			
No	62 (88.6)	24 (96.0)	0.43
Yes	8 (11.4)	1 (4.0)	
Marijuana use			
No	65 (92.9)	20 (80.0)	**0.07**
Yes	5 (7.1)	5 (20.0)	
Crack cocaine use			
No	66 (94.3)	19 (76.0)	**0.02**
Yes	4 (5.7)	6 (24.0)	
Methamphetamine use			
No	69 (98.6)	23 (92.0)	0.17

Yes	1 (1.4)	2 (8.0)	
Injection drug use			
No	68 (97.1)	23 (92.0)	0.28
Yes	2 (2.9)	2 (8.0)	
Inhalant use			
No	69 (98.6)	22 (88.0)	**0.05**
Yes	1 (1.4)	3 (12.0)	
Previously treated for TB			
No (<1 month of treatment, 'new case')	21 (30.0)	2 (8.0)	**0.03**
Yes (≥1 month of treatment, 'previously treated')	49 (70.0)	23 (92.0)	
Knowledge of TB airborne transmission			
No	14 (20.0)	5 (20.0)	1.0
Yes	56 (80.0)	20 (80.0)	
Knowledge that TB is curable* **			
No	5 (7.3)	0	0.32
Yes	64 (92.8)	25 (100.0)	
Social stigma by preference to treat a family member with TB in secrecy			
No	61 (87.1)	18 (72.0)	**0.08**
Yes	9 (12.9)	7 (28.0)	
Ever saw or felt discrimination against TB			
No	44 (65.7)	12 (48.0)	0.12
Yes	23 (34.3)	13 (52.0)	
Ever felt fear related to TB			
No	34 (48.6)	9 (36.0)	0.27
Yes	36 (51.4)	16 (64.0)	
BCG vaccination* **			
No	17 (24.3)	0	**0.01**
Yes	53 (75.7)	25 (100.0)	
History of family TB			
No	41 (58.6)	19 (76.0)	0.12
Yes	29 (41.4)	6 (24.0)	
Close contact with a TB patient			
No	38 (54.3)	13 (52.0)	0.84
Yes	32 (45.7)	12 (48.0)	
Place first learned about TB			
Health clinic, doctors, hospital	52 (77.1)	18 (72.0)	0.61
Other (family, public media, school, books)	16 (22.9)	7 (28.0)	
Use of alternative remedies/therapies to treat TB	53 (94.6)	22 (91.7)	0.63
No	3 (5.4)	2 (8.3)	
Yes			
Marital status			
Single, divorced, separated, widow	37 (52.9)	12 (48.0)	0.68
Married, free union	33 (47.1)	13 (52.0)	
Household income per 15 days (pesos)			

<2,000	22 (38.9)	8 (33.3)	0.99
2,001 – 5,999	33 (50.8)	12 (50.0)	
6,000 or higher	10 (15.4)	4 (16.7)	
Current socioeconomic status****			
Highest, Upper-Middle	17 (24.3)	8 (32.0)	0.26
Middle	41 (58.6)	10 (40.0)	
Lowest, Low-Middle	12 (17.1)	7 (28.0)	
Running water inside home			
No	8 (11.4)	4 (16.0)	0.55
Yes	62 (88.6)	21 (84.0)	
Normal mode of transportation			
Personal car	15 (21.4)	8 (32.0)	0.29
Other (public bus, metro, taxi, bike)	55 (78.6)	17 (68.0)	
Residence in MMA municipalities (SES groupings based on geospatial analysis)			
San Pedro, San Nicolás (high)	7 (10.0)	1 (4.0)	0.59
Monterrey, Guadalupe, García, Santa Catarina (medium, medium-low)	42 (60.0)	14 (56.0)	
Apodaca, Escobedo, Juarez (very low)	12 (17.1)	7 (28.0)	
Outside the MMA	9 (12.9)	3 (12.0)	
Ever been a resident in prison			
No	62 (88.6)	23 (92.0)	0.63
Yes	8 (11.4)	2 (8.0)	
Ever been a resident of a homeless shelter***			
No	68 (97.1)	25 (100.0)	1.0
Yes	2 (2.9)	0	

*Individual genetic ancestry estimated from 291,917 single nucleotide polymorphisms (SNPs)
**Total pack years calculation: (#cigarettes per day * years of smoking)/20
***Insufficient data for approximation
***Calculated from the AMAI Mexican 10-item survey (2008)

In multivariable logistic regression containing these six patient characteristics, only crack cocaine use remained an independent risk factor for drug resistance. We combined marijuana and inhalants into one category and re-ran the model, which resulted in identical final results (Table 4.3). After adjusting for all potential risk factors, age, previous TB treatment, social stigma, and marijuana/inhalant use fell out of the final model and were no longer significant predictors of DRTB. To summarize our final results among this clinic-based sample of TB patients in the MMA, the odds of using crack cocaine were over 5 times greater for DRTB patients compared to drug-susceptible TB patients (OR 5.21, 95% CI: 1.33, 20.4, $p = 0.02$).

Table 4.3. Crude and adjusted logistic regression analysis to assess predictors of drug-resistant TB (N = 95)

Variable	**Drug-sensitive** n = 70 **N (%)**	**Drug-resistant** n = 25 **N (%)**	**Crude Odds Ratio (95% CI)**	**Crude *p*-value**	**Adjusted* Odds Ratio (95% CI)**	**Adjusted *p*-value***
Age in years	48.9 ± 17.7	39.2 ± 13.9	0.97 (0.94, 1.00)	0.05		
Previous TB treatment						
No	21 (30.0)	2 (8.0)	Reference	--		
Yes	49 (70.0)	23 (92.0)	4.93 (1.06, 22.8)	0.03		
Social stigma						
No	61 (87.1)	18 (72.0)	Reference	--		
Yes	9 (12.9)	7 (28.0)	2.64 (0.86, 8.07)	0.08		
Marijuana and/or inhalant use						
No	65 (92.9)	19 (76.0)	Reference	--		
Yes	5 (7.1)	6 (24.0)	4.11 (1.13, 14.95)	0.03		
Crack cocaine use						
No	66 (94.3)	19 (76.0)	Reference	--	Reference	--
Yes	4 (5.7)	6 (24.0)	5.21 (1.33, 20.39)	0.02	5.21 (1.33, 20.4)	0.02

*Odds ratios are adjusted for all variables in the table

Due to the fact that crack cocaine use is often embedded in co-existing adverse behavioral and socioeconomic conditions, we assessed the associations of crack cocaine use with other patient characteristics using Fisher's exact tests (Appendix B, Supplementary table B.1). We found a strong association between crack cocaine and socioeconomic factors, such that a significantly higher proportion of crack cocaine users

had lower educational attainment, non-professional and unemployed status, history of alcohol problems, hepatitis, other drug use, and several poor housing characteristics, compared to those who had never used crack cocaine.

DISCUSSION

Knowledge of patient-specific factors that predict DRTB is important in Mexico where routine DST screening is not obtained for diagnostic purposes due to limited healthcare resources. It is well-known that demographic, health, and socioeconomic characteristics can be predictive of people at risk for TB disease, but these broad characteristics are not consistently associated with DRTB [133, 144]. As a result, patients with increased risk for DRTB can be difficult to identify. The goal of this study was to explore patient risk factors that were predictive of drug resistance in order to help healthcare providers identify patients potentially at risk of DRTB [136].

In crude analyses, DRTB patients were more likely than drug-susceptible TB patients to be younger, use illicit drugs (marijuana, inhalants, and crack cocaine), have previously been treated for TB, and prefer to treat a family member in secret. After controlling for these variables in multivariable analysis, only crack cocaine use remained an independent predictor of DRTB. It is important to note that crack cocaine use may be a proxy for more complex social variables that were not accounted for in this preliminary study that need more attention in future studies, especially considering that crack cocaine use was significantly associated with other adverse behavioral and socioeconomic conditions, and the confidence intervals were wide showing substantial variability.

Individuals who use illicit drugs present high-risk groups for having DRTB for several reasons [17, 138]. First, illicit drug use (herein referred to as “drug use”) challenges TB care from direct immune impairment [145, 146]. For example, crack cocaine inhalation can weaken pulmonary function through suppressed alveolar macrophage antimicrobial activity and cytokine production [146, 147]. Second, drug use is embedded in adverse health and social factors that can affect patient loss to follow-

up (i.e., treatment default) [133]. Several of these interconnected risk factors for DRTB that make drug users a vulnerable population include alcohol abuse, homelessness, low education and income, impoverished and crowded housing conditions, unfavorable patient-provider relations, financial burdens of treatment, and limited access to health services [4, 137-141]. The combination of immune impairment and treatment barriers associated with drug use may increase risk for DRTB, although not all studies have found an association [142, 144, 148].

Specifically, crack cocaine use among TB patients has previously been linked with distrustful patient-provider relationships and non-adherence [141], leading to poor treatment outcomes, patient loss to follow-up, and higher treatment costs, all of which increases risk for DRTB [137, 147]. Studies that have shown an association between DRTB and crack cocaine use also acknowledge other associations with race, low income neighborhoods, and other co-morbidities [141]. In addition, the risk of transmission of drug-resistant strains at crack houses may place crack users at increased risk of DRTB [141]. In our study, the association between crack cocaine was independently associated with DRTB, even after controlling for various other patient factors, but its correlations with these characteristics suggests that it is still embedded in a larger contextual model of risk. Overall, TB control efforts in Mexico may benefit from ascertaining crack cocaine history as a risk assessment for DRTB, and conducting DST in new and recurrent TB patients with a positive crack cocaine history.

Previous treatment for TB tends to be a strong predictor of drug resistance [135, 136], although this variable dropped out in our multivariable analysis. This may be due to the fact that previously treated TB cases includes different “types” of patients, including relapse cases (patients previously cured or treatment completed), patients who began treatment but then abandoned it, and patients who failed initial treatment, possibly because they were infected with a drug-resistant isolate [133]. Previously treated cases are a heterogeneous group with respect to risk factors, such as low income, alcohol abuse, HIV co-infection, inadequate TB knowledge, previous treatment abandonment, herbal medication use, male sex, difficult access to hospital services, and

poor supervision for adherence [149-151]. In addition, it is possible that accurate reporting of prior treatment may be difficult to obtain due to patient's fear of shame, reprisals, or not being offered treatment, again. Future surveillance should separate previously treated TB cases into the three sub-groups of relapse, treatment default, and initial treatment failure, and explore risk factors specific to each group [133].

A strength of this study was that we screened all TB patients for drug resistance. Additionally, our questionnaire assessed comprehensive information on individual, clinical, household, and neighborhood variables, with broad measures of socioeconomic characteristics. The questionnaire included a nationwide socioeconomic survey [53], providing a standardized measure for comparative studies in other regions of Mexico. The validation of co-morbidities and TB-related data through medical record reviews helped to counter possible recall bias among participants. This exploratory secondary analysis has several limitations, one of which was the small sample size of drug-resistant cases, leading to wide confidence intervals in our final results. Additionally, participants were recruited from a single public hospital in Monterrey that was a catchment site for the MMA, so results may not be generalizable to patients that attend clinics in areas of higher socioeconomic status, or in less urbanized areas throughout Mexico. As mentioned above, the complexity of social and behavioral factors that influence DRTB may not have been captured with our questionnaire, notably with factors that predispose patients to drug use and consequently poor disease management. We recognize the potential for residual confounding in our findings, such that if confounding variables that influence DRTB were omitted or not accurately captured, the inclusion of these variables in future models could diminish the magnitude of the effect of crack cocaine use on DRTB that we reported. Importantly, this study focused on patient risk factors and did not include community risk factors, such as the quality of health care services. The exploratory nature of this study is recognized with crack cocaine use a possible proxy for more complex interrelated risk factors, and our findings can guide future work on DRTB in the MMA.

Conclusion

DRTB is increasing in Mexico, especially along the U.S.-Mexico border, and presents substantial challenges to TB care. Usual predictors of TB disease were not associated with risk for drug resistance in this sample, and therefore might be inadequate predictors of people at risk for drug resistance in the MMA. Crack cocaine use, however, clearly distinguished drug-resistant from drug-susceptible TB patients and may help identify higher risk individuals for prioritized DST. The correlations between crack cocaine use and adverse behavioral and social conditions highlights the co-existing risk factors for drug users, presenting a complex set of challenges for TB prevention and treatment. In regions where DST is not routinely administered, healthcare providers should consider testing for drug resistance in all new and recurrent TB cases who have a positive history of crack cocaine use.

CHAPTER 5. SUMMARY AND CONCLUSIONS

Summary of findings

This dissertation was an integration of detailed genetic and environmental data to explore causes of TB disease variation in the MMA. The project addressed a complex suite of risk factors for active TB and DRTB to better understand the variation in rates across populations. The broader goal of this study was to explore potential reasons that TB disparities have persisted among people of non-European descent, and to know what role ancestry played in this persistence from genetic and social perspectives. To our knowledge, no previous study has formally tested the association of genetic ancestry with active TB. The key findings of the dissertation are summarized as follows.

Chapter 2 findings were based on a case-control study from individuals with confirmed latent TB infection and active pulmonary TB. We carefully selected controls to represent the source population of cases. Overall, we found no support for an independent association between genetic ancestry and active TB status. The main predictors for active TB included diabetes (OR 2.48, 95% CI: 1.0, 6.8), single/divorced/separated status (OR 2.89, 95% CI: 1.3, 6.6), and not having a computer in the home (OR 2.28, 95% CI: 1.0, 5.1). These correlates of TB disease support growing recognition of the importance of assessing individual *and* social factors that are specific to a population. The roots of TB disease disparities will be better understood by assessing population-specific social conditions that affect individual-level exposures.

In Chapter 3, we addressed the need to investigate whether ancestry could serve as a useful proxy for the genetic variants of risk of multifactorial disease across the parental populations of admixed groups. Genetic associations between ancestry and disease-causing alleles cannot be found under a one-time admixture model, so it is important to know the admixture history of a population prior to conducting a disease association study. Given our lack of an association between ancestry and TB status described in Chapter 2, it was surprising that our sample did not fit a one-time admixture model. Instead, the substantial genetic heterogeneity among 86% of individuals was consistent with a more complex model of admixture, partially fleshed

out by evidence of indigenous immigration and assortative mating based on European ancestry and socioeconomic status. Given its wide range of variation in the MMA, genetic ancestry has the potential to be informative about genetic differences in TB risk between the parental populations. However, in our recent study of the correlates of active pulmonary TB in the MMA, we found no association between TB-disease status and genetic ancestry, suggesting that: 1) genetic differences in TB-causing alleles do not exist between the ancestral populations that formed the Monterrey population, 2) any genetic differences that do exist contribute proportionately little to variation in TB disease compared to sociocultural factors, or 3) power was too low to detect existing associations. In sum, results suggest that in countries throughout the Americas with limited healthcare resources, public health strategies for disease prevention may be more effective by addressing the social determinants that have larger effects on disease disparities.

Finally, our analysis in Chapter 4 of DRTB in the MMA gave insight into an important risk factor for DRTB. Even though typical risk factors for TB did not separate drug-resistant from drug-susceptible cases, the use of crack cocaine was a robust predictor of DRTB (OR 5.21, 95% CI: 1.33, 20.4). Given increasing rates of DRTB in Mexico and along the U.S.-Mexico border, targeting higher risk patients for prioritized drug susceptibility testing is useful for prevention and adapted treatment policies. The fact that crack cocaine use is embedded in complex social factors related to treatment barriers makes crack cocaine users a special population for TB treatment, and warrants further attention on modified treatment policies for helping detect and cure DRTB.

Strengths and limitations

Several notable strengths of this study include the fact that all cases and controls had confirmation of TB status, whether it was active pulmonary disease, latent TB infection, or DRTB. Our careful selection of controls to reflect the source population of cases was a critical component of a high-quality case-control study, even though this meant that well-matched controls were harder to identify and recruit, leading to

relatively small sample sizes for sub-analyses. Additional strengths of this dissertation include our comprehensive assessment of individual and residential variables, many of which were taken from Mexican surveys that can be used for future comparisons. Our findings found population-specific predictors of active TB and DRTB that were unique to the MMA. Lastly, our use of genomic data to test the effects of genetic ancestry on TB status was novel.

Several limitations of this dissertation are important to note. Power may have been limited due to small sample sizes within each chapter. TB is a complex disease, and it is likely that we did not capture all environmental risk factors in the MMA, and even those that we did collect may not have been perfectly measured. Lastly, our findings may be limited to urban areas in developing countries and may be less applicable for TB control efforts among rural populations.

Conclusions

In conclusion, we did not find an association with genetic ancestry and active TB or DRTB. Diabetes, marital status, and computer ownership were the main predictors of active pulmonary TB in our MMA sample. Crack cocaine use was a robust predictor of DRTB among our subset of participants with drug resistant and drug sensitive pulmonary TB disease. Overall, health, behavioral, and residential variables were the main predictors of TB status in the MMA, despite variation in genetic ancestry estimates throughout the population. It is long argued that genetic factors explain TB variation between populations, but our results suggest that there is no support for a genetic basis of disease risk that varies between parental populations that contributed to the MMA, or that genetic effects are low compared to environmental factors. Variation in TB rates across populations may be better understood by addressing contextual factors of socioeconomic and health conditions that have larger effects on active TB and DRTB susceptibility.

APPENDIX A

SUPPLEMENTARY TABLES FOR CHAPTER 2

Supplementary Table A.1. Crude associations between active pulmonary TB and individual characteristics (N=137)

Variable	Cases (n = 97)	Controls (n = 40)	Odds Ratio (95% CI)	*p*-value
	Mean ± s.d.	**Mean ± s.d.**		
Age in years	44.8 ± 16.9	41.4 ± 12.9	1.01 (0.99, 1.04)	0.25
European genetic ancestry %*	37.2 ± 10.8	39.4 ± 12.4	0.16 (0.004, 6.12)	0.32
Native American genetic ancestry % *	55.7 ± 12.3	53.1 ± 13.1	6.17 (0.20, 186.2)	0.30
African genetic ancestry % *	7.1 ± 2.3	7.5 ± 2.4	0.01 (<0.01, >999.9)	0.41
Mean total pack years**	6.7 ± 15.8	2.7 ± 6.1	1.03 (0.99, 1.08)	0.12
Second-hand smoke exposure (hours per day)	1.4 ± 3.6	0.9 ± 2.2	1.06 (0.92, 1.23)	0.41
	N (%)	**N (%)**		
Sex				
Female	44 (45.4)	15 (37.5)	Reference	--
Male	53 (54.6)	25 (62.5)	0.72 (0.34, 1.54)	0.40
Indigenous ethnicity				
Indigenous heritage	18 (18.6)	10 (25.0)	0.68 (0.28, 1.65)	0.41
Non-indigenous	79 (81.4)	30 (75.0)	Reference	--
Educational attainment				
Less than primary through secondary	70 (72.2)	20 (50.0)	2.35 (1.1, 5.0)	0.03
Commerical, high school, or higher	27 (27.8)	20 (50.0)	Reference	--
Principal lifetime employment				
Professional, semi-professional, student	26 (26.8)	18 (45.0)	Reference	--
Non-professional or unemployed	71 (73.0)	22 (55.0)	2.24 (1.04, 4.82)	**0.04**
Diabetes				
No	69 (71.1)	34 (85.0)	Reference	--
Yes	28 (28.9)	6 (15.0)	2.42 (0.92, 6.38)	**0.07**
History of alcohol problems				
No	84 (86.6)	39 (97.5)	Reference	--
Yes	13 (13.4)	1 (2.5)	6.04 (0.76, 47.79)	**0.06**
Asthma				
No			Reference	--
Yes	3 (3.1)	1 (2.5)	1.25 (0.13, 12.34)	0.85
Hypertension				
No			Reference	--
Yes	9 (9.3)	7 (17.5)	0.48 (0.17, 1.40)	0.17
Knowledge of TB airborne transmission				
No	21 (21.7)	3 (7.5)	3.41 (0.96, 12.16)	**0.06**
Yes	76 (78.4)	37 (92.5)	Reference	--
Knowledge that TB is curable				
No	5 (5.2)	3 (7.5)	0.68 (0.15, 2.98)	0.61
Yes	91 (94.8)	37 (92.5)	Reference	--
Marijuana use				
No	86 (88.7)	37 (92.5)	Reference	--
Yes	11 (11.3)	3 (7.5)	1.58 (0.42, 5.98)	0.50

Stimulant use (cocaine, methamphetamine)				
No	85 (87.6)	40 (100.0)	Reference	--
Yes	12 (12.4)	0 (0.0)	N/A***	N/A
Injected drug use				
No	92 (94.9)	40 (100.0)	Reference	--
Yes	5 (5.2)	0 (0.0)	N/A***	N/A
Inhalant use				
No	93 (95.9)	40 (100.0)	Reference	--
Yes	4 (4.1)	0 (0.0)	N/A***	N/A
Ever had BCG vaccination				
No, don't know	18 (18.6)	5 (12.8)	1.60 (0.55, 4.64)	0.39
Yes	79 (81.4)	35 (87.5)	Reference	--

*Individual genetic ancestry estimated from approximately 25,000 single nucleotide polymorphisms (SNPs)

**Total pack years calculation: (#cigarettes per day * years of smoking)/20

***Insufficient data for approximation

Supplementary Table A.2. Crude associations between active pulmonary TB and social characteristics (N=137)

Variable	Cases (n = 97)	Controls (n = 40)	Odds Ratio (95% CI)	*p*-value
	Mean ± s.d.	**Mean ± s.d.**		
Lifetime household crowding*	2.3 ± 1.6	2.5 ± 1.4	0.93 (0.74, 1.17)	0.55
Total number of windows in the house	4.8 ± 2.7	5.6 ± 2.8	0.91 (0.79, 1.03)	0.14
Current socioeconomic status**	151.2 ± 61.6	164.4 ± 59.8	1.0 (0.99, 1.00)	0.25
Lifetime socioeconomic status**	134.6 ± 66.3	145.9 ± 72.9	1.0 (0.99, 1.00)	0.38
Travel time to UANL Hospital (minutes)	67.8 ± 71.6	54.0 ± 30.8	1.01 (1.00, 1.01)	0.24
	N (%)	**N (%)**		
Household income per 15 days (pesos)				
<2,000	32 (35.2)	12 (30.8)	1.54 (0.51, 4.55)	0.43
2,001 – 5,999	45 (49.5)	19 (48.7)	1.35 (0.49, 3.76)	0.53
6,000 or higher	14 (15.4)	8 (20.5)	Reference	--
Marital status				
Single, divorced, separated, widow	50 (51.6)	12 (30.0)	2.48 (1.13, 5.44)	**0.02**
Married, free union	47 (48.5)	28 (70.0)	Reference	--
Current socioeconomic status**				
Highest, Upper-Middle	25 (25.8)	14 (35.0)	Reference	--
Middle	52 (53.6)	21 (52.5)	1.39 (0.61, 3.17)	0.44
Lowest, Low-Middle	20 (20.6)	5 (12.5)	2.24 (0.69, 7.28)	0.18
Windows in the bedroom				
No	11 (11.3)	0 (0)	N/A***	N/A
Yes	86 (88.7)	40 (100.0)	Reference	--
Running water inside home				
No	12 (12.4)	6 (15.0)	0.80 (0.28, 2.30)	0.68
Yes	85 (87.6)	34 (85.0)	Reference	--
Known close contact with someone with TB				
No, don't know	51 (52.6)	17 (42.5)	Reference	--
Yes	46 (47.4)	23 (57.5)	0.67 (0.32, 1.40)	0.28
Frequency of going to bed hungry (lifetime)				
Never	76 (78.4)	31 (77.5)	Reference	--
Sometimes, frequently	21 (21.7)	9 (22.5)	0.95 (0.39, 2.31)	0.91
Number of rooms in house (not including bathrooms, hallways, patios, rooftops)**				
1-4	53 (55.2)	21 (52.5)	1.12 (0.53, 2.34)	0.77
5 or more	43 (44.5)	19 (47.5)	Reference	--
Number of complete bathrooms with shower and toilet exclusive to members of household**				
0	8 (8.3)	1 (2.5)	3.54 (0.43, 29.3)	0.24
1 or more	88 (91.7)	39 (97.5)	Reference	--
Presence of functioning shower in the house**				
No	11 (11.5)	1 (2.5)	5.05 (0.63, 40.48)	**0.09**
Yes	85 (88.5)	39 (97.5)	Reference	--
Number of lights in house (on ceiling, walls, floor lamps, desk lamps, etc.)**				
0-5	31 (32.3)	10 (25.0)	1.40 (0.48, 4.03)	0.54

6-10	45 (46.9)	21 (15.4)	0.96 (0.38, 2.47)	0.94
11 or more	20 (20.8)	9 (22.5)	Reference	--
Material of household floor**				
Earth or cement	58 (60.4)	23 (57.5)	1.13 (0.53, 2.39)	0.75
Other (e.g., tile)	38 (39.6)	17 (42.5)	Reference	--
Number of cars at house (excluding taxis)**				
0	49 (51.0)	19 (47.5)	1.13 (0.40, 3.17)	0.82
1	31 (32.3)	14 (35.0)	0.97 (0.33, 2.88)	0.95
2 or more	16 (16.7)	7 (17.5)	Reference	--
Number of functioning color televisions in house**				
0	2 (2.1)	1 (2.5)	0.95 (0.08, 10.93)	0.97
1	29 (30.2)	8 (20.0)	1.73 (0.71, 4.22)	0.23
2 or more	65 (67.7)	31 (77.5)	Reference	--
Number of personal computers**				
0	59 (62.1)	17 (42.5)	2.22 (1.05, 4.70)	**0.04**
1 or more	36 (37.9)	23 (57.5)	Reference	--
Presence of gas or electric stove in house**				
No	2 (2.1)	1 (2.5)	0.83 (0.07, 9.42)	0.88
Yes	94 (70.6)	39 (97.5)	Reference	--
Educational attainment of highest income earner in household				
Less than primary through secondary	65 (68.9)	20 (50.0)	2.32 (1.1, 5.0)	0.03
Commerical, high school, or higher	28 (30.1)	20 (50.0)	Reference	--
Residence as a child				
Ranch or small town	31 (32.0)	9 (22.5)	Reference	--
City	66 (68.0)	31 (77.5)	0.62 (0.26, 1.46)	0.27
Normal mode of transportation				
Car	23 (23.7)	11 (27.5)	Reference	--
Public bus	63 (65.0)	26 (65.0)	1.16 (0.50, 2.72)	0.73
Other (taxi, metro, bike, moto)	11 (11.3)	3 (7.5)	1.75 (0.41, 7.58)	0.45
Factory within 10 blocks of house				
No	74 (76.3)	32 (80.0)	Reference	--
Yes	23 (23.7)	8 (20.0)	1.24 (0.50, 3.07)	0.64
Residence in MMA municipalities (SES groupings based on geospatial analysis)				
San Pedro, San Nicolás (high)	8 (8.3)	6 (15.0)	Reference	--
Monterrey, Guadalupe, García, Santa Catarina (medium, medium-low)	57 (58.8)	20 (50.0)	2.14 (0.66, 6.91)	0.20
Apodaca, Escobedo, Juarez (very low)	19 (19.6)	10 (25.0)	1.43 (0.39, 5.26)	0.59
Outside the MMA	13 (13.4)	4 (10.0)	2.24 (0.52, 11.4)	0.26
Ever been a resident in prison				
No	86 (88.7)	39 (97.5)	Reference	--
Yes	11 (11.3)	1 (2.5)	5.00 (0.62, 40.00)	**0.10** (0.18 fishers)
Ever been a resident of a homeless shelter				
No	95 (97.9)	40 (100.0)	Reference	--
Yes	2 (2.1)	0 (0.0)	N/A***	N/A
Ever been a resident in a nursing home				
No	97 (100.0)	40 (100.0)	Reference	--
Yes	0 (0.0)	0 (0.0)	N/A***	N/A

*Household crowding index: Number of people living in house / Number of rooms for sleeping ; higher numbers mean more crowding, any number over 1.0 is considered crowding
**Taken from the AMAI Mexican socioeconomic 10-item survey (2008)
***Insufficient data for approximation

Supplementary table A.3. Significant associations of 'computer in the home' with socioeconomic variables

Variable	**No Computers** (n = 76)	**1+ Computers** (n = 59)	***p*-value**
Educational attainment			
Less than primary through secondary	64 (84.2)	25 (42.4)	**<0.0001**
Commerical, high school, or higher	12 (15.8)	34 (57.6)	
Principal lifetime employment			
Professional, semi-professional, student	11 (14.5)	33 (55.9)	**<0.0001**
Non-professional or unemployed	65 (85.5)	26 (44.1)	
Household income per 15 days (pesos)			
<2,000	35 (48.0)	7 (12.7)	**<0.0001**
2,001 – 5,999	43 (46.6)	30 (54.6)	
6,000 or higher	4 (5.5)	18 (32.7)	
Current socioeconomic status**			
Highest, Upper-Middle	5 (6.6)	34 (57.6)	**<0.0001**
Middle	47 (61.8)	25 (42.4)	
Lowest, Low-Middle	24 (31.6)	0 (0.0)	
Educational attainment of highest income earner in household			**<0.0001**
Less than primary through secondary	60 (81.1)	24 (41.4)	
Commerical, high school, or higher	14 (18.9)	34 (58.6)	

APPENDIX B

SUPPLEMENTARY TABLE FOR CHAPTER 4

Supplementary table B.1. Associations of crack cocaine use among drug-resistant and drug-sensitive TB cases with individual and social factors (N=95)

Variable	No crack cocaine use N=85	Yes, history of crack cocaine use N=10	*p*-value
Age in years	45.5 ± 17.7	39.4 ± 8.2	0.07
European genetic ancestry %	30.9 ± 11.5	35.9 ± 7.1	0.21
Native American genetic ancestry %	60.9 ± 12.9	54.5 ± 7.9	0.15
African genetic ancestry %	8.1 ± 3.0	9.7 ± 2.3	0.14
Mean total pack years*	61 ± 16.1	12.1 ± 13.7	0.22
		N (%)	
Sex			
Female	41 (48.2)	3 (30.0)	0.33
Male	44 (51.8)	7 (70.0)	
Self-reported indigenous ethnicity			
Indigenous heritage	16 (18.2)	1 (10.0)	0.68
Non-indigenous	69 (81.2)	9 (90.0)	
Educational attainment			
Less than primary through secondary	58 (68.2)	10 (100.0)	**0.058**
Commerical, high school, or higher	27 (31.8)	0 (0.00)	
Principal lifetime employment			
Professional, semi-professional, student	25 (29.4)	0 (0.0)	**0.058**
Non-professional or unemployed	60 (70.6)	10 (100.0)	
Diabetes			
No	59 (69.4)	7 (70.0)	1.0
Yes	26 (30.6)	3 (30.0)	
History of alcohol problems			
No	78 (91.8)	5 (50.0)	**0.003**
Yes	7 (8.2)	5 (50.0)	
Asthma			
No	83 (97.7)	10 (100.0)	1.0
Yes	2 (2.4)	0 (0.0)	
Hypertension			
No	76 (89.4)	10 (100.0)	0.59
Yes	9 (10.6)	0 (0.0)	
Hepatitis			
No	83 (97.7)	8 (80.0)	**0.05**
Yes	2 (2.4)	2 (20.0)	
Marijuana use			
No	82 (96.5)	3 (30.0)	**<0.0001**
Yes	3 (3.5)	7 (70.0)	
Methamphetamine use			
No	85 (100.0)	7 (70.0)	**<0.0001**
Yes	0 (0.0)	3 (30.0)	
Injection drug use			

No	85 (100.0)	6 (60.0)	**<0.0001**
Yes	0 (0.0)	4 (40.0)	
Inhalant use			
No	84 (98.8)	7 (70.0)	**0.003**
Yes	1 (1.2)	3 (30.0)	
Previously treated for TB			
No (<1 month of treatment, 'new case')	22 (25.9)	1 (10.0)	0.44
Yes (≥1 month of treatment, 'previously treated')	63 (74.1)	9 (90.0)	
Knowledge of TB airborne transmission			
No	18 (21.2)	1 (10.0)	0.68
Yes	67 (78.8)	9 (90.0)	
Knowledge that TB is curable			
No	5 (5.9)	0 (0.0)	1.0
Yes	80 (94.1)	9 (100.0)	
Social stigma by preference to treat a family member with TB in secrecy			0.36
No	72 (84.7)	7 (70.0)	
Yes	13 (15.3)	3 (30.0)	
Ever saw or felt discrimination against TB			
No	49 (59.8)	7 (70.0)	0.73
Yes	33 (40.2)	3 (30.0)	
Ever felt fear related to TB			
No	40 (47.1)	3 (30.0)	0.50
Yes	45 (52.9)	7 (70.0)	
BCG vaccination			
No	16 (18.8)	1 (10.0)	0.68
Yes	69 (81.2)	9 (90.0)	
Close contact with a TB patient			
No	43 (50.6)	8 (80.0)	0.10
Yes	42 (49.4)	2 (20.0)	
Place first learned about TB			
Health clinic, doctors, hospital	62 (72.9)	10 (100.0)	0.11
Other (family, public media, school, books)	23 (27.1)	0 (0.0)	
Use of alternative remedies/therapies to treat TB			
No	65 (92.9)	10 (100.0)	1.0
Yes	5 (7.1)	0 (0.0)	
Travel time to UANL Hospital (minutes)	65 ± 65	57 ± 30	0.50
Marital status			
Single, divorced, separated, widow	42 (49.4)	7 (70.0)	0.32
Married, free union	43 (50.6)	3 (30.0)	
Household income per 15 days (pesos)			
<2,000	25 (31.3)	5 (55.6)	0.28
2,001 – 5,999	41 (51.3)	4 (44.4)	
6,000 or higher	14 (17.5)	0 (0.00)	
Frequency of going to bed hungry (lifetime prevalence)			0.69
Never	67 (78.8)	7 (70.0)	
Sometimes, frequently	18 (21.2)	3 (30.0)	
Running water inside home			
No	9 (10.6)	3 (30.0)	0.11
Yes	76 (89.4)	7 (70.0)	
Windows in bedroom			
No	7 (8.2)	4 (40.0)	**0.01**

Yes	78 (91.8)	6 (60.0)	
Normal mode of transportation			
Personal car	23 (27.1)	0 (0.0)	0.11
Other (public bus, metro, taxi, bike)	62 (72.9)	10 (100.0)	
Residence in MMA municipalities (SES groupings based on geospatial analysis)			
San Pedro, San Nicolás (high)	8 (9.4)	0 (0.0)	0.62
Monterrey, Guadalupe, García, Santa Catarina (medium, medium-low)	49 (57.7)	7 (70.0)	
Apodaca, Escobedo, Juarez (very low)	18 (21.2)	1 (10.0)	
Outside the MMA	10 (11.8)	2 (20.0)	
Ever been a resident in prison			
No	78 (91.8)	7 (70.0)	0.07
Yes	7 (8.2)	3 (30.0)	
Ever been a resident of a homeless shelter			
No			
Yes	84 (98.8)	9 (90.0)	0.20
	1 (1.2)	1 (10.0)	
Number of rooms in house (not including bathrooms, hallways, patios, rooftops)**			
1-4	44 (51.8)	7 (77.9)	0.17
5 or more	41 (48.2)	2 (22.2)	
Number of complete bathrooms with shower and toilet exclusive to members of household**			
0	7 (8.2)	1 (11.1)	0.57
1 or more	78 (91.8)	8 (88.9)	
Presence of functioning shower in the house**			
No	9 (10.6)	2 (22.2)	0.28
Yes	76 (89.4)	7 (77.8)	
Number of lights in house (on ceiling, walls, floor lamps, desk lamps, etc.)**			
0-5	22 (25.9)	7 (77.8)	**0.007**
6-10	44 (51.8)	1 (11.1)	
11 or more	19 (22.4)	1 (11.1)	
Material of household floor**			
Earth or cement	49 (57.7)	7 (77.8)	0.30
Other (e.g., tile)	36 (42.4)	2 (22.2)	
Number of cars at house (excluding taxis)**			
0	40 (47.1)	7 (77.8)	
1	30 (35.3)	1 (11.1)	0.26
2 or more	15 (17.7)	1 (11.1)	
Number of functioning color televisions in house**			
0	1 (1.2)	1 (11.1)	**0.02**
1	23 (27.1)	5 (55.6)	
2 or more	61 (71.8)	3 (33.3)	
Number of personal computers**			
0	50 (59.5)	8 (88.9)	0.15
1 or more	34 (40.5)	1 (11.1)	
Presence of gas or electric stove in house**			
No	2 (2.4)	0 (0.0)	1.0
Yes	83 (97.7)	9 (100.0)	
Educational attainment of highest income earner			

in household**			
Less than primary through secondary	55 (67.1)	8 (88.9)	0.27
Commerical, high school, or higher	27 (32.9)	1 (11.1)	
Current socioeconomic status***			
Highest, Upper-Middle	24 (28.2)	1 (10.0)	**0.01**
Middle	48 (56.5)	3 (30.0)	
Lowest, Low-Middle	13 (15.3)	6 (60.0)	

*Total pack years calculation: (#cigarettes per day * years of smoking)/20
**Individual questions taken from the AMAI Mexican socioeconomic 10-item survey (2008)
***Compiled index of the AMAI Mexican socioeconomic 10-item survey (2008)

APPENDIX C

LETTER OF ACCEPTANCE FOR COLLABORATION AT UANL

UANL

July 22, 2009

Keith L. Hunley
Assistant Professor
Department of Anthropology
University of New Mexico

Thanks for your kind invitation to be members of the investigator group of the study entitled "Effecst of genetic ancestry and socio-cultural factors on susceptibility to tuberculosis in Mexico".

Adrian Rosas and I are committed to perform any duty we will be assigned. Our institution will support this project allowing us to use its facilities and having access to the patients at the TB Clinic. Beside we will receive any person who may need to visit our institution.

We have performed research for many years and we are aware of the Good Clinical Practices standards and the Ethic regulations we must follow. We are sure this collaborative research project will be very productive to better understand the TB behavior.

Sincerely,

Adrian Rosas, PhD

Adrian Rendon, M.D.

CIPTIR

APPENDIX D

PROTOCOL FOR DNA EXTRACTION

Name: _____
Date: _____
Extractions: _____

Puregene DNA Isolation Kit Protocol
Modified by Molecular Epidemiology Lab, MSKCC
Used by Dr. Keith Hunley's Lab in the UNM Department of Anthropology

DNA Isolation from Buccal Cells in Mouthwash
Expected DNA Yield Range: 4 µg – 40 µg, Mean: 16 µg
Estimated Time to Process Four Samples: 2hrs, 45 mins – 3hrs

Buccal Cell collection and Cell Lysis
1. Dispense 10 ml Original Mint Scope Mouthwash into a 50 or 15 ml tube.
2. Collect buccal cells by swishing orally the 10 ml of mouthwash for 1 minute (time it if needed) and spitting back into the 50 ml tube.
Notes: i) For best results, wait at least one hour after eating or drinking to collect buccal cells.
ii) Buccal cells are stable at room temperature for at least 7 days in the mouthwash solution if mouthwash contains ethanol up to 15%; otherwise add 2 ml of 70% ethanol solution to collected sample. That increases storage time while controlling for bacterial and fungi growth
iii) If samples are frozen, allow to thaw to room temperature (1-2 hours)
3. Centrifuge at 2,000 x g (4,000 rpm in lab centrifuge) for 10 minutes to concentrate the cells. Immediately pour off supernatant leaving behind 100 µl of residual liquid. *Loose pellets will result if samples sit too long after centrifugation. Repeat this step if that occurs and pour supernatant immediately after spinning.*
4. Vortex vigorously to resuspend the cells in the residual supernatant (up to 1 minute may be necessary).
5. Add 3 ml **Cell Lysis Solution** to the resuspended cell and vortex 5 seconds at medium speed to maximize contact between cells and Cell Lysis Solution.
6. Add 15 µl of **Proteinase K** (20 mg/ml) and incubate at 56ºC for 1 hour.

Protein Precipitation
1. Cool sample to room temperature. (10-15 minutes)
2. Add 1 ml **Protein Precipitation Solution** to the cell lysate.
3. Vortex samples at high speed for 20 seconds to mix the **Protein Precipitation Solution** uniformly with the lysate.
4. Place tube in an ice bath for 10 minutes to ensure a tight pellet in Step 5 below.
5. Centrifuge at 2,000 x g (4,000 rpm in lab centrifuge) for 10 minutes. The precipitated proteins should form a tight, green pellet.

DNA Precipitation
1. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 50 or 15 ml tube containing 3 ml 100% **Isopropanol** (2-propanol) and 5 µl **Glycogen Solution** (20 mg/ml). *(Once you add the glycogen you MUST finish entire protocol.)

2. Mix the sample by inverting gently 50 times and keep tube at room temperature for at least 5 minutes.
3. Centrifuge at 2,000 x g (4,000 rpm in lab centrifuge) for 10 minutes. The DNA may or may not be visible as a small white pellet, depending on yield.
4. Pour off the supernatant and drain tube briefly on clean absorbent paper. (Drain upside down on absorbent paper for a few minutes.) Add 3 ml cold 70% **Ethanol** by gently pipetting down the side of the tube to wash the DNA pellet. Do not shake or agitate the tube.
5. Centrifuge at 2,000 x g (4,000 rpm in lab centrifuge) for 3 minutes. Carefully pour off the Ethanol.
6. Invert and drain tube on clean absorbent paper and allow to air dry for 1-2 hours. Leave space so air can help evaporate the ethanol. ALL the ethanol MUST be completely gone.

DNA Hydration
1. Add 200 µl of **DNA Hydration Solution.** Vortex briefly and spin down the tubes on "quick run" (about 800 rpm) in lab centrifuge.
2. Allow DNA to rehydrate by incubating at 65 ºC for 1 hour.
3. For storage, sample may be vortexed, centrifuged briefly and transferred to a 1.5 or 2 ml tube. Store DNA at 2-8ºC. For long term storage, store at -20ºC or -80ºC.

Reading the DNA Results
1. Can read DNA yield at the 260/280 ratio after waiting 1 night of DNA at room temperature in hydration solution on a rotator (shaken gently).
    a. Want 260/280 ratio between 1.6-1.8. If > 1.8 then getting too much residual alcohol and phenols. If < 1.6 then bacteria or some other contaminants in sample.
    b. For DNA, want a nucleic acid concentration of minimum of 400 (if you are getting super high yields, e.g., >1,000, it could be indicative of infection by bacteria or fungus).
2. When prepping the nanodrop, use the SAME hydration solution to "blank" as is in the samples. Use only 2 µl of solution to read nanodrop
    a. Nanodrop machine should be re-calibrated every year
    b. Check results page- "Factor" should be at 50.0
    c. Want 260 to be similar for samples, want 280 to be similar for samples

APPENDIX E

QUESTIONNAIRE FOR FACE-TO-FACE INTERVIEWS (ENGLISH VERSION)

"The Effects Genetic Ancestry and Socio-Cultural Factors on the Susceptibility of Active Tuberculosis in Mexico"

Questionnaire: Face-to-face interview form

GENERAL INFORMATION

Participant Name: _____

Medical record number (UANL): _____

Participant ID#: _____

Participant's Phone Number: _____

Interviewer Name: _____

Date of interview: _____ (mm/dd/yy)

Patient registered clinic:

_____

Time interview began: _____ Time interview finished: _____

Researcher validated:

Type of participation: [ ] Latent [ ] Active [ ] Unknown

Date of diagnosis: _____ year or _____ age

Type of TB: [ ] Pulmonary [ ] Other: _____

Drug-resistant: [ ] Yes [ ] No [ ] Unknown

SOCIOECONOMIC AND DEMOGRAPHIC INFORMATION

Sex: [ ] Female [ ] Male

In what month and year were you born? _____ (mm/yy) [ ] Don't Know (1)

How old are you now? _____ (years) (1)

What is your current marital status? (1)

[ ] Civil union
[ ] Married
[ ] Widowed
[ ] Divorced
[ ] Separated
[ ] Single

What was the highest level of school that you completed?

[ ] No studies	[ ] Primary incomplete
[ ] Primary completed	[ ] Middle school incomplete
[ ] Middle school complete	[ ] Commercial degree
[ ] Technical degree	[ ] High school incomplete
[ ] High school complete	[ ] Bachelors incomplete
[ ] Bachelors complete	[ ] Specialist, Masters degree
[ ] Doctoral degree	[ ] Don't know /no answer

Where are you from? _____ (12) (city)

Currently, do you live in Monterrey? [ ] Yes [ ] No

If "yes," how much time have you lived in Monterrey? (1)
[ ] ____ years

If "no," in what locality do you live? (1;2)
City: _____
State: _____
Country: _____
[ ] N.R.

When you were younger, we will say until around 12 years old, did you live the majority of the time on a ranch, in a pueblo/small town, or in a city? (1)
[ ] Ranch [ ] Pueblo/small town [ ] City [ ] N.R.

What is your current home address?

_____

Do you consider yourself indigenous? (10)
[ ] Yes
[ ] No
[ ] N.R.
[ ] Don't know

Do you speak an indigenous language? [ ] Yes [ ] No (10)
If "yes," which language?

[ ] Nauhuatl	[ ] Maya	[ ] Zapoteco	[ ] Mixteco
[ ] Tzotzil/tzetzal	[ ] Otomi	[ ] Totonaca	[ ] Mazateco
[ ] Chol	[ ] Huasteco	[ ] Chinanteco	[ ] Mazahua
[ ] Mixe	[ ] Other (specify): _____		

Do your parents or other family members speak (or did they speak) an indigenous language?
[ ] Yes [ ] No

If "yes," which language?

[ ] Nauhuatl	[ ] Maya	[ ] Zapoteco	[ ] Mixteco
[ ] Tzotzil/tzetzal	[ ] Otomi	[ ] Totonaca	[ ] Mazateco

[ ] Chol [ ] Huasteco [ ] Chinanteco [ ] Mazahua
[ ] Mixe [ ] Other (specify): _____

Are your parents from Mexico? [ ] Yes [ ] No

If "no," where is your _____ from? _____ (country)

Are your grandparents on both sides from Mexico? [ ] Yes [ ] No

If "no," where is your _____ from? _____ (country)

Are your greatgrandparents on both sides from Mexico? [ ] Yes [ ] No

If "no," where is your _____ from? _____ (country)

Are you currently employed? [ ] YES [ ] NO

If "YES," what is your current occupation?

_____

How many years have you had this occupation? _____ years

What was your occupation before this job?

_____ occupation

For how many years did you have this occupation? _____ years

What type of work have you had for most of your life? (International Labor Organization)

[ ] Professional (manager, senior officials, professionals)

[ ] Semi-professional (technicians, office workers, skilled laborers)

[ ] Nonprofessional (service and sales workers, farmers, unskilled workers, homemakers)

SOCIOECONOMIC LEVEL <u>NOW</u>

What is the total number of rooms in the house, bedrooms? Please don't include bathrooms, half bathrooms, hallways, courtyards, or rooftops. (5)

Response	Points
[ ] 1	0
[ ] 2	0
[ ] 3	0
[ ] 4	0
[ ] 5	8
[ ] 6	8
[ ] 7 or more	14

How many bathrooms are complete with a shower and W.C. are exclusive to the use of the members of your household? (5)

Response	Points
[ ] 0	0
[ ] 1	13
[ ] 2	13
[ ] 3	31
[ ] 4 or more	48

In the house, is there a shower that functions in one of the bathrooms? (5)

Response	Points
[ ] No	0
[ ] Yes	10

Counting all the sources used to illuminate your house, including those on the ceiling, walls and floor lamps, desk, etc., tell me, how many lights are there in your house? (5)

Response	Points
[ ] 0-5	0
[ ] 6-10	15
[ ] 11-15	27
[ ] 16-20	32
[ ] 21 or more	46

Is the floor of your house predominately earth, cement, or of another type of finish? (5)

Response	Points
[ ] Earth or cement	0
[ ] Another type of material or finish	11

What type? _____

How many cars do you own, excluding taxis, do you have at home? (5)

Response	Points
[ ] 0	0
[ ] 1	22
[ ] 2	41
[ ] 3 or more	58

How many televisions (color) that are functioning do you have en your house? (5)

Response	Points
[ ] 0	0
[ ] 1	26
[ ] 2	44
[ ] 3 or more	58

How many personal computers, including desk tops, lap-tops, are running in your house? (5)

Response	Points
[ ] 0	0
[ ] 1	17
[ ] 2 or more	29

In your house, is your stove gas or electric? (5)

Response	Points
[ ] Not gas or electric	0
[ ] Yes gas or electric	20

If its not gas or electric, what type of stove is it? _____
If you use (or have used) wood, for how long? _____

Thinking about the person that is the major income earner for the house, what was the highest level of school that he/she completed? (5)

Response	Points
[ ] No studies	0
[ ] Primary incomplete	0
[ ] Primary completed	22
[ ] Middle school incomplete	22
[ ] Middle school complete	22
[ ] Commercial degree	38
[ ] Technical degree	38
[ ] High school incomplete	38
[ ] High school complete	38
[ ] Bachelors incomplete	52
[ ] Bachelors complete	52
[ ] Specialist, Masters degree	72
[ ] Doctoral degree	72
[ ] Don't know /no answer	

Table of points per level:

Level	Points
E	Until 60
D	61-101
D+	102-156
C	157-191
C+	192-241
A/B	242 and more

Research calculated AMAI SES:
_____ Total Points
_____ Final Level

Approximately what is the 15-day salary (income) total in pesos that is earned by your house? (3)

[ ] Less than 2,000
[ ] 2,001-5,999
[ ] 6,000-9,999
[ ] 10,000-13,999
[ ] 14,000-17,999
[ ] 18,000 or more
[ ] N.R.
[ ] Don't know

What type of health insurance do you have?

[ ] None
[ ] Marina/Defensa
[ ] ISSSTE Estatal
[ ] Pemex
[ ] Private
[ ] IMSS
[ ] ISSSTE (public)
[ ] Seguro Popular (SSA)
[ ] Servicio Médico de la Universidad (UANL)
[ ] Other Institution (specify): _____
[ ] N.R.
[ ] Don't know

What socioeconomic level did your parents have when you lived with them (when you were growing up)?

[ ] Low resources
[ ] Medium resources
[ ] High resources
[ ] N.R.

HOUSING QUESTIONS <u>NOW</u>

Thinking in the house where you are living now:

Do you live in house that you: [ ] own [ ] rent [ ] is your family's [ ] other: _____

For how many years have you lived there? _____ years

How many people live with you- including kids and adults? _____

Of these people that live with you, how many of them work and receive a salary? _____ people

How many rooms in the house are used to sleep? _____ rooms to sleep (1)

Does the house have a place to cook? (1)
[ ] Yes [ ] No

If "Yes," for what other purposes is the space used for?
[ ] The place is only for cooking
[ ] Other uses of the space: _____
[ ] Don't know

Approximately how many windows are in the house? _____ total windows

Does your bedroom have windows? (9) [ ] Yes [ ] No

If "yes," how many? _____
How many hours in the day are the windows open? (mark all that apply)
[ ] _____ hours during the winter
[ ] _____ hours during the summer
[ ] Don't know

How is your house heated?
[ ] Gas
[ ] Electricity
[ ] Coal
[ ] Wood furnace
[ ] Other: _____
[ ] N.R.
[ ] No heater in house

What material are the majority of the walls of your home made out of? (1)
[ ] Cardboard sheet
[ ] Palm or bamboo or Carrizo
[ ] Embarro o bajareque
[ ] Wood
[ ] Asbestos sheet or metallic sheet
[ ] Adobe
[ ] Stone, brick, cement block
[ ] N.R.
[ ] Don't know

Do you have a refrigerator in your house? (9)
[ ] YES [ ] NO [ ] Don't know

Does your house have indoor running water? (1)

[ ] Yes, inside the house
[ ] Yes, outside the house but in the same area
[ ] No
[ ] N.R.

Is there a factory or industry less than 10 blocks from your house? (9) [ ] Yes [ ] No [ ] N.R.

If "yes," what does the factory produce?

[ ] Cement [ ] Metals (iron, copper, bronze)
[ ] Beer [ ] Soda (Coca-Cola, Pepsi)
[ ] Food (Bimbo, Cookies) [ ] Other: _____

With what frequency can you see or smell pollution from this factory?

[ ] Never o a few times each year
[ ] A few times each month
[ ] A few times each week
[ ] Ever day

How long does it take you to travel to this hospital? _____ [ ] hours _____ [ ] minutes

Researcher calculated total minutes: _____ minutes

How do you normally travel around Monterrey?

[ ] Public bus
[ ] Car
[ ] Taxi
[ ] Metro
[ ] Motorbike
[ ] Walk
[ ] Bicycle
[ ] Other: _____

SOCIOECONOMIC LEVELS <u>IN THE PAST</u>

In the 5-10 years previous to the TB diagnosis (or if you have never had TB, in the 5-10 years previous to your positive TB skin test) have you changed your house (living situation)?
[ ] Si [ ] No

If "yes," how many times have you changed your house in the 5-10 years before you had the TB diagnosis (or if you never had TB, in the 5-10 years before your positive test)?
_____ times

If "yes," please think about the house where you lived the majority of the time around the 5-10 years before you had the TB diagnosis (or if you never had TB, 5-10 years before your positive test):

What material was the majority of the walls of your home made out of? (1)

[ ] Cardboard sheet
[ ] Palm or bamboo or Carrizo
[ ] Embarro o bajareque
[ ] Wood
[ ] Asbestos sheet or metallic sheet
[ ] Adobe
[ ] Stone, brick, cement block
[ ] N.R.
[ ] Don't know

Did you have a refrigerator in your house? (9)

[ ] YES [ ] NO [ ] Don't know

Did your house have indoor running water? (1)

[ ] Yes, inside the house
[ ] Yes, outside the house but in the same area
[ ] No
[ ] N.R.

What was the total number of rooms in the house, bedrooms? Please don't include bathrooms, half bathrooms, hallways, courtyards, or rooftops. (5)

Response	Points
[ ] 1	0
[ ] 2	0
[ ] 3	0
[ ] 4	0
[ ] 5	8
[ ] 6	8
[ ] 7 or more	14

How many bathrooms were complete with a shower and W.C. were exclusive to the use of the members of your household? (5)

Response	Points
[ ] 0	0
[ ] 1	13
[ ] 2	13
[ ] 3	31
[ ] 4 or more	48

In the house, was there a shower that functioned in one of the bathrooms? (5)

Response	Points
[ ] No	0
[ ] Yes	10

Counting all the sources used to illuminate your house, including those on the ceiling, walls and floor lamps, desk, etc., tell me, how many lights were there in your house? (5)

Response	Points
[ ] 0-5	0

[ ] 6-10 15
[ ] 11-15 27
[ ] 16-20 32
[ ] 21 or more 46

Was the floor of your house predominately earth, cement, or of another type of finish? (5)

Response	Points
[ ] Earth or cement	0
[ ] Another type of material or finish	11

What type? _____

How many cars did you own, excluding taxis, did you have at home? (5)

Response	Points
[ ] 0	0
[ ] 1	22
[ ] 2	41
[ ] 3 or more	58

How many televisions (color) that were functioning did you have in your house? (5)

Response	Points
[ ] 0	0
[ ] 1	26
[ ] 2	44
[ ] 3 or more	58

How many personal computers, including desk tops, lap-tops, were running in your house? (5)

Response	Points
[ ] 0	0
[ ] 1	17
[ ] 2 or more	29

In your house, was your stove gas or electric? (5)

Response	Points
[ ] Not gas or electric	0
[ ] Yes gas or electric	20

If it was not gas or electric, what type of stove was it? _____
If you used wood, for how long? _____

Thinking about the person that was the major income earner for the house, what was the highest level of school that he/she completed? (5)

Response	Points
[ ] No studies	0
[ ] Primary incomplete	0
[ ] Primary completed	22
[ ] Middle school incomplete	22
[ ] Middle school complete	22
[ ] Commercial degree	38
[ ] Technical degree	38
[ ] High school incomplete	38

Table of points per level:

Level	Points
E	Until 60
D	61-101
D+	102-156
C	157-191
C+	192-241
A/B	242 and more

[ ] High school complete 38
[ ] Bachelors incomplete 52
[ ] Bachelors complete 52
[ ] Specialist, Masters degree 72
[ ] Doctoral degree 72
[ ] Don't know /no answer

Research calculated AMAI SES:
_____ Total Points
_____ Final Level

How many people were living with you- including kids and adults? _____

How many rooms in the house were used to sleep? _____ rooms to sleep (1)

How often did you go to sleep feeling hungry because there wasn't food?
[ ] Never [ ] Sometimes (1-3 nights/wk) [ ] Frequently (4-6 nights/wk) [ ] Every night

TB HISTORY

[***Cross-validated with clinical records***]

At any time have you received the skin test for tuberculosis (PPD)?
[ ] Yes [ ] No [ ] Don't know

Date of application of PPD: _____ (mm/dd/yr)

Date of reading of PPD: _____ (mm/dd/yr)

Result: [ ] Positive [ ] Negative [ ] Don't know

At any time, have you be given a vaccine- BCG- en the arm against tuberculosis? (the one that leaves a scar) (1)?
[ ] Yes [ ] No [ ] Don't know

Do you have a scar on your arm (right or left)? [ ] Yes [ ] No [ ] Don't know

Have you had close contact (e.g., lived) with anyone who is/was sick with tuberculosis?
[ ] YES [ ] NO [ ] Don't know

If "yes," when? _____ (year) or _____ (age)

Is there anyone in your family that has or has had tuberculosis? (12)
[ ] Yes [ ] No [ ] Don't know

If "yes," who? _____

Have you ever had to take medications for tuberculosis?
[ ] YES [ ] NO [ ] Don't know

If "YES," how many months were you on the tuberculosis medications? _____

How many pills did you take per day? _____

Did you completely finish your treatment? [ ] YES [ ] NO [ ] Don't know

If "No," why not?

[ ] Transportation to the clinic was difficult

[ ] I felt bad when I took them

[ ] The clinic did not have the medications

[ ] I forgot to take them

[ ] There were too many medications to take in one day

[ ] I didn't understand why I was taking them

[ ] Other reasons: _____

Date of initiation of treatment: _____ (month/yr)

Date of end of treatment: _____ (month/yr)

Besides those medications from the doctor for tuberculosis, have you used other medicines or tried other things for the treatment of tuberculosis- for example, natural therapies or medicines, herbs?

[ ] Yes [ ] No [ ] Don't know

If "yes," what else have you used or tried?

_____

How is tuberculosis spread from one person to another, in your opinion? *CHECK ALL THAT APPLY:* (4)

[ ] Through the air by coughing or sneezing
[ ] Through sharing utensils
[ ] Sharing towels
[ ] Through touching a person with tuberculosis
[ ] Through sexual contact
[ ] From the genes of the parents
[ ] Supernatural
[ ] Other: _____
[ ] Working too much
[ ] Cold weather
[ ] Malnutrition
[ ] Sharing food
[ ] Through mosquito bites
[ ] By the mind
[ ] Don't know

Do you believe that tuberculosis has a cure? (4)

[ ] Yes [ ] No [ ] Don't know

If a member of your family got tuberculosis, would you want it to remain a secret or not? (4)

[ ] Yes, remain a secret
[ ] No
[ ] Don't know/depends on : _____

Have you felt any discrimination in your community because you have tuberculosis?

[ ] Yes [ ] No [ ] Don't know

If "yes," what types of discrimination?

Do you have any fears in relation to tuberculosis?

[ ] Yes [ ] No [ ] Don't know

If "yes," what fears do you have?

How did you learn about tuberculosis?

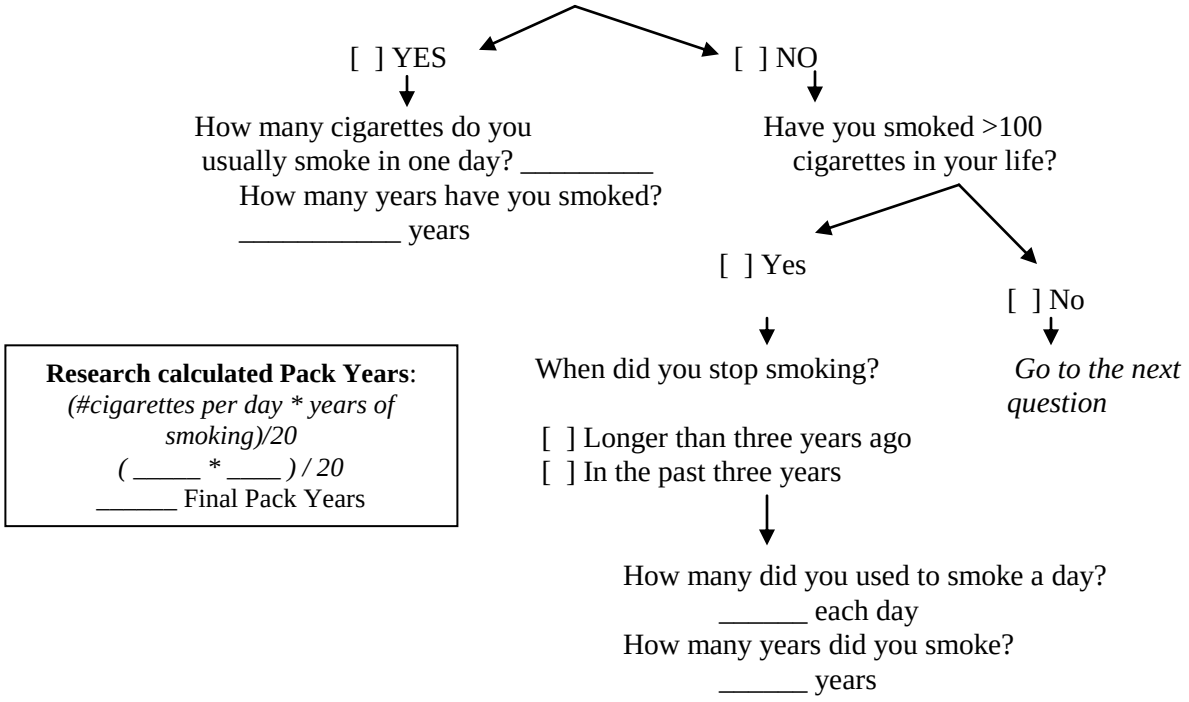
[ ] Clinic, hospital [ ] Doctors
[ ] Family, friends [ ] TV, movies
[ ] Radio [ ] Magazines, newspapers
[ ] Internet [ ] Other sources: _____

HEALTH PROFILE

[Cross-validate from clinical records]

(For Women) Are you currently pregnant? [ ] Yes [ ] No [ ] Don't know
If "YES," how many weeks are you in the pregnancy? _____ weeks

Do you currently smoke cigarettes or use tobacco?



Research calculated Pack Years:
*(#cigarettes per day * years of smoking)/20*
*(_____ * ____) / 20*
_____ Final Pack Years

What (other) type of tobacco do you currently use, if any? (2)

[ ] None
[ ] Pipe
[ ] Chewing tobacco
[ ] Snuff
[ ] Cigars
[ ] Other: _____

Are you around cigarette smoke even if you're not smoking? For example, is there anyone in your house that smokes? [ ] YES [ ] NO

If "YES," where? _____
If "YES," how many hours are you around it? _____ hrs/day _____ hrs/week

Do you drink alcohol? [ ] Yes [ ] No

If "yes," please respond to the following questions:

When you drink, what do you drink? _____

When you drink _____, how much do you drink? _____

With what frequency do you drink? _____ times/day _____ times/week
_____times/month

Do you have or have you ever had any medical conditions or problems that require some treatment? (7; 8)
[ ] YES [ ] NO

If "YES," check all that apply:

[ ] Hypertension (high blood pressure)

[ ] Diabetes: [ ] Gestational [ ] Type I [ ] Type II

[ ] Crohn's disease

[ ] Thyroid disorder

[ ] Asthma

[ ] Cancer: _____

[ ] Silicosis

[ ] Alcoholism

[ ] Organ transplant: _____

[ ] Depression

[ ] Rheumatoid arthritis

[ ] Kidney disease

[ ] Worms, helminths

[ ] Heart disease

[ ] Hepatitis

[ ] Malnutrition

[ ] HIV or AIDS

[ ] Other(s):
_____

Co-morbidities from the patient's medical record:

_____ _____
_____ _____
_____ _____

Do you take a vitamin, multivitamin, or mineral supplement regularly (4+ times per week), for example, iron, calcium, folic acid, etc.?
[ ] YES [ ] NO

If "YES," what vitamin and/or mineral supplement?

Single nutrient: _____
Multivitamin: _____
Single mineral: _____

When did you begin using the vitamin(s) and/or mineral supplements? _____ (mm/yy)

Out of the past 7 days, how many days did you actually take them? _____ days

How often do you eat:

Food	Never or a few times a year	Each month (monthly)	Each week (weekly)	Every day (daily)
Fruit				
Vegetables				
Red meat				
Poultry				
Fish				

How often do you go to sleep feeling hungry because there wasn't food?
[ ] Never [ ] Sometimes (1-3 nights/wk) [ ] Frequently (4-6 nights/wk) [ ] Every night

Have you ever been a resident, employee, or volunteer at: [CHECK ALL THAT APPLY]

[ ] Prison- [ ] never [ ] resident [ ] employee [ ] volunteer; time of stay: _____

[ ] Nursing home- [ ] never [ ] resident [ ] employee [ ] volunteer; time of stay: _____

[ ] Homeless shelter- [ ] never [ ] resident [ ] employee [ ] volunteer; time of stay: _____

Have you ever taken any recreational drugs? [ ] YES [ ] NO

If "yes," have you ever used a drug by injecting it with a needle/syringe? [ ] YES [ ] NO

If "YES" to drug use, complete:

Check if taken:

[ ] Marijuana/Hashish: [ ] How long have you used this? _____

[ ] Heroin: [ ] How long have you used this? _____

[ ] Cocaine/Crack: [ ] How long have you used this? _____

[ ] Inhalants (glue, solvent): [ ] How long have you used this? _____

[ ] Methamphetamines: [ ] How long have you used this? _____

[ ] Other: _____ [ ] How long have you used this? _____

[ ] Other: _____ [ ] How long have you used this? _____

Height: _____ cm
(Conversion to feet: _____ft)

Weight: _____ kg
(Conversion to pounds: _____lbs)

Researcher calculated BMI: _____
(Source: www.cdc.gov)
Mexican classification: _____
(NORMA MX 2005)

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