Reasons for the Low Asthma Prevalence among Adult Chinese Immigrants in Boston

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Chapter 1. Introduction

In 2011, I began working with Professor Doug Brugge on a study of risk factors for asthma, a very common immune disease with respiratory symptoms, among children attending the Museum of Science in Boston. We asked parents about their children's demographic information, medical history, and exposure to a variety of environmental factors. We had a fairly homogenous middle class sample. In conducting this study, I also became interested in potential cross cultural differences in asthma risk factors. To explore the potential risk factors in a very different study population, I did an independent study project using essentially the same survey but with an indigenous Chilean population in Temuco, Chile. I interviewed almost every family with an asthmatic child in the region about their experiences of living with asthma and how asthma was managed through a combination of traditional and Western medicine in their community.

Throughout my time in Chile, Professor Brugge was both advising me on my independent study project and communicating some of the initial findings from his work on the health risks of living near freeways. Professor Brugge is the Principal Investigator of the Community Assessment of Freeway Exposure and Health (CAFEH) study. CAFEH is a large, cross-sectional study designed to understand the cardiovascular health risks faced by Boston residents who live close to freeways. Deena Wang, a member of the CAFEH study team, noticed in her review of the study data the low asthma prevalence among the Chinatown participants compared with the participants from other Boston neighborhoods. Given my interest in studying asthma, Professor Brugge suggested that I study why the asthma prevalence might be unusually low among the Chinese immigrants in Boston for my thesis. He emphasized that the low prevalence among the Chinese immigrants was striking in large part because of the demographic characteristics, such

as relatively low educational attainment and low family income, of the Chinese participants in CAFEH. The conventional wisdom regarding asthma is that individuals with lower family incomes and less educational attainment are more likely to have asthma than individuals with a higher socioeconomic status (SES). Therefore, it might be expected that the Chinese immigrants would have a higher asthma prevalence, rather than a lower asthma prevalence, than the general population.

I was curious why first generation immigrants, and specifically the Chinese immigrants participating in the CAFEH study, would be 80% less likely to have asthma than their higher SES, U.S. born neighbors. As far as I am aware, there are few other studies of asthma prevalence among adult Chinese immigrants residing in a city in the U.S. with which we could compare our estimate. However, foreign born Asian children residing in Boston are also three times less likely to have asthma than children born in the U.S. (1) and Chinese adolescent immigrants residing in Canada have a lower asthma prevalence than Chinese Canadians (2).

These findings do not seem unique to Chinese immigrants. Similar trends of a "healthy immigrant effect" exist among other immigrant groups whereby recent immigrants tend to have prevalence levels of chronic diseases more similar to others in their home country than others in their host country (3). After immigrants acculturate, there does seem to be some convergence of health status for some immigrant groups (4). This convergence of health status was not observed among the Chinese immigrants in the CAFEH sample with regards to asthma although it was observed for cardiovascular risk factors such as cholesterol levels and blood pressure. Therefore, I decided to explore whether the observed low prevalence of asthma among first generation Chinese immigrants could be understood in terms of the broader and ongoing investigation into the etiology and pathobiology of asthma. In particular, I was interested in examining theories of

immigrant health and asthma etiology in association with the social, environmental and clinical characteristics of a subset of the CAFEH participants.

Since CAFEH was designed to consider the relationship between highway related air pollution and cardiovascular health, the clinical and survey questions focused on risk factors for cardiovascular outcomes, not immunological or respiratory outcomes. Therefore, the project did not collect all the information that a study focused strictly on asthma would have. Nonetheless, the survey questions were quite comprehensive. Many of the factors for cardiovascular disease are also risk factors for asthma and participants were asked whether they had been diagnosed with asthma. I was thus able to explore the differences between environmental, occupational, and lifestyle exposures between the Chinese immigrants and the U.S. born whites and between the non-asthmatic and asthmatic groups within the CAFEH study.

I had to analyze the trends by nationality and asthma status separately because there were only eight Chinese participants with asthma. The small number of Chinese asthmatics is a testament to the fact that the asthma prevalence was quite low since the sample size of the Chinese immigrants was not small (n= 189), representing at least five percent of the Chinese immigrant community in Boston (5). However, only descriptive statistics could be reported among this group and simple measures of association were used in all of my other analyses. My goal in comparing populations such as Chinese immigrants and the U.S. born whites in the CAFEH was to explore the differences in the risk factors for asthma between groups with quite disparate asthma prevalence not explained by socioeconomic differences.

Specifically, I considered differences between the Chinese immigrants and the U.S. born whites with regards to exposure to cigarette smoke, proximity to highways, occupational exposure to dust and chemicals, perceived stress, and the presence of high levels of

immunological biomarkers. Each of these factors has been associated with poor respiratory health outcomes in other studies. The Chinese immigrants were less likely to be current smokers or to be exposed to secondhand smoke but were more likely to be former smokers than the U.S. whites. The Chinese immigrants were less likely to report occupational exposure to dust, asbestos, and chemicals. The trends for two of the inflammation biomarkers were also consistent with the low asthma prevalence among the Chinese immigrants compared to the U.S. born whites but the other two biomarkers did not suggest differences in the immunological profile of the Chinese immigrant and white participants that would explain the disparate asthma prevalence. None of the other exposures considered were associated with asthma or country of origin or the direction of the association for the other exposures was not consistent with the lower asthma prevalence among the Chinese immigrants. Notably, proximity to highways was not associated with asthma status and it is possible that using proximity was an inadequate proxy for exposure to highway related air pollution. This will be tested within the primary CAFEH analysis once air pollution exposure models are developed.

The risk factors for which the CAFEH study collected data are not the only ones of interest in studying variations in asthma rates. I thus supplemented my analysis of the CAFEH data with a comprehensive literature review. In this review, I considered studies of both pediatric and adult populations since there is far more literature on childhood onset asthma and there are likely to be at least some shared mechanistic pathways with adult onset asthma. The literature clearly suggested that multiple other genetic, epigenetic, and environmental exposures likely interact to cause asthma or at least affect the development of the immune system which could prime individuals for developing asthma later in life.

In particular, I considered the emerging literature on genetic loci associated with asthma and epigenetic influences largely within the context of the hygiene hypothesis framework. The hygiene hypothesis is a theory that considers how the immune system has evolved over time to fight environmental pathogens. It suggests that immigrants from lower income nations may have been exposed to a set of environmental exposures as young children that served as protective factors against later development of asthma, such as a different set of microorganisms or infectious diseases. The trend of higher asthma prevalence in more developed areas has been observed globally with asthma prevalence following a geographic gradient in a large, international multicenter study (6,7) and is consistent with the differences in asthma prevalence observed among the CAFEH participants. I could not directly test the influence of early life exposures for the CAFEH participants and the scope of my analyses was limited in part due to the minimal data available on health outcomes in the Chinese immigrant population more generally in the literature. Future work with these populations could help clarify the complex set of environmental, genetic, and social factors that lead to asthma. I also included a brief discussion of other later life environmental exposures, such as exposure to pests and mold in the home, which the literature suggests might be associated with asthma but were not directly tested in the CAFEH study because they are not necessarily risk factors for cardiovascular outcomes.

In the final chapter, I suggest potential study designs that could more completely address reasons why the asthma prevalence was so much lower among the Chinese immigrants than among the U.S. born white participants. Even if financial, human, and technical resources were virtually unlimited, it probably would not be possible to fully answer this question. Therefore, I compared the relative strengths and weaknesses of three study types. A larger, representational cross sectional sample would be feasible to collect and could be used to ask participants about

risk factors associated with respiratory and immunological outcomes that were not assessed in the CAFEH study. However, this type of study would probably not contribute substantially more information than I was able to glean from the initial CAFEH study since I would need a larger sample size than is possible within the Chinese immigrant population residing in Boston. A second option I propose is a matched pair prospective cohort study that would consider the differences in exposures and risk of asthma between Chinese residents, Chinese immigrants, and U.S. born whites. Given the extended average length of time the Chinese immigrants have resided in Boston (19 years), it seems unlikely that following this sample would yield much information about the difference in asthma risk due to later life exposures and following a different cohort of potential immigrants in China would present too many logistical and potential ethical complications to be reasonably considered. Early life exposures would also still be quite difficult to ascertain, although I do discuss how certain biomarkers may reflect genetic, epigenetic, and environmental influences. If these biomarkers could serve as reasonable proxies for early life exposures, a much less complicated and less resource intensive alternative study design would be a 2x2 case control study that recruited participants based on asthma status and nationality plus ethnicity. Regardless of the specifics of the study design, I would hope that future work in the field could more comprehensively consider the complex interplay between genetic, epigenetic, and environmental factors and that eventually a more thorough understanding of asthma etiology could lead to evidenced-based policies focused on asthma prevention.

Chapter 2. Asthma Prevalence and Risk Factors in the CAFEH Study

Asthma and Immigrant Health Trends

In 2010, more than one in every eight adults in the United States had been diagnosed with asthma, representing a steady increase in prevalence over the past decade; in 2000 only one in ten adults were diagnosed with asthma (8). In Massachusetts, the prevalence is even higher with over 15.3 percent of adults reporting that they had been diagnosed with asthma by 2010 up from 11.9 percent of adults reporting the same in 2000 (8,9). Not everyone is equally likely to have asthma (see Figure 1). Over 19 percent of Massachusetts adults who identify as Hispanic had ever had asthma compared to 17.5 percent of Massachusetts residents who identify as black and 15 percent of Massachusetts residents who identify as non-Hispanic white (8). Additionally, asthma is more common among those in the lower income brackets. Nearly one quarter of Massachusetts residents with an income below $15,000 annually have been diagnosed with asthma compared to 13.6 percent of Massachusetts residents with an income above $75,000 (8). In contrast, a recent study of adult asthma in 70 countries found China had the lowest current doctor-diagnosed asthma prevalence (0.19%) of any country (10), a number concordant with investigations into asthma prevalence in China (11). Given such disparities and the increasing prevalence of asthma globally, it seems critical to understand what factors are contributing to this condition that costs the U.S. over $60 billion per year between direct medical costs and lost workplace productivity (12).

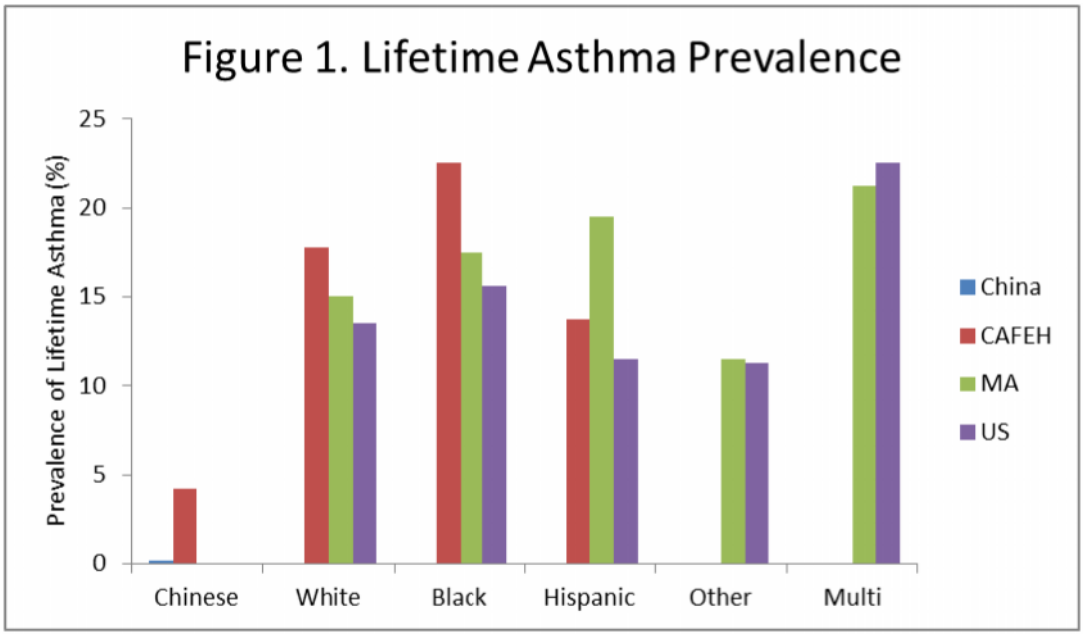
One method to discover factors that affect asthma onset is to compare groups with typical or high prevalence of asthma to groups that have much lower prevalence of asthma. This could be done with a variety of different populations but one comparison of interest is between immigrant and native groups living in a particular geographic region. Due to the generally lower

socioeconomic status and potentially limited access to health services, it could be expected that first generation immigrants would have worse overall health. Additionally, linguistic and cultural barriers could prevent effective communication which might worsen the quality of care received. Potential barriers may also disproportionately affect immigrants with chronic conditions or may affect the health of immigrants in complex ways. For example, asthmatic children of immigrant families residing in California were more likely than asthmatic children of nonimmigrant families to report worse perceived health status, more delays to health care, and a lack of a usual source of care. However, these children were also less likely to have been hospitalized for asthma in the previous year and were less likely to report asthma symptoms (13). This apparent paradox reflects a more general situation in which new immigrants tend to have better health than native born residents.

The trend can be observed in relation to asthma prevalence for immigrants of many backgrounds. For example, in Boston, foreign-born black adults were only about one third as likely to report having asthma as U.S. born black adults (14). Other high-income nations have also observed this trend of lower asthma prevalence among immigrants coming from lower-income nations. A large cross sectional study of asthma prevalence among Chinese born and Canadian-born Chinese adolescents in Canada suggested that the asthma prevalence was lower among Chinese immigrants from mainland China than among Chinese immigrants from Hong Kong and that the prevalence of asthma among both of these groups was lower than that of the Canadian Chinese (2). Furthermore, the risk of asthma increases with an increased duration of residency in a higher-income country. For example, individuals born outside of Australia, a country with a very high asthma prevalence compared to most of the world, were over twice as likely to develop asthma after living there for five to nine years (15).

There are several potential explanations for the discrepancies in health status between recent immigrants and native born residents. Some self-selection bias likely exists for who is physically and financially able to immigrate so that at least initially immigrants could be relatively healthy compared to the host population. Certain countries have immigration policies that screen out would-be immigrants for certain health problems. Immigrants may also be underdiagnosed or health conditions could be underreported in immigrants. Additionally, immigrants may have healthier behaviors from their birth country than are common in the host country. Individuals could also have varying susceptibility to certain conditions and these genetic tendencies could be more common in different regions of the world. There could also be differences in environmental or other exposures that affect disease onset. Most likely, some combination of these factors affects the apparent healthy immigrant effect (16).

We also observed this trend of better health status among Chinese immigrants compared to U.S. born whites in the Community Assessment of Freeway Exposure and Health (CAFEH) Study. Moreover, Asian children residing in these communities who were born outside of the U.S. were three times less likely to have asthma than children born in the U.S. (1). However, there are no studies to date reporting the prevalence of asthma among first generation adult Chinese immigrants with which to compare the CAFEH data. While numerous studies have been conducted exploring the risk factors associated with asthma exacerbation, this chapter attempts to consider possible reasons for the low asthma prevalence among the adult Chinese immigrant population residing in Boston by examining the association between demographic, environmental and medical characteristics of the CAFEH participants.



Study Methods and Demographics

CAFEH is a five year cross sectional study of the relationship between highway related air pollution and cardiovascular health within a community based participatory research model. A more detailed explanation of the methods for the CAFEH study has been given elsewhere (17). Briefly, community partners included Somerville Transportation Equity Partnership, the Committee for Boston Public Housing, the Chinese Progressive Association and the Chinatown Residents Association. Academic partners included researchers from Tufts, Harvard, Brown and Boston Universities. Participants were recruited from three proximity strata within each study area. Participants within 100m of either I-90 or I-93 were considered to be in the near highway exposure group. Participants who lived greater than 1000m from either highway were included in an urban background group. An intermediate group lived between 100m and 400m from the nearest highway. A stratified random sample of addresses was obtained and participants were chosen from these homes. A convenience sample was also recruited from each neighborhood. Exposure to traffic related air pollution was estimated by proximity to major highways. While

the distance between the home and highway was initially measured using geocoding, more accurate distances were assigned using Orthophotos (scaled aerial images). The distance was measured from the center of the Ortho-photo corrected location of the home to the edge of the nearest highway lane, excluding ramps to the highway (18). The Orthophoto corrected distances were available for all 704 participants in the full CAFEH study. The sample considered in this paper reflects a subset of these participants from across the three communities.

The study team collected data in a variety of formats including participant questionnaires, clinical biomarkers (blood samples), and monitoring of ultra-fine particulate and other traffic related air pollutant concentrations. I only used the questionnaire and clinical data that had been previously collected in my thesis. The questionnaire was administered in a variety of languages including English, Cantonese, and Mandarin in participants' homes. Questionnaires asked participants about basic demographic information, health status and medications, health behaviors (such as smoking history), social factors (such as perceived discrimination), diet, a time-activity log of work and non-work days, other exposures (such as SHS), and risk perception of air pollution. I was able to use data from the full CAFEH study that had already been entered into a Microsoft Access database and checked for accuracy.

From this database, I identified the subsample to be included in my analysis based on the questions pertaining to country of origin, race, and asthma status. Only participants who self-identified as white, non-Hispanic and born in the U.S. (n = 264) or who stated that they were born in China were included (n = 189). All participants eligible based on nationality and race also had data on their asthma status. I transferred information collected from each of these participants to SPSS (Version 20) for data analysis. Descriptive statistics and bivariate associations were examined for each relevant risk factor. The primary outcome variables of

interest were yes or no answers to doctor diagnosed asthma and Chinese born or U.S. born whites. I assessed bivariate associations with odds ratios. All variables were dichotomized for analysis of bivariate associations. Although the CAFEH study analyzed the three proximity strata separately, I collapsed the 100-400m strata with the >1000m strata. Analyses excluding the intermediate strata yielded the same trends as those reported within this chapter. Additionally, certain variables were derived from multiple survey questions. For example, exposure to secondhand smoke (SHS) was categorized as yes or no based on whether participants reported that they were exposed for any amount of time in either the home or car.

In a larger sample size, multiple linear regression models would have been utilized in the data analysis to discern the relative effects of the various potential risk factors for asthma between the Chinese immigrants and U.S. born whites. However, due to the relatively small sample size, particularly of asthmatic cases among the Chinese immigrants, this was not useful. I instead generated separate analyses to evaluate whether specific factors were associated with asthma diagnosis and place of birth. For some variables, I could only use descriptive statistics due to the small sample size.

Full CAFEH Demographics

While my main analysis was between the Chinese immigrants and the white U.S. born participants, ethnic and racial minorities more generally constituted the majority of the CAFEH participants so I will briefly consider the larger trends in the data. Of all 704 CAFEH participants, 328 were born in the U.S. (46.6%). Of the 328 U.S. born participants, 264 reported that they were white, non-Hispanic (80.5%), 50 reported that they were not white (15.2%), 40 reported that they were black (12.2%), and two reported that they were Hispanic (0.6%). None of the U.S. born participants reported an Asian race or Chinese ethnicity. Of the 376 non-U.S. born

participants, 233 were from Asian or South Asian countries (63.0%), 50 were from the Caribbean, Central, or South American countries (5.1%), nine were from European countries (7.1%), and one was from an African country (0.3%). Among the Asian and South Asian participants, 189 were Chinese (81.1%) and six of those who did not identify China as their country of birth identified as being of Chinese ethnicity. Since there were only six U.S. born participants who identified as Chinese, it was not feasible to compare Chinese first generation immigrants to U.S. born ethnic Chinese participants. I did compare Chinese immigrants to other Asian immigrants as well as the Chinese immigrants to the black U.S. born participants.

The comparison of the Chinese immigrants to other sub-populations in the larger CAFEH sample suggests that the asthma prevalence of the Chinese immigrants differed from both U.S. born minority groups and other immigrant groups. The overall asthma prevalence among all CAFEH participants was 13.4 percent (n = 94). Among the 40 U.S. born participants who identified as black, nine participants had asthma (22.5%). This means that the blacks in this sample were 6.57 times as likely to have asthma as the Chinese immigrants (95% CI = 2.35 - 18.18). This was true despite the fact that the U.S. born blacks were 9.32 times as likely to have graduated high school (95% CI = 4.15 - 20.92) and less likely to live within 100m of the major highway I-90 (OR 7.75, 95% CI = 1.03 - 58.8). However, at least some of this difference could potentially be explained by the difference in smoking prevalence. The U.S. born blacks were 6.91 times as likely as the Chinese immigrants to be current smokers (45.0% compared to 10.6%, 95% CI = 3.18 - 15.13).

Differences also existed between the Chinese immigrants and the immigrants from Caribbean, Central, and South American countries, although the magnitude of the difference was not as great as between the Chinese and US born participants. Among the 49 Caribbean, Central,

and South American born participants with data on asthma status, five participants had asthma (10.2%). This means that the Caribbean, Central, and South American immigrants in this sample were 2.57 times as likely to have asthma as the Chinese immigrants (95% CI = 0.80 - 8.24). The Caribbean, Central, and South American immigrants were 2.50 times as likely to have graduated high school (95% CI = 1.32 - 4.74) but there was no significant difference between the groups for current smoking prevalence (OR = 1.86, 95% CI = 0.79 - 4.37). The Caribbean, Central, and South American participants were more likely to live within 100m of I-93 (OR = 8.57, 95% CI = 3.48 - 21.13) but were less likely to live within 100m of I-90 (OR = 0.84, 95% CI = 0.79 - 0.89).

Four of the 44 non-Chinese Asians had asthma (9.09%). This was not a statistically significant difference in asthma prevalence compared to the Chinese immigrants (OR = 4.23, 95% CI = 0.65 - 7.87). However, non-Chinese Asians were 3.25 times as likely to have graduated from high school (95% CI = 1.65 - 6.37). Additionally, there was a non-statistically significant trend showing non-Chinese Asians were approximately twice as likely to be current smokers (95% CI = 0.91 -5.18). Non-Chinese Asian immigrants were more likely to live within 100m of I-93 (20.5% vs. 4.8%), but Chinese immigrants were more likely to live near I-90 (16.6% of Chinese vs. 4.5% of other Asians).

Subset of CAFEH Participants for Full Analysis

Throughout the rest of this chapter, I will only analyze data from the 453 participants in the CAFEH study who were either U.S. born whites (n=264) or Chinese born immigrants (n=189). Of these participants, 58.9 percent were female (n=267). Participants ranged in age from 40 to 91 (mean 61.42 years, standard deviation 12.75 years). Almost 60 percent had a high school diploma (n=273) and 20 percent had a bachelor's degree (n=92). There were 55 people with asthma in this sample, representing a prevalence of 12.1 percent. Of all of the asthmatics in the

sample, 63.6 percent were female. At least 70 percent of the white asthmatics were diagnosed as adults. Of the eight Chinese asthmatics, six were male, and all were between the ages of 67 and 82. At least six of these individuals were diagnosed with asthma since coming to the United States and all had adult onset asthma. The following table summarizes this demographic information about the CAFEH participants:

Table 1. Demographic Characteristics of CAFEH Sample

	Total	U.S. Born White	Chinese	All White and Chinese Asthmatics	Chinese Asthmatics
Sample	453 (100%)	264 (58.3% of sample)	189 (41.7% of sample)	55 (12.1% of sample)	8 (4.2% of Chinese)
Female	410 (58.2%)	157 (59.5%)	110 (58.2%)	35 (63.6%)	2 (25.0%)
Mean age	61.4 (range 40-91)	59.8 (range 40-89)	63.7 (range 40-91)	62.4 (range 42-88)	75.5 (range 67-82)
High School Graduate	273 (60.3%)	175 (66.3%)	98 (51.9%)	39 (70.9%)	4 (50.0%)
College Graduate	92 (20.3%)	65 (24.6%)	27 (14.3%)	14 (25.5%)	1 (12.5%)
Income <$25,000	234 (51.7%)	111 (42.0%)	123 (65.1%)	29 (52.7%)	7 (87.5%)
Asthmatic	55 (12.1%)	47 (17.8%)	8 (4.2%)		

As Table 1 shows, Chinese immigrants were more likely to have an annual family income below $25,000 than U.S. born white participants (OR = 2.35, 95% CI = 1.57 - 3.51). Additionally, Chinese immigrants were significantly less likely to have a high school diploma (OR = 0.56, 95% CI = 0.38 - 0.82) or a bachelor's degree (OR = 0.49, 95% CI = 0.30 - 0.81). Of perhaps greatest relevance, Chinese immigrants were significantly less likely to have asthma than U.S. born whites (n=8, OR = 0.204, 95% CI= 0.09 - 0.44). There were non-statistically

significant trends for participants with asthma to be less likely to hold a high school diploma (OR = 1.69, 95% CI = 0.91 - 3.13) or a bachelor's degree (OR = 1.42, 95% CI = 0.74 - 2.74). There was no statistically significant difference in the likelihood of having a low annual income (OR = 1.05, 95% CI = 0.58 - 1.90).

One of the primary reasons to consider asthma in adults, and particularly older adult populations, is the increased likelihood of complications from co-morbidities. The following table shows the prevalence of four conditions (diabetes, prior heart attack, high blood pressure, and high cholesterol) among the CAFEH participants.

Table 2. Percent of Participants with Other Medical Conditions

	Total (n = 453)	U.S. Born White (n = 264)	Chinese (n = 189)	All White and Chinese Asthmatics (n = 55)	Chinese Asthmatics (n = 8)
Diabetes	13.5	13.6	13.2	16.4	25
Heart Attack	5.7	7.2	3.7	9.1	25
High Blood Pressure	38.4	38.3	38.6	52.7	75
High Cholesterol	30.2	36.7	21.2	54.5	50
Any of the Above	53.9	56.1	50.8	72.7	100

While there was not a statistically significant difference in overall co-morbidity prevalence between U.S. born whites and Chinese immigrants (OR = 0.81, 95% CI = 0.56 - 1.18), Chinese immigrants were significantly less likely to have high cholesterol (OR = 0.46, 95% CI = 0.30 - 0.71). Participants with asthma were 2.54 times as likely as non-asthmatics to have at least one of the co-morbid conditions (95% CI = 1.36 - 4.74). In particular, the participants with asthma were significantly more likely to have high cholesterol than participants without asthma (OR =

3.24, 95% CI = 1.82 - 5.76). Participants with asthma were also more likely to have high blood pressure (OR = 1.92, 95% CI = 1.09 - 3.39).

Differences in perceived stress were also observed. Individuals with asthma reported greater feelings of stress on the validated four question Perceived Stress Scale (PSS) (mean of 5.38 versus 3.40, t = -3.07, $p = 0.002$). Perhaps contrary to expectations that immigrants would have more stress, CAFEH participants born in China had lower perceived stress than the U.S. born white participants (mean of 3.15 versus 3.99, t = 3.93, $p < 0.0005$).These results should be interpreted cautiously, however, as there could also be cultural differences in common coping mechanisms or tendencies to self-report stress. The CAFEH participants reported lower overall perceived stress than would be expected for adults ages 55-64 (mean 3.9 versus 4.2, t= -2.13, $p = 0.033$) (19). However, while the mean age of the CAFEH participants fell within that age bracket, not all participants did and thus the comparison maybe somewhat biased since reported stress tends to decrease with age on the PSS. Nevertheless, the relatively low perceived stress among the Chinese immigrants could serve as a protective factor since among adults, the more perceived stress individuals face, the greater the risk of developing an atopic disorder (20).

Results and Discussion for Air Quality Factors

To attempt to explain why U.S. born whites were 4.9 times as likely to have been diagnosed with asthma as Chinese immigrants in their lifetime (95% CI = 2.3-10.6), I examined environmental factors that have commonly been associated with asthma prevalence and severity. Air quality measures included in the analysis were cigarette smoke exposure, occupational exposure to asbestos, chemicals, and dusts, and proximity to highways.

<u>Tobacco Smoke Exposure</u>

As shown in Table 3, 51.2% of all participants were either former or current smokers. The Chinese immigrants were not any more or less likely than whites to be former smokers (OR = 0.98, 95% CI = 0.64 - 1.49). However, the U.S. born whites were more likely to be current smokers (OR = 1.67, 95% CI = 1.01 – 2.70). Among the Chinese participants with asthma, one was a prior smoker and another was a current smoker. In this sample, there was no statistically significant difference between exposure to cigarette smoke and asthma (OR = 1.17, 95% CI = 0.58 - 2.40). Among all participants, 10.6 percent were exposed to SHS in their homes and 11.7 percent were exposed in cars. In total, 17.9 percent of CAFEH participants were exposed to SHS in either their homes or cars. About three quarters of participants who were exposed to SHS were only exposed in one of their homes or cars, but not both. Chinese immigrants were less likely to be exposed to cigarette smoke in the home (OR = 0.48, 95% CI = 0.25 - 0.94) but not overall (OR = 0.75, 95% CI = 0.45 - 1.23). No other statistically significant differences were observed for exposure to SHS by nationality or asthma status. No Chinese immigrants with asthma reported exposure to SHS.

Table 3. Exposure to Cigarette Smoke for CAFEH Sample

	Total (%, n)	U.S. Born White (%, n)	Chinese (%, n)	Asthmatics (%, n)	Chinese Asthmatics (%, n)
Current Smoker	19.2% (87)	22.4% (59)*	14.8% (28)*	16.4% (9)	12.5% (1)
Former Smoker[a]	39.9% (145)	40.6% (82)	39.4% (63)	38.6% (17)	14.3% (1)
Smoker Ever	51.2% (232)	53.4% (141)	48.4% (91)	48.1% (26)	25.0% (2)
Smoke Exposure in Home	11.0% (48)	13.8% (35)*	7.1% (13)*	15.1% (8)	0.0% (0)
Smoke Exposure in Car	11.8% (53)	12.2% (32)	11.2% (21)	9.1% (5)	0.0% (0)
Smoke Exposure	18.5% (81)	20.3% (52)	16.0% (29)	20.8% (11)	0.0% (0)

Inside[b]			

[a]Former smoker counts do not include current smokers
[b]Secondhand smoke exposure in the home, the car, or both home and car
* *p* value of 0.05-0.099

In the U.S., 19 percent of all adults were current smokers in 2010, including 20.6 percent of white adults and 9.9 percent of non-Hispanic Asian adults (21). The national statistics may not accurately represent the prevalence among the older Chinese immigrants as this relatively small group's contribution to the average is likely diluted. Smoking, exposure to SHS, and exposure to traffic related air pollution are associated with worse respiratory outcomes (22). According to the U.S. Surgeon General's 2010 report, "the evidence is sufficient to infer a *causal conclusion* between smoking and asthma-related symptoms (i.e., wheezing) in childhood and adolescence, all major respiratory symptoms among adults, including coughing, phlegm, wheezing, and dyspnea, [and] poor asthma control" (23). A large retrospective study of Swedish adults found that smokers were 50 percent more likely to develop asthma (IR = 1.6, 95% CI = 1.2 - 2.0) than never-smokers and that the effect was stronger in women than men (24). Given the strong evidence implicating smoker status with asthma, it would be expected that among groups with lower smoking rates, the asthma prevalence would tend to be lower.

U.S. born whites in the CAFEH study were more likely to be current smokers. Previous research has shown that as Chinese immigrants acculturate, their attitudes towards smoking and smokers becomes more negative (25). This could reflect the differences in prevalence between the two countries. In China in 2010, 28.1 percent of all adults over 15 years of age smoked. There are clear gender differences as over half of all men smoked (52.9%) compared to only 2.4 percent of women (26). However, in the U.S., fewer than one in five adults were current smokers (19.2%) in 2010 and there was a more even distribution of smokers by gender (53.8% of smokers

were male) (27). Among the Chinese immigrants in the CAFEH sample, 52.7 percent of females had ever smoked and 41.8 percent of males had ever smoked.

Smokers are more likely to develop asthma (28) and current smoking status is more predictive of current asthma severity and control than former smoking status (29). However, this alone cannot explain the difference in asthma prevalence between the two groups. Data was not collected in the CAFEH study to ascertain whether asthma onset occurred before or after individuals quit smoking. Additionally, as part of the risk assessment process, it could be informative to know if a dose response relationship exists in which an increased prevalence of asthma is seen among individuals who smoke more per day. However, one factor alone cannot completely explain the increase in asthma incidence over the past several decades. For example, while smoking is certainly linked to asthma exacerbations and quite possibly linked to asthma onset, cigarette use has declined in the US, although not in China, to almost 50 percent of the levels of the peak consumption of 640 billion cigarettes in 1981 (30).

Unfortunately, the adverse effects of smoking are not limited to the smokers themselves. Among the most comprehensive and authoritative reviews of the literature on the effects of exposure to SHS are the Surgeon General reports. According to the Surgeon General, “there is no risk-free level of exposure to SHS … a smoke-free environment is the only way to fully protect nonsmokers from the dangers of SHS” (31). Among the specific dangers of SHS, the Surgeon General has concluded that there is causal relationship between parental smoking and asthma among children of school age (32). Postnatal exposure to SHS has been associated with increased rates of asthma (33). In children, exposure to SHS has been linked the development of asthma in a dose response manner (34). Furthermore, among people who were exposed to SHS

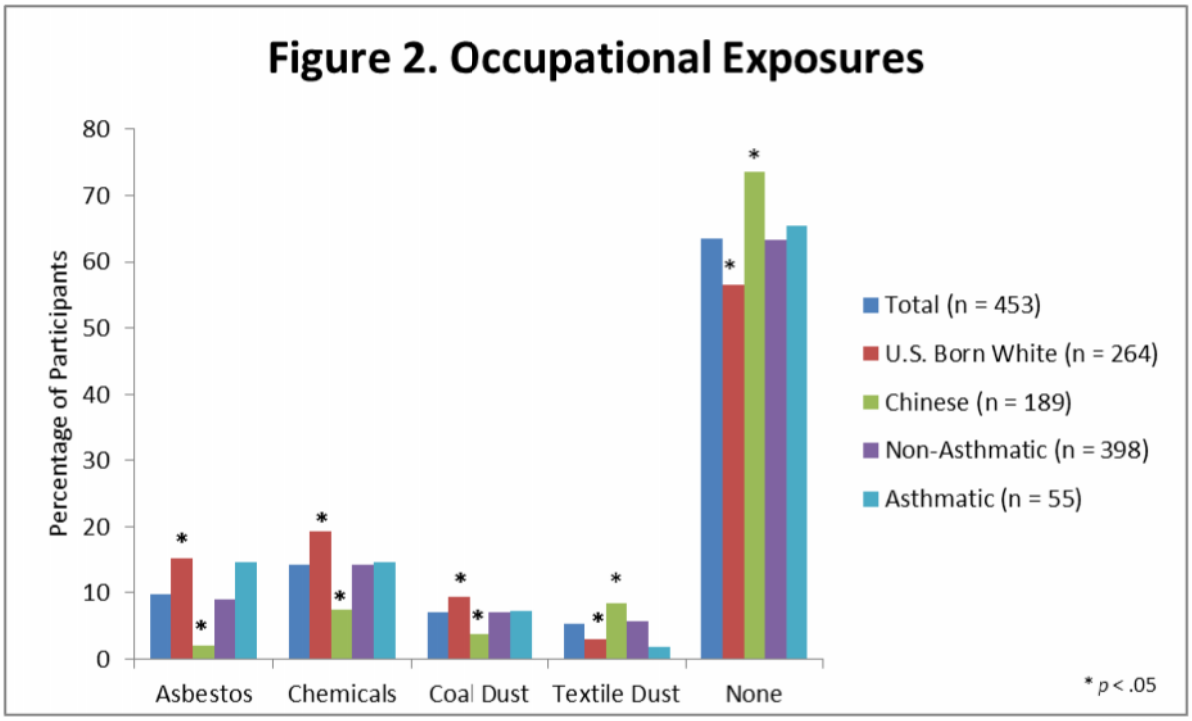
within the previous year, the attributable risk of SHS on asthma incidence over the 2.5 year study period was nearly 50 percent (95% CI = 16.0 - 69.2) (35).

However, with regards to adult onset asthma, the data are less clear. According to the Surgeon General, "the evidence is suggestive but not sufficient to infer a causal relationship between SHS exposure and adult-onset asthma" (36). Among the difficulties in assessing the relationship between SHS exposure and adult-onset asthma are the challenges in distinguishing true adult-onset asthma from pediatric-onset asthma that was not diagnosed until later in life (37). Nevertheless, one of the strongest observational studies conducted was the Swiss study on Air Pollution and Lung Disease in Adults (SAPALDIA). SAPALDIA was a large cohort study that considered the cardiovascular outcomes associated with air quality. The SAPALDIA researchers found that even after controlling for a variety of factors such as a family history of asthma, adults who had never smoked but were exposed to SHS were significantly more likely to have doctor diagnosed asthma (odds ratio = 1.39, 95% CI = 1.20 – 1.86) and asthma symptoms than adults without exposure to SHS in the home or workplace (38). Another longitudinal study of 3914 nonsmoking adults found that not only are individuals significantly more likely to develop asthma over a ten year period if they were exposed to SHS in the workplace, occupational SHS exposure was the strongest occupational predictor of asthma onset (39). Of note, this study considered data collected from 1977 to 1987– prior to the implementation of strong policies protecting workers from the dangers of inhaling SHS in the U.S. and Europe. This study is particularly relevant to an interpretation of the CAFEH results because the average age of participants in this study at time of enrollment was 56.5 years and the mean age among CAFEH participants was 61.4 years.

Occupational Exposures to Asbestos, Chemicals, and Dusts

Another major category of indoor air quality exposures includes occupational exposures to asbestos, chemicals, coal dust, textile dusts, wood dusts, and other dusts. These exposures could be especially relevant in the CAFEH study since it focuses on older adults who primarily have late-onset asthma which could have been affected by occupational exposures. Participants were asked about their job status and about exposure to these compounds. Of note, seven of the eight Chinese asthmatics reported that they were retired and the eighth did not have an occupational status listed. None had any current indoor or outdoor occupational combustion exposure. Indoor and outdoor occupational combustion exposure was a derived variable created from participants' current and past occupation and self-reported occupational exposures. The larger CAFEH study did ask participants about previous jobs or previous occupational exposures.

Among the 47 white asthmatics, 19 (40.4%) reported occupational exposure to at least one of asbestos, chemicals, coal dust, textile dusts, wood dusts, and other dusts. Six (12.8%) also reported indoor occupational combustion exposure and one (2.1%) reported outdoor occupational combustion exposure. In comparison, among the 398 non-asthmatic Chinese and white participants, 146 (36.7%) reported exposure to at least one of asbestos, chemicals, coal dust, textile dusts, wood dusts, and other dusts. Additionally, 27 (6.8%) non-asthmatic participants reported indoor occupational combustion exposure and 7 (1.8%) reported outdoor occupational combustion exposure. Figure 2. summarizes the types of occupational exposures participants reported.



Between the Chinese and white participants, self-reported occupational exposure to asbestos, chemicals, coal dust, and textile dust exposures were all statistically different ($p < 0.02$). The Chinese were less likely to be exposed to asbestos (OR = 0.34, 95% CI = 0.04 - 0.35), chemicals (OR = 0.33, 95% CI = 0.18 - 0.62), coal dust (OR = 0.37, 95% CI = 0.16 - 0.87), and to at least one exposure overall (OR = 0.47, 95% CI = 0.31 - 0.70). However, the Chinese immigrants were significantly more likely to have been exposed to textile dust at work (OR = 2.96, 95% CI = 1.24 - 7.07). Although there were no statistically significant differences in exposure for the asthmatics compared to the non-asthmatics, the fact that the Chinese immigrants and U.S. born whites had such different occupational exposures could have affected the groups' relative health status. Chinese immigrants were less likely to be exposed to these factors despite having a lower average income and educational attainment. There could be recall bias or potential knowledge barriers for self-reporting occupational exposures. Workers may not be aware of chemicals or agents they or other employees are using. Additionally, while worker protection processes are

theoretically supposed to be utilized, workers may be inadvertently using protective gear incorrectly or there could be inadequate protection to start so workers may be exposed to more agents than they could self-report.

The differences in exposure could also reflect the statistically significant older mean age of the Chinese immigrants (t = -3.167, p = 0.002) if the data on previous job exposures was less complete than the data on the most recent job exposures. Additionally, since most participants did not have newly diagnosed asthma and it is likely that some subset of the participants changed jobs multiple times since asthma diagnosis, the reported exposures may have been irrelevant to asthma onset for the CAFEH participants and earlier exposures that could have been relevant to the analysis may have been missed.

Occupational exposures can account for up to 15 percent of adult onset asthma cases (40). Occupational factors could stimulate previously quiescent asthma to become active (41) and exposure to asbestos, dust, and fumes has been associated with the development of respiratory symptoms (42,43). Some occupational exposures, such as chlorine and ammonia, are associated with almost immediate onset of asthma symptoms while other chemicals affect asthma symptoms after chronic exposure. Moreover, some occupational exposures may affect asthma through IgE dependent mechanisms similarly to other forms of allergic asthma while other irritants are not thought to work through these atopic mechanisms (40).

Proximity to Highways

The CAFEH study was primarily designed to measure the cardiovascular and other health effects of highway related air pollution. Exposure to traffic related air pollution was estimated by proximity to major highways. Chinese immigrants were significantly less likely to live within 100m of I-93 (OR = 0.153, 95% CI = 0.074 - 0.316) but were more likely to live within 100m of

I-90 (OR = 12.75, 95% CI = 4.42 – 36.81). No participants with asthma lived within 100m of I-90. There was no statistically significant association between individuals who lived within 100m of I-93 and asthma status (OR = 1.33, 95% CI = 0.65 – 2.71).

There are several problems with using proximity to highways as a proxy for particulate matter exposure. First, exposure to traffic related air pollution from nearby roadways other than highways is ignored. Second, using proximity to highways in Chinatown is complicated by the presence of both major highways and many high rise buildings that could affect actual exposure. Additionally, proximity to the highway does not take into account weather patterns or the presence of large buildings that could result in different distributions of particulate matter within a region near a highway. Additionally, if there are any differences in the effects of different types of particulate matter, these cannot be readily accounted for using only proximity data. To correct for some of these limitations, including time spent outside of the home, actual exposure models are currently being developed by others on the CAFEH team based on measured particulate levels measured at multiple time points each day over a period of several months accounting for weather patterns and other variables. Once these models are complete, a more sophisticated analysis of the association between exposure to highway related air pollution and asthma will be possible. However, short of actually monitoring the exposure to particulate matter for individual participants, the analysis will still be somewhat incomplete. One such factor not included here that affects exposure to traffic related particulate matter is activity level near areas of high traffic density. Outdoor physical activity near highways and other roads with high traffic density represents a greater risk to individuals than riding in a car along these same roads. For example, bicyclists are exposed to over four times the amount of vehicular emissions as passengers in cars (44). Despite the limitations of using proximity as a proxy for exposure, using proximity as a

proxy is common in the literature. People who live in cities (45) or within 400 meters of roads in which more than 5000 trucks and 30,000 cars pass per day (46) are more at risk of developing respiratory problems.

Given the small number of cases of asthma in the Chinese immigrant population, it was not possible to detect a statistically significant difference in exposure between the Chinese immigrants with and without asthma. On an ecologic level, within the last fifteen years, doctors in China have noted a 40 percent increase in the prevalence of asthma to almost 4 percent in some areas which they think may be at least partially attributable to the increase in air pollution (47). The toxicity of traffic related air pollution may not be equivalent among different racial and ethnic groups. For example, non-Hispanic blacks seemed to be more susceptible to the adverse effects of traffic related air pollution than Hispanics or non-Hispanic whites. Non-Hispanic blacks were 1.73 times as likely to have current asthma with increased fine particulate matter exposure (95% CI = 1.17 – 2.56). However, among the total 110,000 participants, there was not a significant association between an increase of 10 microns of fine particulate matter per cubic meter and current asthma (48).

Inhalable traffic related air pollutants may be an exposure of greater concern for children than adults based on their increased metabolic rate, increased likelihood of being active outdoors, and overall likelihood of higher exposure per kilogram of bodyweight (49). It has been found that inflammation and bronchoconstriction biomarker levels are higher in children when ambient $PM_{2.5}$ levels are higher (50). Traffic related air pollution as measured by NO_2 monitors in participants' homes during a prospective cohort study has been significantly associated with asthma onset in children of 10-18 years of age (51). However, the Harvard Six Cities Study, a large prospective cohort study, found that total suspended particle and sulfur dioxide levels were

not associated with doctor diagnosed asthma or persistent wheeze in children. They also found no association between PM_{15} and pulmonary function in the children (52,53), although the researchers reported that exposure to particulate matter can affect acute exacerbation of respiratory symptoms in an influential review article (54).

Nevertheless, many studies do report an increased likelihood of asthma in adults with increasing exposure to highway related air pollution. For example, SAPALDIA researchers suggest that adults who have never smoked are 1.3 times as likely (95%CI = 1.05-1.61) to develop asthma for every micron per cubic meter increase in empirically measured traffic particulate matter near the home over a several year period (55). The SAPALDIA project's results represented an important contribution to the literature because prior to this study, associations between pediatric asthma and traffic related air pollution were well documented but the relationship was less well established for adult onset asthma (56). Additionally, the European Community Respiratory Health Survey (ECRHS) of 4394 participants found that for every 10 $\mu g \cdot m^{-3}$ of NO_2, adults had a ratio of mean asthma score of 1.23 fold higher (95% CI = 1.09-1.38). The authors suggested that the risk of developing asthma was also positively associated with exposure to NO_2 (ratio of mean asthma symptoms = 1.25, 95% CI = 1.05–1.50) (57).

Beyond the potential for immediate effects of air pollution on asthma onset and exacerbation, climate change resulting from increased air pollution may disproportionately affect people with allergic asthma and people at risk for asthma development. Some research questions whether increased atmospheric carbon dioxide and average temperatures is increasing the amount of pollen and mold spores which in turn could lead to asthma exacerbation or potentially asthma onset (58,59). The extreme weather such as increased frequency and intensity of thunderstorms may also indirectly affect asthma exacerbations as aeroallergens can be released

in thunderstorms which has previously been associated with asthma exacerbation outbreaks (60–63).

Other purported effects of air pollution include increased risk for low birth weight and low birth weight is associated with an increased risk of developing asthma (64,65). One study examining the effect of components of particulate matter ($PM_{2.5}$) on birth weight revealed that maternal exposure to zinc, elemental carbon, silicon, aluminum, vanadium, and nickel is associated with lower birth weight. This study added to the literature suggesting that particulate matter is not a homogenous pollutant so specific chemical constituents and chemical mixtures may need to be studied individually. A limitation of the study, like much work on the health effects of air pollution, was that only county level exposure was considered and this provides an inexact proxy for actual exposure (66).

Hospitalization discharge data can be used to evaluate whether proximity to highways affects asthma prevalence. There were 479,000 hospitalizations due to asthma with an average length of stay of 4.3 days in the U.S. during 2009 (67). Based on hospitalization records in the Tufts Initiative for Forecasting and Modeling of Infectious Diseases database, between 1991 and 2006, zip codes within a mile of interstate highways in New England had a statistically significant higher number of mean hospitalizations of residents over 65 years of age than zip code areas farther from interstate highways ($t = -2.28$, $p = 0.012$) (68). However, only a cautious interpretation of this trend is possible. For example, although regions with low population density could have high hospitalization rates, a very small number of absolute cases could make it appear that certain regions are hotspots when in fact they are not. This is especially true since the analysis of hospitalization rate was based on individuals' residence, not based on where the hospitalization actually occurred. Additionally, proximity to highways varies within a given zip

code. Individual residents may live closer to or farther from interstate highways than their zip code alone would suggest. Nevertheless, the general trend is consistent with the idea that proximity to highways could increase the risk of having uncontrolled asthma.

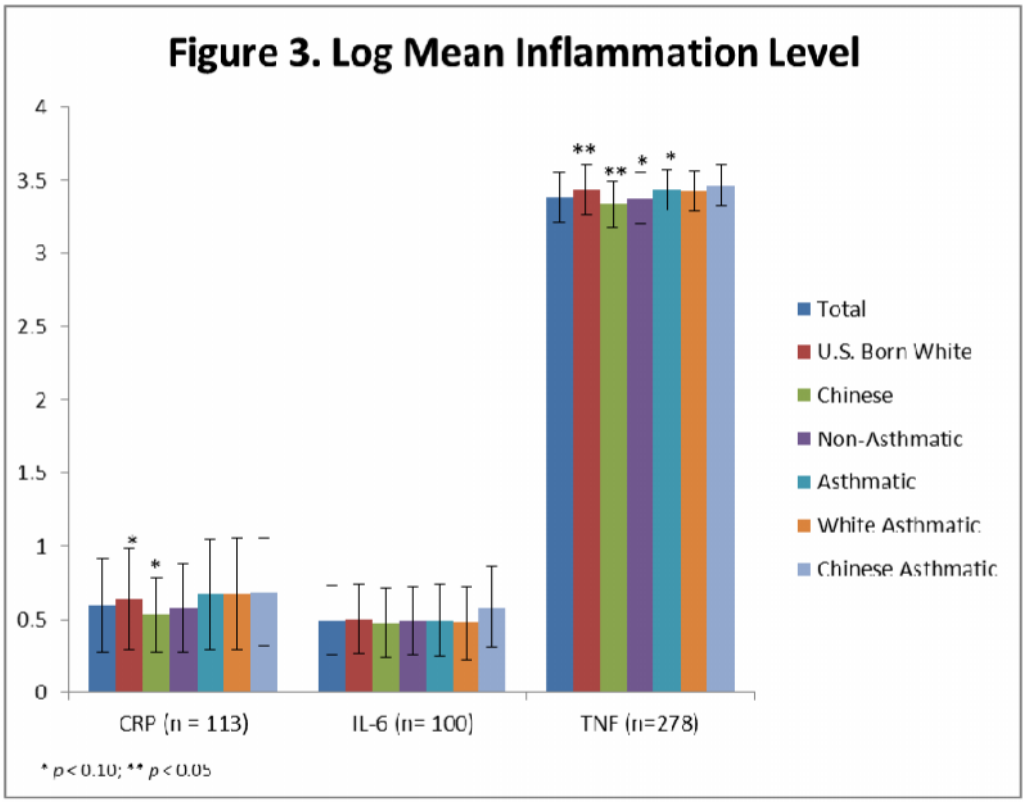
While it seems fairly clear that air pollution is associated with an exacerbation of respiratory symptoms and possibly even with asthma onset (69) or more severe asthma outcomes, neither the CAFEH study nor others have conclusively determined whether this exposure increases the risk of developing late-onset asthma. Given the complexity of the immune system and heterogeneity of asthma, this is perhaps unsurprising and suggests that an analysis of non-respiratory risk factors is also warranted to attempt to understand why the asthma prevalence of the Chinese immigrants is less than one-fourth that of the U.S. born whites.

Results and Discussion for Biomarkers

Among the categories of factors that have been associated with asthma, genetic and early life influences are thought to be quite important. However, theories that the development of the immune system is altered early in life in such a way that protects against later asthma onset cannot be tested directly from the available CAFEH data. CAFEH was designed to consider factors associated with cardiovascular disease. Since asthma was only an after-the-fact analysis, no information was collected about the participants' early life residences, exposures, or family history of disease. This lack of information about early life exposures and medical history is a major limitation of my analysis. Nevertheless, CAFEH did collect clinical biomarkers for molecules relevant to cardiovascular disease and several of these molecules are also associated with immunological function. Appendix A explores some of the basic pathobiology of asthma, including a discussion of asthma subtypes.

I was interested to see if there were physiological differences between the Chinese immigrants and U.S. born whites that would suggest differences in immune system function. While I cannot assess what led to these differences and I only have cross sectional data, the differences in expression of specific biomarkers may be a result of differences in immune system development. Among these biomarkers were blood levels of CRP, IL-6, TNF, and fibrinogen. CRP, IL-6, and TNF have been associated with inflammation while fibrinogen has been associated with coagulation. In the CAFEH study, participants in each of the communities were invited to a clinic where blood samples were taken and other physiological signs were recorded. The total number of participants with I had clinical data for was 454 and the total number of participants with clinical data for my analysis ranged from 100 for IL-6 to 278 for TNF.

Based on the distribution of values, log values of CRP, IL-6, and TNF levels were analyzed (Figure 3). Chinese immigrants had lower log mean levels of TNF ($t = -5.0$, $p < 0.0005$) and a trend for lower log mean levels of CRP ($t = 1.9$, $p = 0.06$). Asthmatics had a trend of higher log mean levels of TNF RII ($t = -1.8$, $p = 0.07$).



C-Reactive Protein

The first of the inflammation biomarkers, C-reactive protein (CRP) may be associated with airflow obstruction and airway inflammation. While the evidence is not conclusive, one study of non-smoking adults indicated that high levels of serum CRP may be negatively associated with pulmonary function and positively associated with sputum eosinophil number. This result was only found for asthmatic subjects who were not taking inhaled corticosteroids. Additionally, the control subjects were statistically significantly younger than the asthmatic subjects in this study (70). The association between CRP and asthmatic symptoms may only be present for individuals with certain subtypes of asthma. The European Community Respiratory Health Survey was a multicenter study with 1289 subjects including 189 subjects with asthma. High CRP levels were associated with increased age, increased BMI, current smoker status, presence of respiratory symptoms, and increased IgE level. Non-allergic asthmatics had statistically higher levels of

CRP than allergic asthmatics. The researchers found that controlling for age, sex, smoking, BMI, and center, high CRP levels were associated with non-allergic asthma but not with allergic asthma. In this study, allergic asthma was defined as atopic asthma indicated by IgE levels of at least 0.35kU/l against specific allergens (71). Similarly, among middle aged adults participating in a British birth cohort, CRP levels were positively associated with non-atopic asthma but not with atopic asthma (72). The lack of association between CRP and asthma in the CAFEH data could be affected by the aggregation of all asthmatic subtypes into one asthma category.

CRP levels may be associated with a number of other factors that could potentially explain the trend of lower CRP levels for the Chinese immigrants compared to the U.S. born whites found in the CAFEH data. Interestingly, much of the previous literature on factors associated with high levels of CRP would suggest that the Chinese immigrants could be at risk for increased, not decreased CRP levels compared to the generally higher SES U.S. born white participants. Low socioeconomic status, as defined by employment type, even controlling for age, sex, smoking status, and BMI is associated with higher CRP levels (73) and the majority (65.1%) of the Chinese immigrants in CAFEH had very low family incomes (<$25,000). A prospective study found that smoking status and fruit and vegetable intake mediated the effects of low education and income on CRP levels (203). Additionally, independent of income and educational attainment, community factors such as living in less advantaged neighborhoods may also increase both CRP and IL-6 levels (74).

Interleukin-6

There were no significant differences between the Chinese and white participants or between the asthmatic and non-asthmatic participants in the levels of the second inflammation biomarker, IL-6. These results were somewhat surprising. There have been specific genetic polymorphisms

associated with the degree of expression of IL-6 which has been associated with juvenile arthritis (75). Ethnic differences in the distribution of alleles have also been observed in relation to the expression of cytokines. A small study found that Asians were more likely to express IL-6 than whites, were less likely to express IFN-γ, and were no more or less likely to express TNF (76). Additionally, it might have been expected that the log mean IL-6 levels would have been higher in the asthmatic participants. One study of adults with mild to moderate allergic asthma found that IL-6 levels were correlated with IL-13 and IL-13 has been consistently associated with the Th2 pathway. This study also found that IL-6 was inversely correlated with lung function (77). The mechanisms by which IL-6 functions have been investigated in animal models of allergic asthma. IL-6 likely induces the proliferation of Th2 cells, damages lung tissue, and signals to cells involved in the inflammation response (78).

The lack of association between asthma and IL-6 levels could be partially attributable to the heterogeneity of asthma since IL-6 has more commonly been associated with allergic asthma and even with allergic asthma, not all studies have found statistically significant differences (79). Moreover, the fact that the log mean level of IL-6 was not statistically different between the Chinese immigrants and the U.S. born whites despite the difference in asthma prevalence could suggest that the immunological profile or susceptibility to asthma differs between the groups. This would not be the only physiological characteristic that differentially affects Chinese and U.S. born individuals. For example, Chinese individuals may be more at risk from the health effects of obesity at a lower body mass index than Caucasians (80). However, this explanation seems discordant with the data on the other inflammation biomarkers for the CAFEH participants and it could be that other factors such as medication use, co-morbid conditions, or other risk factors I did not control for were confounding the relationship.

Tumor Necrosis Factor α-RII

The results for the third biomarker I considered, TNF, were most consistent with the original hypotheses that the immunological profiles would differ between the U.S. born whites and Chinese immigrants in such a way that could partially explain the difference in asthma prevalence between the two groups. The association between TNF and asthma has been consistently demonstrated in the literature (81–83) and even with the relatively small sample size I had available when I did the analysis of clinical variables, participants with asthma had a trend of higher log mean TNF levels and Chinese immigrants had lower log TNF levels than U.S. born whites. If TNF did in fact play a role in the differences in asthma prevalence, it would make sense that in groups with lower TNF levels, a lower prevalence of the disease would be observed.

While no conclusions can be drawn about the temporal relationship between TNF and asthma diagnosis based on the CAFEH study, biologically, the positive association between TNF levels and asthma makes sense. TNF is important for both inflammation pathways and oxidative responses by depleting the antioxidant glutathione and stimulating reactive oxygen species. Many immune system related cell types, such as eosinophils, mast cells, T cells, and lympochytes can produce the cytokine TNF. The precise role of TNF in asthma is not yet understood but it is thought to damage bronchi epithelial, endothelial and smooth muscle cells (84) as well as contribute to an increased histamine response in sensitized individuals (85). Mast cell derived TNF has been particularly associated with IgE dependent inflammation for allergic asthma (86). The effects of TNF may be at least partially independent of the type of TNF expressed since a genetic polymorphism that up-regulates TNF has not been associated with an increase in asthma likelihood or severity (87) and a prospective birth cohort did find any association between different TNF genotypes and asthma onset (88). This is especially relevant

because of racial and ethnic differences in the single nucleotide polymorphisms expressed for TNF receptor types (89,90).

Fibrinogen

The final biomarker considered in the CAFEH study, fibrinogen, has been associated with asthma in previous studies. Fibrinogen is a glycoprotein that is part of the blood coagulation cascade. As part of this network, fibrinogen is converted into a fibrin network (91). The structure of fibrin networks look qualitatively different in mice models of asthma compared to control mice (92). The finding that fibrinogen levels are associated with asthma has been seen in humans as well. One study of over 1500 Finnish men 45-74 years old found a dose-response relationship between both CRP levels and fibrinogen levels with bronchial asthma (93). In contrast to the other biomarkers which have been associated predominantly with the pathways leading to an asthmatic response, fibrinogen levels may be high in individuals with asthma due to the repeated epithelial damage and resultant need for increased levels of coagulation related molecules (94). The relationship between fibrinogens and asthma may vary by subtype. Individuals with adult onset asthma have been shown to have higher levels of fibrinogens than adults without asthma but this relationship was not found for individuals with earlier onset asthma (95). Among the 31 asthmatic CAFEH participants with clinical data, there was a trend for mean fibrinogen levels to be higher in participants with later onset asthma ($t = 1.612$, $p = 0.118$). While the interpretation of this may be quite ambiguous given the small sample size and imprecise classification method for early and late onset asthma based on ranges of times since diagnosis, it would be consistent with previous studies. However, there was no overall association between fibrinogen level and asthma status.

Fibrinogen levels may vary by ethnicity but the differences between Chinese and white individuals may not be as large as the differences in fibrinogen level between other groups. A study in England found that blacks had lower levels of fibrinogen than whites even after controlling for smoking status, BMI, age, and hormone exposure. However, they found that South Asians did not have statistically different levels of fibrinogens than the whites (96) and a different study found that fibrinogen levels between Chinese and European participants were not statistically different (97). These results seem concordant with the CAFEH data that found no significant difference in the mean fibrinogen levels between the Chinese immigrants and the U.S. born whites. However, as with the IL-6 data, the interpretation of the differences in biomarker levels, or lack thereof, between the Chinese immigrants and U.S. born whites is further complicated by the potential for differential impact from similar absolute levels of the biomarkers. A large multi-ethnic cohort study found that cardiovascular events were able to be predicted by CRP or fibrinogen levels for whites and IL-6 or fibrinogen levels for blacks but none of CRP, IL-6, or fibrinogen levels predicted cardiovascular events in the Chinese participants (98).

CAFEH within the Context of the Healthy Immigrant Effect

The previous discussion of various demographic, environmental, and physiological factors depicts a complicated picture of asthma and ultimately, it remains quite difficult to accurately determine precisely why different individuals or groups may be more susceptible to asthma. Nevertheless, the risk factors discussed within this chapter suggest that the low asthma prevalence observed among the Chinese participants compared to the U.S. born whites may be partially attributable to environmental and lifestyle factors in the participants' current country of

residence. This can perhaps be best evaluated within the framework of the healthy immigrant theory that recent immigration could be a protective factor against certain health outcomes.

Given the long average length of time the Chinese immigrants in the CAFEH study had resided in the U.S. (approximately 19 years on average), the healthy immigrant theory might suggest that the health status of the immigrants would be approximately the same as that of native-born U.S. citizens or potentially slightly worse due to the increased likelihood that the immigrants face other challenges to their health status such as poverty and that newer immigrants would have better health status. Length of time in the host country and age at immigration would be relevant if it results in fewer years of exposure to the lifestyle and environmental conditions of the host country and these exposures contributed to disease risk. In this case, chronic conditions that take many years to develop would be less likely in older immigrants than younger immigrants (3). For example, among older adult immigrants to Canada the healthy immigrant effect is observed (99). Interestingly, the average age of the Chinese immigrants in the CAFEH study when they came to the U.S. was 45.4 years (median 46, range 6-74, 0.01% immigrated as children under 18 years of age) compared to the older average age of migration for the asthmatic Chinese at 57.3 years (range 47-65). While this is not entirely consistent with the predictions that could be made from the healthy immigrant effect for chronic diseases, asthma is not a typical adult chronic disease such as heart disease or diabetes that would be expected to result from years of certain health behaviors.

Additionally, although the Chinese were less likely to have high cholesterol, Chinese immigrants and U.S. born whites did not have a significantly different prevalence of co-morbidities overall. Moreover, there were more differences in health status between Chinese participants who had immigrated more recently (in 2005 or later, n = 35) compared to those who

immigrated before 2005 (n = 153) than between Chinese immigrants who had resided in the U.S. for longer than the mean of the immigrants (immigrated prior to 1992, n = 86) compared to Chinese immigrants who immigrated later (n = 102). Specifically, as might be expected, newer immigrants were significantly less likely than immigrants who had been in the U.S. since before 2005 to have high blood pressure (OR = 0.32, 95% CI = 0.13 - 0.78), to have high cholesterol (OR= 0.18, 95% CI = 0.04 -.79), and to have at least one co-morbid condition (OR = 0.32, 95% CI = 0.14 -.70). Despite these trends, there were no statistically significant differences in the prevalence of any of the co-morbid conditions considered for Chinese immigrants who had resided in the U.S. for longer than the mean of the immigrants compared to Chinese immigrants who immigrated later.

Furthermore, the trends for asthma status were not completely clear. While either six or seven of the eight asthma cases among the Chinese immigrants were diagnosed since the individuals came to the U.S., suggesting that time in the U.S. might have been a factor, the average amount of time that the Chinese immigrants in the CAFEH sample with asthma had lived in the U.S. was 18 years and 11 months which is almost identical to the average amount of time across all of the Chinese immigrants in the sample had lived in the U.S. Additionally, there was no difference in asthma status between immigrants who came to the U.S. in 2005 or later (OR = 1.27, 95% CI = 0.56 - 2.85) or between immigrants who had resided in the U.S. for longer than the mean amount of time (OR = 1.43, 95% CI = 0.33 – 6.15). However, based on the small number of cases of asthma in this group and the inexact ranges for diagnoses date, there is not enough evidence to draw conclusions about the association between time in the U.S. and asthma onset for this population.

Nevertheless, the general trend has been observed before. For example, in Canada, new immigrants also report better health status initially but worse health status than native born Canadians after about five years. While some of the difference could be due to the natural worsening in health status with increasing age, the authors suggest that the health status of the immigrants increasingly resembles the health status of native Canadians the longer they remain in Canada. Among immigrants, the overall asthma prevalence was 5.8 percent initially but increased to 6.4 percent after 10 years in Canada compared to the asthma prevalence among native born Canadians of 8.9 percent (100). This is an effect referred to as convergence of health status.

Beyond an analysis of outcome prevalence, the healthy immigrant theory would predict that exposure status to various environmental exposures associated with health outcomes of immigrants who have been in their host country for several years should be approximately the same as residents born in the host country unless genetic or early life exposures could have long-term effects. Alternatively, if there are persistent differences in exposures even within the host community, the health status of the immigrants should remain better than the health status of the native-born residents. The latter situation seems more consistent with the CAFEH data. There were no statistically significant differences for any demographic or environmental variables between Chinese participants who had immigrated before or after 1993 or between Chinese participants who immigrated before or after 2005. Moreover, the exposure profile of the Chinese immigrants and the U.S. born whites did differ significantly on many of the variables considered and the Chinese immigrants may have had heavier highway related air pollution exposure. In support of the idea that later life protective factors may affect the asthma prevalence, the Chinese immigrants were significantly less likely to be exposed to occupational chemicals and dust, less

likely to be exposed to SHS in the home, and were less likely to be current smokers. While it seems that the CAFEH data are at least partially consistent with prediction from the healthy immigrant theory, the trends observed for later life exposures only addresses one component of the difference in prevalence. Varying susceptibility is clearly an important factor. Although no data was collected about CAFEH participants' family medical history or early life environment, there are almost certainly genetic and epigenetic differences that make individuals more or less likely to develop immune disorders. I will review the relevant literature on several early life risk factors in the following chapter.

Limitations

Using the CAFEH data afforded me the opportunity to consider the association of multiple putative risk factors with asthma in the Chinese immigrant and U.S. white population residing in Boston. However, there were several important limitations to my analysis, many of which I have noted previously. Since there were only eight Chinese asthmatic participants in the study, the statistical power and ability to control for a large number of variables was limited. The number of participants with asthma may also have been underreported as Chinese immigrant children in Boston may have a relatively high prevalence of undiagnosed asthma although previous work has found that the prevalence is quite low (101). This may be partially attributable to translation difficulties of the word 'wheeze' from English to Cantonese (102). Additionally, the CAFEH study only asked whether participants had ever been diagnosed with asthma, not whether they currently have active asthma. This would primarily be a concern if asthma symptoms were significantly different between prior and current living and occupational situations. Furthermore, since limited information was available about age of onset of asthma in this sample, I could not consider whether the various exposures occurred prior to or after the individuals were diagnosed

with asthma. Without clear temporal data, it would not possible to assess causality. Exposure assessment was complicated as no data was collected about participants' former residences in China or the time frame they lived in China. This type of information, along with information of whether participants have a family or personal history of immune conditions, if they have had infectious diseases previously, or whether individuals are exposed to allergens such as pest or mold in the home would have been useful in assessing the influence of genetic, epigenetic, and environmental factors associated with asthma in previous research (103,104).

Despite these limitations, the asthma prevalence found for the Chinese immigrants is similar to that of other immigrant groups and the relatively low asthma prevalence among the Chinese immigrants compared to the U.S. born whites can be at least partially explained by the lower current smoking rates, lower exposure to SHS, and lower exposure to occupational dust and chemicals. Future work with this sample should incorporate the particulate matter exposure models currently being developed rather than using proximity to highways as a proxy for outdoor air quality. Additionally, participants' exposure to combustibles within the home based on different heating and ventilation systems could be included in future analyses. Ideally, future studies would have larger numbers of Chinese asthmatics to allow for an analysis of the relative importance of the various factors associated with asthma. Other methods for investigating this research question will be explored in the final chapter.

Chapter 3. Genetic, Epigenetic, and Environmental Risk Factors

Methods for Literature Review

From previous work I have done with risk factors for asthma and while reading the literature pertaining to my analysis of the CAFEH subsample, I knew that I would need to explore risk factors beyond those included in the CAFEH study since CAFEH was not designed to answer questions about immune or respiratory outcomes and my central question was whether the observed low prevalence of asthma among Chinese immigrants could be understood in terms of the broader and ongoing investigation into the etiology and pathobiology of asthma. Multiple genetic, epigenetic, and environmental exposures likely interact to cause asthma. How these factors may lead to asthma onset is as yet unknown, however. Any theory of asthma etiology must explain the observed global temporal and geographic trends of asthma prevalence as well as the biologic mechanisms that are associated with asthma. In this chapter, my goal is to present several categories of risk factors, including genetic, epigenetic, and environmental influences not already considered within the CAFEH analysis, that if present in some combination, could potentially lead to asthma onset.

I reviewed several hundred journal articles between April 2012 and March 2013. Most of these I identified through Google Scholar searches. I gave priority to articles that were highly cited, were from journals with high impact factors, were recent, or seemed particularly pertinent to the research question. Beyond basic keyword searches, I found a substantial minority of the articles by reviewing the bibliographies of the studies I cited. Some of the key words included most often in my searches were: adult, asthma, biomarkers, Chinese, cytokines, environment, epigenetic, ethnicity, genetic, hygiene hypothesis, inflammation, immigrant, immune, maternal, methylation, microbiome, nationality, pollution, prevalence, respiratory, risk, and vitamin D.

After identifying relevant articles exploring the relationship between the putative risk factor and asthma, I separately searched for articles considering differences by ethnicity and nationality.

Genetic Influences

The first category I considered within my literature review was the influence of various genetic factors on asthma prevalence and risk. While much of the literature focuses on genetic differences between groups of individuals, 99.9% of the human genome is shared by everyone and the differences that are observed neither reflect any inherently good or bad distinctions nor any meaningful differences that would distinguish racial groups (105). However, there could still be heritable differences between groups of individuals that make them more or less susceptible to a given disease and examining these differences can help us learn more about the disease pathobiology. Thus, while in this section, potential genetic differences by nationality and by asthma status will be considered using the language that is typically used in the literature, it should in no way be taken as a statement about differences between the population groups that mean anything beyond potential differences in the expression of specific proteins.

Genetic factors may have substantially influenced the differences in asthma prevalence between the Chinese immigrants and U.S. born whites but no family medical history was obtained from the CAFEH participants, so I could not consider genetic influences directly. Epidemiological twin studies indicate a strong genetic component in asthma etiology. While these types of studies have to be interpreted cautiously given the potential for shared environmental characteristics, one Australian twin study on asthma and hay fever found that the correlation between monozygotic twins for disease occurrence was 0.65 while the correlation between dizygotic twins was only 0.25. The correlation was particularly strong between the 567 male monozygotic twin pairs at 0.75 (106). Another, larger study of 11,688 Danish twin pairs

suggested that up to 73 percent in the variation of asthma prevalence may be due to genetic factors, even accounting for similarities in environmental factors (107).

While the role hereditary factors play in asthma incidence is rather uncontroversial, determining the precise genetic loci that are involved is challenging. The association between family history of immune disorders and asthma may be mediated by certain genetic polymorphisms in genes encoding elements of the immune system, specifically those associated with the major histocompatibility complex, cytokines, and chemokines (108). However, given the heterogeneity of asthma subtypes and the complexity of the pathophysiology of the disorder, it is perhaps unsurprising that recent genome wide association hypothesis generating studies have found that genetic loci on several chromosomes (2, 4-7, 9, 11-17, and 22) are associated with asthma (109,110). Additionally, some of the observed variation in asthma risk may have to do with which particular variant of a gene is expressed. This is true for variants of the gene that code for IL-17 as some variants have been found to be protective while others have been associated with asthma among Chinese participants (111). A recent meta-analysis has suggested that only certain haplotypes for IL-10 are associated with asthma (112).

Among the loci with strong associations with asthma in one study were alleles of genes that encode for the IL-18 receptor 1, the IL-1 receptor ligand 1, and the major histocompatibility complex. In this study, some chromosomal regions may have been more closely associated with adult onset asthma than childhood onset asthma, such as the single nucleotide polymorphisms present in one region of chromosome 17 and the major histocompatibility complex. This study did not find any statistically significant associations with chromosomal regions and either occupational or severe asthma. The authors were able to create a predictive model for asthma based on seven genetic markers that had a specificity of 75 percent and a sensitivity of 35

percent (110). Another study found that an allelic variant in the region on chromosome 17 is associated with people who were diagnosed with asthma after 40 years of age but not with people who were diagnosed between 20 and 40 years of age or who were diagnosed at less than 20 years of age (113).

It would be especially relevant to the CAFEH analysis if genetic factors at least partially determined TNF and CRP levels. TNF seems to be associated with a genetic locus on chromosome 6 (109). A genetic polymorphism in a certain intron has been shown to down regulate TNF levels (114). CRP is also associated with certain polymorphisms. The Framingham Heart Study found that clinical indicators such as age, sex, BMI, cholesterol levels, and blood pressure accounted for 26 percent of the variability in CRP levels between individuals while a tri-allelic single nucleotide polymorphism accounted for only 1.6 percent of the variability in CRP levels. Two of the three haplotypes were associated with higher CRP levels than the third, and most common haplotype (115). These genetic associations have been shown to be important in other immune disorders. Certain genotypes associated with each of high levels of TNF and low levels of CRP have been found in individuals with the autoimmune disease systemic lupus erythematosus (116,117). TNF has even been implicated in the pathogenesis of arthritis. By treating transgenic mice with altered TNF genes, arthritis was prevented (118). Furthermore, differing TNF haplotypes have been found between white and Chinese populations (119). It seems plausible, therefore that genetic factors could account for at least some of the observed difference in asthma prevalence between the Chinese immigrants and U.S. born whites.

Of note, the methods for assessing the genetic role of asthma have changed dramatically over the past several decades. In the coming years, sequencing studies are expected to help researchers identify rare alleles associated with asthma that are difficult to identify with genome

wide association studies alone (120). Most likely, the inheritance pattern for asthma is polygenic and that the associated alleles are relatively common (121). Additionally, future research will need to verify the initial findings from the genome wide association studies as the majority of the associations of specific loci with asthma have yet to be replicated (122).

Epigenetic and Early Life Influences

The immune system is not fully developed at birth so postnatal exposures, especially during a critical period immediately after birth, can affect immune function later in life (123). Epigenetics, the field dedicated to studying the "heritable changes in gene expression that are not due to changes in the DNA sequence," has become increasingly important in our understanding of development and disease processes (124). Environmental exposures can affect DNA methylation which in turn down-regulates gene transcription, modify histones to change the accessibility of DNA for transcription, and can up-regulate degradation of mRNA (125).

Some of the early life exposures that had previously been recognized as either risk or protective factors for the development of asthma may affect the development of the immune system through epigenetic mechanisms (126). For example, one recent study suggested that DNA methylation is down-regulated in the promoter region for a specific innate immunity receptor by epigenetic mechanisms in the placentas of pregnant women who lived on farms but not in the placentas of women who did not live on a farm (127). Of potential clinical importance, in one of the first studies considering the epigenetic effects of prenatal exposure to cigarette smoke, it was found that maternal smoking is associated with decreased DNA methylation of specific disease related genes (128).

Genes associated with atopy and asthma are among the genes that have been found to have epigenetic regulation. Specifically, genes associated with certain cytokines such as IL-4, Il-13,

IL-17, and IFN-γ have been implicated (125). The effects of the epigenetic regulation on the expression of asthma symptoms are varied. For example, in animal models lacking a specific regulatory sequence for IL-4 which is required for proper acetylation of relevant histones, asthma responses were substantially inhibited (129). Differentiation of T helper cells into type one and type two cells from naive $CD4^+$ T cells is also affected by epigenetic regulation. Depending on whether the cell will become a Th1 or Th2 cell, different sets of genes are activated and silenced. There is some evidence that the sets of genes are mutually inhibitory. For example, the *Ifng* gene (which expresses IFN-γ) is preferentially transcribed and the *Il4* gene is silenced in Th1 cells while the reverse pattern is seen in Th2 cells (130). While the implications of epigenetic regulation of T helper cell differentiation, and specifically the implications of reciprocal regulation seen with certain elements of the pathways, on asthma and other atopic disorders is not yet completely clear, the field of epigenetics may hold many of the keys to understanding the etiology of asthma and other diseases.

Most of the literature on epigenetic mechanisms for asthma onset has focused on cell or animal models and the studies in humans have focused almost exclusively on pediatric asthma (125). However, one recent epigenetic study of adults over 40 who smoked revealed that more genes were methylated in asthmatic participants than in non-asthmatic participants. When two genes were methylated, one for a specific transcription factor and one for a certain receptor type, there was a synergistically higher likelihood of asthma and when the receptor gene was methylated, the individual was likely to have a more severe form of asthma (131). Additionally, children with asthmatic mothers are more at risk of developing asthma than children of asthmatic fathers (132). This observation could be partially explained by hereditary epigenetic effects that become part of the epigenome (133). One study in mice showed that the severity of asthma

symptoms could be affected transgenerationally through *in utero* exposure to methyl donors. Specifically, eosinophil counts, IgE levels, and IL-13 levels were all higher in mice with prenatal exposure to methyl donors. The authors emphasized that the influence on the Th1/Th2 balance may have been incorporated into the germ line, although the effects were reduced in the subsequent generation (134). This could have clinical implications as folic acid is a source of methyl donors. However, the literature on the association between maternal folic acid supplementation and development of asthma in children is inconsistent (135,136). In the coming years, the field of epigenetics may very well help elucidate the role of the various risk factors in the pathogenesis of asthma. In particular, epigenetic research is lending support to the Hygiene Hypothesis by explaining the mechanisms for how *in utero* and early life exposures alter the development of the immune system (137).

Hygiene Hypothesis

The Hygiene Hypothesis is one of the leading frameworks to explain the observed variation in asthma prevalence by national origin. The Hygiene Hypothesis states that the human immune system evolved to fight environmental pathogens but in high-income areas with less exposure to farm animals, intestinal parasites, and other disease agents, T regulator cells overreact to minor threats when they are activated later in life. This process is atopic sensitization (138). Individuals who have at least one positive allergen-specific test and have doctor diagnosed asthma are considered to have had atopic sensitization. Atopic sensitization may be responsible for over half of the cases of asthma (139). According to the Hygiene Hypothesis, early life exposures may influence whether individuals will develop atopic sensitization.

Immigrants from certain regions of the world may have grown up with more exposure to infectious disease agents that serve as protective factors by promoting typical immune system

development. Thus, the decrease in infectious diseases among children in high income nations may be leading to an increase in immune disorders (140). The data on these exposures can be difficult to collect, however. Self-report data collected in two health centers in Boston suggest that Chinese immigrant children did not have more unsanitary conditions (defined as exposure to factors such as intestinal worms, dirt floors, farm animals in the home, and manure) while in China or in Boston that could account for the difference. While this could certainly be true, participants may have underreported unsanitary living conditions due to the public interview site during data collection (141).

On an ecologic level, the idea that atopic sensitization is more common in countries that are more developed is supported by several large epidemiologic studies. In the International Study of Asthma and Allergies in Childhood (ISAAC), prevalence and changes in prevalence over time were assessed in 56 countries. ISAAC found that the prevalence of asthma and other atopic disorders was higher in more developed regions of Australasia and Northern Europe than in Asia and Eastern or Central Europe (142). The ECRHS was a similar large scale study of adults conducted in 22 countries including the U.S. This study used both self-reported measures of asthma and objective measures such as the forced expiratory volume and methacholine challenge. In support of the observation that there is a geographical gradient in which nations in the Northern hemisphere tend to have a lower prevalence of infectious diseases but a higher prevalence of immune disorders compared to nations in the Southern hemisphere (143). The ECRHS also found that asthma prevalence was higher in more developed regions, that there were urban and rural differences within countries, and there was a lower prevalence of asthma in former Soviet bloc countries compared to Western European and English-speaking countries (6,7).

Beyond this distribution pattern, and of relevance to the CAFEH analysis, first generation immigrants have a lower asthma prevalence than the host population in higher income nations as is predicted by the Hygiene Hypothesis (144). This pattern was seen in a study of military recruits in Israel. The researchers found that first-generation immigrants from Ethiopia and the former Soviet Union had a lower asthma prevalence than the Israeli born recruits and that immigrants from Western countries had the highest asthma prevalence (145). The effect seems to be limited to first generation immigrants. In Sweden, for example, foreign-born immigrant children were three to four times less likely to have asthma as international adoptees and children born in Sweden to foreign-born parents. Additionally, this study found that the older the children were at the time of immigration, the less likely they were to have asthma (146). However, not all of the research has been consistent in reporting a decreased asthma and allergy prevalence among migrants from lower income countries to higher income countries. One review found that poor hygiene in the host country, defined as early life exposure to the gram-negative bacteria endotoxin, did not seem to protect against asthma onset (147).

The literature on adult immigrants and asthma status by national origin is sparser. A 2003 study of adult immigrants to Italy found mixed results about the association between country of origin, development status, and asthma and allergy prevalence. Only male immigrants from the Philippines had statistically lower asthma and allergy prevalence than Italian-born residents while male immigrants from Morocco, El Salvador, and Sri Lanka as well as female immigrants from Brazil and Ecuador all had higher asthma prevalence than Italian-born adults. There were no statistically significant differences in asthma and allergy prevalence between immigrants from either Egypt or China and Italian born residents (148). Additionally, time in the host country may be more important than country of origin for adults. Another Israeli study found that adults of

Ethiopian origin who had lived in Israel for 6-13 years were almost three times as likely to have asthma as the general Israeli population (16.9% and 5.8%, respectively). The authors of this study suggested that industrial exposures in Israel may contribute to asthma onset among the Ethiopian immigrants as previous studies had found that at the time of arrival, only 2.5 percent of Ethiopian immigrants had asthma and the longer the immigrants had been in Israel, the more likely they were to have asthma (149). As discussed in the previous chapter, some of the difference seen between immigrant groups residing in the host country for differing lengths of time may be due to a shift in the immunological profile over time. In particular, some of the protection provided by early life exposure to pathogens may be reversible as anthelmintic treatment has been shown to correlate with IgE levels reverting to a more normal level (150). Nevertheless, the Hygiene Hypothesis that early life exposures affect immune system development remains a prominent theory for the disparities in the global burden of asthma and the rising asthma prevalence globally (151–154).

Parasites, Viruses, and Microorganisms

While it will remain extremely difficult to confirm a link between early exposures and asthma onset after an extended latency period with a variety of unknown exposures and interactions over time, studies with animal models have been used to attempt to overcome this limitation. Mice exposed to mycobacterial antigens reduced the development of bronchoconstriction and hyper-responsiveness to methacholine, both characteristic features of asthma (155). However, studies in humans are necessary to validate the laboratory findings. It may be slightly easier to study the Hygiene Hypothesis among children as this reduces the latency period although even this can be quite challenging. Nevertheless, there is a fairly

extensive literature on the influence of parasites, viruses, and microorganisms on immune system development and function.

Populations exposed to more parasites at a young age have been shown to be less likely to have atopic sensitization. For example, a large cross sectional study found that Vietnamese children who had been infected with the Ascaris parasite were less likely to have an allergic response to dust mites (adjusted OR = 0.28, 95% CI = 0.10 - 0.78) (156). Likewise, a case-control study in Italy found that atopy was less common in children exposed to at least one of *Toxoplasma gondii, Helicobacter pylori*, or hepatitis A virus (OR = 0.70 for exposure to one infection, OR = 0.37 for exposure to more than one infection) (157). Moreover, a randomized controlled trial examining the potential protective effect of probiotics showed that at risk children (those with a close relative with an atopic disease) exposed to *Lactobacillus rhamnosus* (*Lactobacillus* GG) prenatally and through the first six months of life were about half as likely to develop atopic eczema. However, the effect of the probiotic was not consistently protective as total IgE antibodies, antigen-specific IgE antibodies, and skin-prick test reactions were not significantly different at any point up to two years between the placebo and experimental groups (154).

Beyond the association with parasites that could contribute to immune system development, early life exposures to respiratory viruses and other pathogens have also been associated with a decreased risk of asthma onset later in life. For example, among a cohort in Guinea-Bissau, children who had had the measles were less likely to develop asthma (158). Some evidence also links exposure to more children at home or in day care to a decreased risk for asthma which may be attributable to an increased risk of infections early in life (159). Given that early life factors likely influence later asthma onset, randomized controlled trials have shown how relatively

simple changes to the early life environment can affect later asthma onset. The intervention group in a Canadian asthma prevention study reduced dust mite exposure from bedding, supported parents in their efforts to reduce or eliminate SHS exposure in the home, advocated avoidance of day care and pets, and encouraged breast feeding for at least four months. Follow up of the children at seven years of age indicated that the children in the intervention group were less likely to have asthma although there were no statistically significant differences in the prevalence of other atopic disorders (160).

Additionally, environmental and dietary exposures can affect the intestinal microbiome and these exposures differ by region (161). A qualitative shift in the microbiome to a different combination of microorganisms in industrial cultures may affect individuals' ability to tolerate different pathogens (152). Starting at a person's birth, the microorganisms that colonize the intestine exist in a symbiotic relationship with the individual and may contribute to the development of the immune system's tolerance to different antigens. Understanding the human microbiome is proving to be a quite involved task. Following the Human Genome Project, the current Human Microbiome Project (162) is attempting to catalogue the microorganisms which constitute about 1-2% of the human body by mass. This undertaking is quite ambitious given both the diversity and sheer number of microbial cells within each individual. There are thought to be at least 100 times as many microbial genes and ten times as many microbial cells as human ones in any given person representing about 7000 strains of microorganisms. Moreover, the net effect of these microorganisms seems to be highly dependent on the total ecology of the microbiome and the microbiome seems to change over time. Nevertheless, if this project can be successfully carried out, researchers hope that interindividual differences, as measured in fecal bacteria profiles, will yield insight into disease etiology (163).

While the microbiome ecology changes at least in infants reflects changes in dietary patterns (164), ecological patterns have emerged that can be associated with asthma status. In a study of adults, distinct categories of microorganisms were associated with asthma by sequencing over 70% of the microorganisms in the airway and analyzing the subsequently derived phylogenetic trees of the microorganisms. Specifically, they found that the presence of increased amounts of proteobacteria was associated with asthma and they thought that three microorganisms in this phyla, *Haemophilus*, *Moraxella* and *Neisseria,* may be pathogenic (165). These microorganisms have been previously associated with an increased risk of asthma. For example, *Haemophilus* is associated with increased levels of IgE antibodies (166).

Additionally, microorganisms in the intestine may affect T regulatory cell development and the Th1/Th2 balance (167). Animal models have even shown that specific microorganisms, such as *Bacteroides fragilis*, can help correct for an imbalance between Th1 and Th2 cells (168). Moreover, in a cross sectional study on pediatric asthma, researchers found children who grew up on farms had exposure to greater diversity of microorganisms in the environment and that this diversity was associated with a decreased likelihood of having asthma (169). However, another study found that the composition of microorganisms is more important than the diversity in the hygiene hypothesis (170). As the field develops, it will be interesting to see which classes or combinations of microbes emerge as the most relevant to asthma etiology.

Later Life Environmental and Lifestyle Influences

Genetic and early life factors are likely not the only influences on the development of asthma. Environmental and lifestyle factors later in life are likely contributors to, or are at least triggers of, asthma pathogenesis, especially for adult-onset asthma. In this section, I present a

discussion of a variety of different types of exposures that have been associated with asthma in the literature.

Exposure to Allergens

While several measures of air quality were collected within the CAFEH study, certain measures of indoor air quality, such as mold or visible water damage in the home, were not considered in CAFEH since these factors have little to do with cardiovascular outcomes. However, exposure to allergens such as mold, have been associated with more severe forms of asthma. At least one-fifth of asthmatics may have positive skin-prick results for sensitivity to *Aspergillus* (171–174). Furthermore, exposure to each of mold and SHS in the home has been associated with more than twice the risk of having adult-onset asthma (OR of 2.2 and 2.4, respectively) (175). Rodent, cockroach, and dust mite allergens have each been associated with asthma risk as well (45). In particular, children sensitized to inhaled allergens before the age of seven are at increased risk of developing asthma if they have a family history of asthma or allergy (176). Exposure to each of these allergens is more common in substandard housing and research has shown that immigrants and minorities, especially Hispanics and blacks, are more likely to live in substandard or overcrowded housing (177,178). With potentially high exposure levels and low ability to mitigate exposures, the second generation Chinese immigrants living in the neighborhoods of the CAFEH sample may be at increased risk of asthma compared to the U.S. born whites. This may be especially true given that their average income and educational attainment was lower and the fact that the protective factors associated with first generation immigrants are not typically observed in later generations of immigrants (179).

Obesity and Diet

Lifestyle factors may also influence asthma risk. For example, obesity is a fairly well known risk factor for asthma. In the large (n= 85,911) prospective Nurses' Health Study which found a cumulative incidence of asthma of approximately 0.5 percent per year, body mass index (BMI) was found to be a strong, positive predictor of asthma independent of other risk factors. Weight gain as an adult was also positively associated with asthma incidence (180). Interestingly, the association may be limited to women, according to a study that used the Canadian National Population Health Surveys (181). However, the association between obesity and asthma has been seen in some studies that include men such as the Finnish Twin Cohort study which found that a BMI of greater than 30 at the baseline of a nine year study tripled the risk of asthma onset (182). Additionally, other research indicates that the relationship between obesity and asthma can vary by asthma sub-type. Individuals with allergic asthma are less likely to be obese than individuals with non-allergic asthma, although some work has found a positive association between obesity and asthma in both sub-types (183).

Multiple mice models have supported the epidemiological observations that obesity leads to an increased risk of developing asthma as indicated by the presence of airway hyper-responsiveness to methacholine. The mechanisms for this association are not yet known but it is not caused by lung inflammation or by a difference in macrophage count. The hormone leptin is also thought to be uninvolved since the ob/ob mouse model that is deficient in leptin still shows an association between obesity and airway hyper-responsiveness. Instead, the authors propose that common pathways including those involving adipokines and IL-6, co-morbidities such as disrupted breathing during sleep, or mechanical factors such as the strain on airway tissues may be responsible (184). There could also be a shared genetic component to asthma and obesity. One study estimated that 8 percent of the heritability of obesity is common to asthma (185).

Other work has identified at least 11 loci on four chromosomes that could link obesity to asthma (186). Nevertheless, BMI was not associated with asthma in the CAFEH sample as the mean BMI between the two groups was almost identical at 30.0 and the differences between the Chinese immigrants and the U.S. born whites was not statistically significant ($t = -1.601$, $p = 0.113$). This is consistent with a previous case control study showing that BMI is not associated with asthma in Asian immigrant children attending a Boston hospital (187).

Dietary factors could also be important to asthma onset. In the Nurses' Health Study, among 77,866 women aged 34 to 68 years, vitamin C and E supplements were significantly associated with asthma onset although after the authors adjusted for age and smoking status, vitamin E was not associated with asthma. Carotene was inversely associated with asthma onset especially among smokers, although the relationship was not statistically significant (188). Moreover, in both the U.S. and the U.K., a population-wide increase in dietary linoleic acid (a polyunsaturated fat) consumption has slightly preceded a rise in asthma prevalence. Linoleic acid could potentially contribute to asthma onset as it is a biochemical precursor to arachidonic acid, a component of cell membranes that gets converted to a prostaglandin which ultimately reduces IFN-γ levels and thus indirectly increases IgE levels. Oily fish, in contrast, has eicosapentaenoic acid which inhibits prostaglandin production and can be a protective factor against allergic sensitization (atopy) that can lead to asthma onset. Regional and temporal trends of oily fish consumption generally support a protective link between eicosapentaenoic acid intake and asthma onset (189).

Randomized control trials have given increased support to the idea that dietary influences affect asthma incidence. Among these is the Australian Childhood Asthma Prevention Study. This randomized controlled study assigned pregnant women whose children had a family history

of asthma to four groups (placebo or active diet intervention and house dust mite reduction or not). The diet intervention included supplemental forms of omega-3 while restricting omega-6. The dust mite intervention used physical and chemical methods to reduce exposure (190). By three years of age, children with atopy, but not non-atopic children, in the diet intervention groups were less likely to have cough symptoms. In the dust mite intervention group, there was a 7.3 percent reduction in sensitization to dust mites. While neither intervention affected wheeze symptoms, these results suggest that certain environmental and lifestyle changes could potentially reduce the burden of asthma among at-risk individuals. (191).

Vitamin D

Another dietary and environmental factor is Vitamin D. Vitamin D insufficiency is recognized as a worldwide problem, although there is not much consensus around precise clinical values for vitamin D insufficiency and deficiency and the current recommended intake values may be too low (192). Vitamin D can be synthesized by the body with sun exposure or can be consumed in the diet. However, since vitamin D is not found in many foods, adequate sun exposure or vitamin D supplements are often necessary. Approximately 40 percent of the population across all age ranges has vitamin D inadequacy (193). Non-white populations tend to be more at risk. The 25-hydroxyvitamin D levels were considered deficient in 94 percent of Asian adults during the winter and 82 percent of Asian adults during the summer in the UK (194). Asthma is among the plethora of conditions potentially linked with both pre and postnatal vitamin D insufficiency (195–197).

Although it was known by the early 1980s that receptors for vitamin D exist in leukocytes (198), interest in the role of vitamin D in asthma is relatively recent. More recent research suggests that vitamin D is implicated in the regulation of both the innate and acquired immune

responses. Targets for immunomodulation by vitamin D may include T cells, antigen presenting cells, and dendritic cells (199). A variety of immune component cells, such as T cells and macrophages, contain receptors for vitamin D. While vitamin D is thought to inhibit the synthesis of cytokines associated with the Th1 pathway, the effects of vitamin D on the Th2 pathway are more complex. Administration of vitamin D to animals has been found to mitigate the symptoms of immune disorders (195), although this effect has not been found consistently (200). Vitamin D may also affect immune system development. An inner-city birth cohort found a weak, but statistically significant correlation between *in utero* 25-hydroxyvitamin D concentration and IFN-γ ($r = 0.11$, $p = 0.01$) as well as a correlation between vitamin D levels and the type of T cells expressed at birth. The correlation between vitamin D and other cytokines was not significant. The researchers found that vitamin D levels varied by city and by season (201). In the CAFEH study, no data were collected about vitamin D status of participants. However, if there were differences in vitamin D levels between the Chinese immigrants and the U.S. born white Boston residents, as seems possible based on the geographic distribution of vitamin D insufficiency, it could contribute to the differences in asthma prevalence (194,202).

Stress

Beyond the dietary factors, chronic stress and acutely stressful experiences have been associated with asthma exacerbation. For example, in a longitudinal study, 90 children ages 6-13 years with episodic asthma were followed for a mean of 620 days. Throughout the study, 22 percent of the children experienced four or more highly stressful life events and these children had almost five times as many asthma exacerbation episodes than the group who experienced fewer major life stressors. The authors also noted that in children with more chronic stress, acutely stressful experiences were also more likely to lead to asthma exacerbations (203). The

effects of stress on asthma may be mediated by the endocrine stress response. In particular, the Th1 and Th2 balance may be affected since glucocorticoids released in response to acute stress are thought to increase the production of cytokines associated with the Th2 pathway (IL-4 and IL-13) while inhibiting the synthesis of molecules associated with the Th1 pathway (IFN-γ and TNF-α) (204). Additionally, chronic stress can also lead to a blunted hypothalamic-pituitary-adrenal axis response which is in turn associated with a shift towards the Th2 pathway (205).

Estrogen

Other endocrine system components may also affect asthma onset. Specifically, adults with higher estrogen levels are more likely to develop asthma. Adult-onset asthma is more common in females, and in particular in females who have previously been pregnant (206,207). Moreover, in the prospective Nurses' Health Study, post-menopausal women who were not taking hormone replacement therapy were less likely than either pre-menopausal women or post-menopausal women who had taken hormone replacement therapy to develop asthma, even adjusting for age, BMI, and smoking status. The authors of this study found a dose-response relationship between both dose of conjugated estrogens and duration of hormone replacement therapy use with risk of asthma (208). In an animal study exploring this idea, estrogen receptor α, but not estrogen receptor β, knockout mice had an increased airway hyper-responsiveness to methacholine. Estrogen receptor α knockout mice sensitized to ovalbumin also showed reduced responsiveness to methacholine compared to both sensitized and non-sensitized wild type mice. Additionally, the researchers found that in estrogen receptor α knockout female mice, ovariectomy reduced but did not extinguish airway responsiveness to methacholine. This effect was not seen in wild-type ovareictomized mice. The various experiments reported in that paper all lend support to the idea that estrogen may have a role in airway hyper-responsiveness (209).

Research Priorities

Clearly, a wide range of factors can affect asthma incidence. In the following chapter, I will consider various research designs that might help us better understand the complex array of risk factors for asthma. It could be quite beneficial to determine whether there are certain critical periods during development during which clinicians could intervene to prevent asthma onset or to prevent the progression of symptoms. It would also be useful to determine if various combinations of risk factors were more likely to result in certain asthma subtypes since this could allow for more individualized and efficient asthma management options. We also need basic research on the mechanistic pathways and to develop our understanding of genetic and epigenetic factors including regulation mechanisms. In particular, the concept of microbiome ecology is quite new.

As a final, yet important point for this chapter, the protective quality that immigrant status seems to confer in the development of asthma does not extend to these groups indefinitely. In particular, among the general U.S. population, lower annual income and less educational attainment is associated with increased prevalence. Second generation immigrants may actually be at an increased risk of developing asthma despite the low asthma prevalence among first generation immigrants (210). Therefore, immigrant health should not be ignored within the context of asthma research despite the finding that first generation immigrants were less likely to have asthma.

Chapter 4. Future Research Directions

While the existing literature on asthma risk factors and etiology is quite extensive, much remains unknown due to the complexity of defining the relationship between genetic, environmental, and social factors and asthma. In this chapter, I will consider three study designs that could potentially address different aspects of my primary research question of why Chinese immigrants would have such a low asthma prevalence compared to U.S. born whites. I will outline the basics of each of a cross sectional, cohort, and case control study, including a discussion of their relative advantages and limitations.

The first and perhaps most straightforward possibility is a larger cross sectional study that builds off of the CAFEH study. Generally, an advantage of a cross sectional design is that a representative sample can be recruited so outcome prevalence can be estimated fairly accurately but the estimates of the asthma prevalence are not likely to change dramatically by simply adding more participants. Nevertheless, gathering data more specific to asthma may be helpful. For example, participants should be asked for the exact date of age of onset and for the asthmatic Chinese immigrants, it would be important to know whether the asthma was diagnosed before or after coming to the U.S. Objective measures of asthma status, asthma severity, and atopy status could ensure that cases were defined homogenously. Participants should also be asked about their family history of asthma and allergies. If data from CAFEH were to be used, the particulate matter exposure models currently being developed should be analyzed rather than proximity to highways as a proxy for outdoor air quality. Additionally, participants' exposure to combustibles within the home based on different heating and ventilation systems should be included in future analyses. Beyond these basic additions, more extensive use of biomarkers specific to asthma could be utilized as discussed within the design for the case control study.

However, a large enough sample size to control for relevant confounding variables and to analyze relative influence of multiple variables in a way that was not possible from the initial CAFEH study is not feasible in Boston. The CAFEH study already had a fairly large sample size, even among Chinese immigrants. According to the 2010 census, there were 3416 Asians living in Chinatown (211). Since not all of these individuals were first generation Chinese immigrants, the sample of Chinese immigrants in the CAFEH study (n = 189) represents well over five percent of the population of Chinese immigrants in Boston. Thus the fact that there were so few cases reflects a low frequency among the population rather than inadequate sample size for the original sub-study. Even if the entire population of Chinese immigrants were included, there would likely be a maximum of about 160 Chinese asthmatics with adult onset asthma and this is still a fairly small sample size to control for several variables simultaneously. Short of recruiting over 6000 participants, including all of the Chinese immigrants in Chinatown, this sample size for Chinese asthmatics would not even be obtained in a cross sectional study. Additionally, as with any cross sectional study, no information about the temporal relationship between exposures and outcomes could be assessed.

One way to address some of these limitations would be to use a matched pair prospective cohort study. The study population would need to be expanded from my original analysis of the subset of CAFEH participants. Two more groups could be formed in a matched pair design with the Chinese participants. These groups would consist of second generation Chinese immigrants residing in Boston Chinatown and Chinese citizens residing in specific communities in China who could be identified through key informant interviews with the first generation Chinese immigrants. All participants should be matched on age and sex. As much as possible, Chinese residents and first generation Chinese immigrants should also be matched on community of

residence. If the communities of origin were heterogeneous, it would be useful to work with community partners and participants to determine which communities may be most representative of the general immigrant population. The purpose of the four groups would be to allow comparisons by country of origin and country of residence to answer questions regarding the relative influence of early life versus later life exposures.

A first step in this study would be to conduct lung function tests and tests for atopic sensitization among each of the four study groups (U.S. born whites, Chinese immigrants, second generation Chinese immigrants born to Chinese parents, and Chinese residents of the native communities) to accurately ascertain the asthma prevalence among each group. Particular care should be taken in assessing both the denominator and numerator for the prevalence among the Chinese resident group. Theoretically, the denominator should account for the population of potential immigrants to the U.S. potentially based on prior immigration trends from certain communities. The numerator would also have to be carefully considered because any differences in the likelihood of being diagnosed with asthma, other than likelihood of actually having asthma, would affect the estimated prevalence. For example, if there were cultural differences in labeling individuals as asthmatic or differences in ability to access diagnostic services, published statistics about asthma prevalence across different populations could be misleading. Given the fairly consistent global trends in asthma prevalence, it would still be expected that individuals living in less developed areas of China would have a lower asthma prevalence than individuals living in Boston but the magnitude of the difference may not be precisely known.

Information on prenatal exposures and early life medical history would likely be very challenging to obtain. Some of this information may be ascertained at a pseudo-ecologic level through key informant interviews. These interviews should be conducted primarily with

individuals who still live in the communities so that they could speak to changes over time. Chinese immigrants and other community partners should be included in the planning, recruitment, and interpretation phases of the key informant interviews. Participants may have the best knowledge of the types of questions they feel are relevant. They can also help determine any culturally appropriate adaptations necessary for proposed interview questions. Additionally, it may be helpful for the participants to take an active role in recruiting friends or relatives who still live in their native community through a snow-ball sampling method.

The goal of the key informant interviews would be to reconstruct an image of approximately what the native communities were like to live in several decades ago and how this changed with time. Some of the information that could be obtained would include: whether the areas were urban or rural, whether there was common exposure to pets or farm animals, whether smoking was common, what common occupations including any equipment or potential exposures were, what materials houses were built out of and what were the type of heating and cooking apparatuses used, how the social support network and access to medical care functioned, what common childhood illnesses were, where water was supplied from, and what typical diets were. Validated questionnaires, such as those used in the large international studies on asthma prevalence like the ECRHS could also be useful in creating individual exposure models (212). Certain basic information such as location of residence for the first several years of life, number of siblings, whether parents smoked, and whether the participant had contact with farm animals or pets could likely be accurately answered even after a period of decades. However, these variables are only proxies and many exposures could not be reliably ascertained at all given the necessity of recalling exposures from decades before and the inability to truly know even current exposures to factors such as certain microorganisms or chemicals.

Beyond the early life exposures and family medical history that could be ascertained to some extent through key informant interviews and surveys of participants, other relevant factors would include all of the factors analyzed in the discussion of the CAFEH sub-study. This would include time and resource intensive assessments for exposure to air pollution for the two new study communities. For the second generation Chinese immigrant group, this may not be too difficult if exposure can be estimated from models that will already be developed within the CAFEH study. However, for the Chinese resident group, this assessment could be quite challenging, especially if participants come from several different communities originally. Even assuming these models could be developed, they would reflect exposure at a later baseline time interval than the CAFEH models do. One alternative to measuring traffic related air pollution is to have participants wear a personal air quality monitoring device for a period of time which could potentially better account for actual exposure to air pollution.

A major advantage to this study design would be in prospectively assessing later life exposures, minimizing the chance of recall bias, by updating the information obtained in CAFEH and including other more asthma specific questions that could then be related to risk of developing asthma over time. If the study population of Chinese residents was least likely to develop asthma, it would be assumed that environmental or social exposures based on country of residence are relevant to asthma onset. This would be further supported if the risk among the other three groups was more similar. However, following the Chinese immigrants in the CAFEH study for an additional period of time may not yield much relevant information about differences in environmental or social factors between the immigrants and the Chinese residents or between the immigrants and the U.S. born population due to the extended average length of time (19 years) that the CAFEH participants have resided in the U.S.

Most studies of disease risk among immigrants consider only up to the first ten or occasionally twenty years after immigration. Beyond this window, it seems unlikely that the change in environmental exposures due to immigration would be the most relevant factor. Presumably, however, the study could include only participants who had immigrated within a more recent interval. This would require finding an entirely new sample of participants. This situation in itself would not be a critical problem. However, the solution could not feasibly be to follow the Chinese residents who subsequently immigrate to Boston.

To do a prospective study that would follow individuals who did and did not immigrate and ascertain their risk of developing asthma, certain communities in China would have to be identified as having individuals with a high likelihood of immigrating. While this is possible, I would be concerned about the potential ethical and legal ramifications of identifying communities this way if it could result in stigmatization or potential for increased government scrutiny into immigration applications than would otherwise be warranted. Additionally, there would have to be some factor or factors that were different between the individuals in these communities if they are more likely to immigrate than others. Assuming that individuals who are more likely to immigrate also have a higher relative SES, the effect would be to underestimate the association between country of origin and asthma prevalence which is not necessarily a major concern. However, if there were other substantial differences between individuals more likely to immigrate and those less likely to immigrate, it would limit the generalizability of the results. A more critical issue would be picking these communities and assuming that they would stay similar enough to each other over a period of decades because the sample size would not be large enough to control for extensive variability in exposures within each country, even if the sample could be large enough to assess the effects of variability in exposures between each country.

Cohort studies can be quite effective when the exposure of interest is rare but when the outcome of interest is rare, cohort studies can be problematic if the sample size is not large enough. Given that asthma is a fairly rare outcome among the Chinese participants, a cohort study may be of limited utility. Assuming that the asthma prevalence among Chinese individuals residing in China is only about a quarter of that among the Chinese immigrants in Boston, there would need to be a proportionately larger sample size among the Chinese resident study group. Otherwise, the conclusions would essentially be limited to a comparison of the asthma prevalence and speculation that some combination of environmental and social factors that differed between the immigrants and the Chinese residents was at least partially responsible for the difference. Given the amount of literature suggesting this hypothesis already, it would probably not be worth the amount of resources required to conduct the study at all unless there were a sufficiently large sample size to control for a variety of potential exposures.

At an even more basic level, if the actual asthma prevalence were more similar between the Chinese immigrant and Chinese resident groups than would be expected *a priori*, I would think that the difference between asthma prevalence among the Chinese immigrants and U.S. born whites was more attributable to early life and genetic exposures rather than the later life exposures. In this case, a prospective design that began in early life and potentially extended several decades into adulthood may be more helpful. The feasibility of such a study on an international scale is quite questionable, although models such as the Framingham Study do exist. Other methods of attempting to objectively ascertain early life exposures in adults are discussed in relation to the case control option. Alternatively, if the asthma prevalence in Chinese residents is substantially lower than that of Chinese immigrants, later life exposures could be more likely to account for at least some of the difference. Given the published statistics

that the asthma prevalence in China is less than about one percent, this scenario seems most likely (10,11). Therefore, a case-control study may adequately address at least a major part of the research question.

A 2x2 case control study could selectively increase the number of Chinese asthmatics within a relatively small sample. Participants could be recruited based on asthma status and country of origin. Specifically, there would need to be four groups consisting of asthmatic Chinese immigrants, non-asthmatic Chinese immigrants, asthmatic U.S. born whites, and non-asthmatic U.S. born whites. Asthmatic Chinese participants should probably be recruited first due to the very small number of individuals who would be eligible for this group. These individuals could perhaps be identified most easily by working with specific clinicians in Chinatown who are likely to encounter Chinese individuals with asthma. From there, non-asthmatic Chinese immigrants who were from similar regions in China originally, immigrated within a several year period, and live in Chinatown could be recruited so the Chinese non-asthmatics can be as representative as possible of the Chinese immigrant population at risk for asthma. U.S. born white individuals could be chosen from similar neighborhoods within Chinatown and asthmatic U.S. born white individuals could be recruited from the same or similar clinical practices as the asthmatic Chinese individuals. Both main effects and interactions could be considered for each of the relevant risk factors.

The risk factors to be included could be similar to those used in CAFEH with some modification as described previously. However, within a case control design, the ability to measure past exposures accurately is critical and case control studies are subject to recall bias. Thus more specific inflammation, genetic, and microbial biomarkers should be included where possible. As discussed in previous chapters, if there were differences in the pathobiology of

asthma between the study groups, it might be expected that there would be an interaction between typical inflammation biomarker levels and country of origin in relation to asthma status. Among the possible inflammation biomarkers that might show such an interaction included in the CAFEH sample are CRP, IL-6, and TNFα-RII (76,89,213–215). Interestingly, these three biomarkers are among the most common in the fairly sparse literature on ethnic differences in immunological profiles. Another immunological marker that may vary with ethnicity includes IFN-α (216). Studies on asthma do not use a standard set of biomarkers but investigating potential differences by nationality could also be useful for immunological markers commonly associated with asthma, such as IL-4, 5, 9, 13, and 17 (79,217,218). It would also be interesting to test the corresponding genetic loci for genes that encode for these proteins to see if expressed blood levels are associated with different genetic loci by nationality or by asthma status.

More specifically, it could perhaps be informative to test for genetic polymorphisms in genes that code for immunologically important molecules such as those on the Th1 and Th2 pathway because this, especially in conjunction with data on participants' family history of asthma and allergies, could more directly suggest the degree genetic differences between the populations can account for the observed difference in asthma prevalence. However, there are far more genetic loci that have been associated with asthma than would be reasonable to test in this type of study, even given unlimited technological and financial capacity. Thus a major question is how to choose which loci to test. Loci that could be included in the analysis could be informed by the allele frequency net database that catalogues the variability in genetic polymorphisms for genes that encode immunologically relevant molecules in a diverse global sample. Data for Chinese populations is only included for the haplotypes for IL-18 but for this molecule, there are substantial differences between US Caucasian populations and Chinese populations for each of

the six possible haplotypes (219) and variants of IL-18 have been associated with asthma status (220). Other potentially important single nucleotide polymorphisms that have been shown to have predictive value for asthma status in genome wide association studies include genes that encode for IL-33 and for the receptors for IL-2 and IL-18 (110).

Regardless of the specific loci chosen, certain variants of the genes that encode for inflammation biomarkers may differ by nationality and that these differences could be partially responsible for immune function differences. If the Chinese participants were less likely to have genetic polymorphisms associated with asthma, it would be expected that they would have a lower asthma prevalence. It would also be expected that Chinese residents, first generation Chinese immigrants, and second generation immigrants born to Chinese parents would all have similar genetic profiles. While this hypothesis would not be able to be tested in the case control study, it could be tested in the cohort study described previously. However, even if this hypothesis were true, it would not explain all of the variation in the asthma prevalence between the groups since the Chinese immigrants are likely to have a substantially higher asthma prevalence than the Chinese residents.

Additionally, as discussed in previous chapters, epigenetic influences are emerging as an increasingly important factor for the risk of developing asthma. Since DNA methylation is thought to reflect epigenetic mechanisms, one way to objectively test the influence of epigenetic factors among an adult population is to consider DNA methylation of specific genetic material. Methylation of the IL-4 receptor gene, the IFN-γ promoter region, and the IL-6 promoter region have each been associated with asthma and could be regions of interest to investigate (221–223). As with the analysis of genetic influences, if early life exposures were in fact important for asthma onset later in life, it would be expected that the individuals who grew up in similar

settings would have more similar DNA methylation patterns and that these patterns would be associated with asthma status. This analysis may be particularly important since early life exposure to microbes, but not later life exposure, has been associated with changes in DNA methylation patterns in specific genes that encode for molecules important in asthma such as natural killer T cells (224). Demethylation of IFN- γ seems to be regulated by epigenetic factors related to early life microbial influences and down-regulation of this molecule is associated with asthma (225). If the Chinese had methylation patterns consistent with early life exposures to microbes but the U.S. born participants did not, this could add support for the Hygiene Hypothesis.

Another way to potentially objectively measure early life microbial exposures is by evaluating the microbiome ecology. It would be interesting to consider whether there were systematic differences in the microbiomes between the Chinese immigrants and U.S. born residents or between asthmatic and non-asthmatic individuals. Given the fairly new techniques of cataloguing the human microbiome, this alone could be a substantial contribution. If this strategy were to be employed with the matched pair cohort study described previously, it would also be interesting to compare the microbiomes of Chinese residents, Chinese immigrants, and U.S. born residents. If there were differences between the Chinese residents and Chinese immigrants, later life exposures could be more indicative of current microbiome ecology. The effects of changes in these later life exposures could be considered in the prospective cohort study design but not in the case-control study. In contrast, if there were more differences between the Chinese immigrants and the U.S. born white residents, it could be indicative of either early life or genetic persistent influences on the development of the microbiome ecology. If these differences were also associated with asthma status, it could lend support to the Hygiene Hypothesis as one factor

influencing the variant asthma prevalence. In this case, doing the far less resource intensive case-control study could be preferable. Either are still plausible explanations and very likely some interaction of the two matters. From a simple technological perspective, even the case-control study could require processing power for information on the order of giga-units if an analogous process to the shotgun genome sequencing techniques were used. It could be possible to limit the technological requirements substantially by only using a subset of all participants and by considering only certain types of microorganisms that are thought to play a critical role in the regulation and development of immune system T cells such as *Lactobacillus casei and Bacteroides fragilis* (168,226,227). Other proteobacteria that have been putatively associated with asthma pathogenicity that could potentially be selectively screened for include *Haemophilus*, *Moraxella* and *Neisseria.*

As this chapter has indicated, there is no one study design that could fully address the interplay between genetic, epigenetic, and environmental factors that affect asthma, even assuming unlimited resources. Table 4 summarizes some of the advantages and disadvantages of each design described.

Table 4. Relative Strengths of Different Study Designs

	Feasible sample size	Early life exposure assessment capacity	Later life exposure assessment capacity	Temporal relationship assessment	Time to complete the study	Control for confounders	Potential to avoid selection bias	Potential to avoid recall bias
Cross-sectional	-	-	+	-	+	-	+	-
Cohort	-	+	+	+	-	-	-	+
Case Control	+	+	+	-	+	+	-	-

Several of the categories' classification, especially for the cohort study option, would depend on the ability to appropriately match Chinese immigrants to Chinese residents. Likewise, the ability to control for confounders in the case control study relies on the ability to find representative

controls within the study communities. Additionally, the early life exposure assessment capacity would be highly dependent on whether the specific biomarkers chosen adequately reflected the pathophysiological mechanisms of asthma which they may not given the still developing literature on asthma pathobiology. The later life exposure assessment would require the resources to objectively measure exposures to air pollution, dust, combustibles, and other relevant factors. Selection bias may be a persistent problem since individuals who are likely to immigrate are probably different from others in their community in ways that could affect their risk of developing asthma. Nevertheless, if I were to choose one of these studies, I would choose to do the 2x2 case control study for its potential to yield at least a comparable level of new insight into the interplay of factors, at least with adequate biomarkers for early life exposures, and with substantially less complexity than the matched pair prospective cohort.

In this chapter, I discussed three study designs and addressed some of the potential limitations of each. This discussion offers more than an intellectual exercise. By comparing foreign born and native born groups living in a specific community, we can improve our understanding of issues in immigrant health such as the relative influence of country of origin and country of residence on health status. As the vast majority of the Chinese participants resided in Boston Chinatown, a community nested between two major highways, later life environmental exposures may be particularly important risk factors for a variety of health conditions. Regardless of the specific methods chosen, future work addressing the differences in asthma prevalence among different population groups such as the Chinese immigrants and U.S. born whites could contribute to our developing understanding of the complex interaction of the various risk factors for asthma.

Appendix A. Basics of Asthma Immunology

A basic understanding of asthma pathophysiology and asthma subtypes is useful to contextualize each of the potential theories for why the asthma prevalence for the U.S. born white participants was over four times as high as the prevalence among the Chinese immigrants. Individuals with asthma can have different responses to exacerbation factors but typical acute outcomes include wheezing, coughing, difficulty breathing, and shortness of breath (228). Previous research has indicated that there are several classes of risk factors associated with asthma exacerbation including environmental and occupational factors, such as SHS (33,229) and allergens (230), and physical factors, such as exercise (231) and respiratory illness (232). The biological pathways leading to inflammation in asthma are not yet precisely understood.

Much of the literature suggests that an imbalance between the T-helper type 2 CD4+ cells (Th2) and the Th1 pathways contributes to asthma. The Th1 pathway is thought to be more closely associated with intracellular pathogens while the Th2 pathway is thought to be more closely associated with extracellular pathogens (233). The Th2 pathway involves immunoglobulin E (IgE) production, eosinophil and mast cells recruitment, and airway hyper-responsiveness (234). The Th1 pathway leads to inflammation through the activation of macrophages (233). These effects are mediated through the release of cytokines. Among the cytokines purportedly associated with the Th2 pathway are interleukins (IL) 4, 5, 9, and 13. The Th1 pathway involves interferon-gamma (IFN-γ), IL-2, IL-12, IL-18, and tumor necrosis factor-beta (TNF-β) (217,218). However, the interactions and precise functions of most of the more than 50 cytokines have yet to be fully understood (235). Additionally, while previous work indicated that the Th2 pathway predominates in asthma and much of the literature to date focuses on this paradigm, the role of Th1 in secreting TNF-α and IFN-γ for severe asthma is becoming

more apparent (234). Many of the immune system components seem critical in several pathways, complicating the research into their physiological roles.

Other related pathways beyond the Th1/Th2 cascade mechanisms may also be important to asthma onset and exacerbation. Increasingly, researchers are considering the potentially protective role of properly functioning T regulatory cells (236). T regulatory cells may have a role in inhibiting allergic responses (218) and in regulating the thickening of the airway in asthma (237). Oxidative stress brought on by exposure to stressful life events or environmental toxicants such as air pollution may also affect the ability for individuals to detoxify reactive oxygen species and this may lead to an atopic response by triggering asthma symptoms (238). Additionally, the relative influence of the innate (non-specific) versus the acquired (specific) immune responses is an area of ongoing study as both have been found to be associated with asthma (239). In particular, the regulation of the innate and acquired immune responses by antigen-presenting cells and T helper cells of subclass 1 and 2, respectively, has been investigated as has the role of the various cytokines associated with each response mechanism (240). To further complicate the matter, each of the regulatory mechanisms is itself regulated. For example, the induction of the Th2 response in acquired immunity may involve toll-like receptors and new research suggests that this could lead to asthma pathogenesis (241).

Early life infections likely affect the developing balance between Th1 and Th2 cell responses in the immune system. Specifically, early life infections may stimulate Th1 cells and thus shift the balance away from the Th2 response that has been linked with an increased risk of atopy. The relationship is not completely straightforward as helminth (parasitic worm) infections are associated with a Th2 response similar to the immune response in asthma but the long term consequences of early life infections are thought to be protective against asthma (153,242).

Certain respiratory infections may also up-regulate the expression of certain cytokines, such as TNF, which could contribute to later asthma onset (243).

Subtypes of Asthma

One of the major complexities in characterizing the pathophysiology of asthma is accounting for the heterogeneity of the disorder. While there are at least two asthma subtypes, less consensus exists regarding the classification of these subtypes, however, three common classification systems do tend to dominate the literature. The first distinguishes between allergic asthma and non-allergic asthma. Allergic asthma is typically considered to be synonymous with atopic asthma. Atopy is defined as a genetic tendency to express an immune disorder as a result of a sensitized immune response (244). Allergic, or atopic, asthma is considered to be the more prevalent subtype but even within this category, there are differences in clinical presentation (245). Some differences in mechanisms have been associated allergic and non-allergic asthma. Non-allergic asthma typically presents without the characteristic increase in IgE levels (246) and has been more commonly associated with occupational asthma (247). This increase in IgE levels in allergic asthma may be mediated by IL-4 activity while non-allergic asthma is more closely related to an IL-2 activation of T cells. Both types have been associated with an IL-5 mediated increase in eosinophil count (248).

The second major classification system for asthma relates to the presence or absence of eosinophilic inflammation. Eosinohilic asthma is the more common sub-type and seems to be more associated with atopic asthma. It has been found that the greater the number of peripheral blood eosinophils, the more severe the asthma is likely to be (249). However, non-eosinophilic (neutrophilic) asthma is increasingly recognized as another common subtype of asthma. In adults the classification of eosinophilic versus non-eosinophilic is relatively stable within individuals

over time (250). Some research indicates that eosinophilic asthma may only account for up to half of the cases (247). The distinction can be quite relevant clinically as patients with non-eosinophilic asthma may not respond as well to corticosteroid medications (251). This classification system does have limitations in explaining the pathophysiology of asthma symptoms, however. A randomized control trial found that airway hyper-responsiveness is not as associated with the presence of eosinophils although the presence of eosinophils did predict a better response to glucocorticoids (252).

A final distinction addressed throughout my thesis is early onset versus late onset asthma. This distinction is among the easiest to classify as it is based on age at diagnoses. The precise age that distinguishes pediatric or early onset from adult or late onset is not standardized. One study found that asthma onset before the age of 12 is associated with a greater likelihood of having allergic sensitization, a family history of immune disorders, and potentially better lung function than individuals with asthma diagnosed after the age of 12 (253–255). Among people with adult onset asthma, occupational factors are likely relevant in up to 15 percent of cases although the latency period after occupational exposures can vary as do the exposures themselves (256). Adult onset asthma should be further distinguished from chronic obstructive pulmonary disease (COPD). COPD primarily occurs in adults who have smoked, results in progressive airway damage, and causes difficulty with expiration. In contrast, asthma can occur in smokers or nonsmokers, typically results in reversible airway obstruction during discrete attacks, and tends to affect inspiration more than expiration (257).

While this discussion of asthma subtypes is far from exhaustive, and in particular leaves out subtypes classified by both severity and exacerbation trigger types, it does immediately suggest a major limitation throughout the asthma literature. Human studies rarely specify which subtype of

asthma they are considering and even if they do, the distinction is not consistent across the literature as specific criteria for clear subtypes have not been established. The one major exception is asthmatic children clearly have early onset asthma. Beyond this limitation, the relationship between the classification systems is still unclear. There could be distinct pathophysiological mechanisms of endotypes of specific asthma phenotypes (258). Alternatively, the classification systems could each be describing only parts of subtypes that are not yet well characterized as much overlap is observed between the systems. However, this basic discussion of asthma pathophysiology and subtype classification should suffice as context for this analysis of CAFEH study data.

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