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Immune Dysregulation and Pancreatic Cancer: Overactivity, Inflammation and Epigenetic Modifications of Immune Function

Author

Huang, Brian

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Immune Dysregulation and Pancreatic Cancer:

Overactivity, Inflammation and Epigenetic Modifications of Immune Function

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by

Brian Huang

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ABSTRACT OF THE DISSERTATION

Immune Dysregulation and Pancreatic Cancer:

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by

Brian Huang

Doctor of Philosophy in Epidemiology

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Professor Zuofeng Zhang, Chair

Background: Pancreatic cancer is a highly lethal malignancy that is often diagnosed at advanced stages where treatment options are limited and prognosis is poor. As the immune system is intricately involved in the detection, elimination, and initiation of cancer, there has been major interest in understanding the role of immune dysregulation in the development of pancreatic cancer. Conditions associated with immune overactivity (e.g. allergies), chronic inflammation (e.g. type 2 diabetes, metabolic syndrome) and the epigenetic modification of immune genes (e.g. cytokines) may perhaps provide insight into the underlying mechanisms of pancreatic carcinogenesis. Past retrospective epidemiological studies have observed inverse associations between allergies and pancreatic cancer risk, but this finding has not been detected in prospective studies. In addition, the relationship between type 2 diabetes onset and pancreatic cancer risk has not been well explored in racially/ethnically diverse populations. Metabolic profiles of diabetes patients and pancreatic cancer risk have similarly not been evaluated in non-white individuals.

Finally, there is limited literature on the influence of methylation in cytokine genes and pancreatic cancer progression.

Objectives and Specific Aims: The objective of this dissertation is to further elucidate the influence of atopic allergic conditions, type 2 diabetes, and methylation of cytokine genes on pancreatic cancer risk and survival. The specific aims were: 1) to conduct a prospective analysis of atopic allergic conditions (AACs) and the treatment of these conditions on pancreatic cancer risk in a multiethnic population; 2) to investigate the association between type 2 diabetes onset and pancreatic cancer risk in a racially diverse patient-based cohort, and to establish a metabolic profile of diabetes patients who are at high risk of developing pancreatic cancer; and 3) to evaluate the relationship between the methylation of cytokine genes in pancreatic cancer tumors and overall survival.

Methods: For specific aim 1, we analyzed prospective data from 187,266 participants from the Multiethnic Cohort (MEC). Information on AACs and antihistamine medication use was assessed via a baseline questionnaire when individuals joined the MEC in 1993-1996. Adjusted risk ratios (RRs) and 95% confidence intervals (CIs) for pancreatic cancer incidence by AACs and antihistamines were estimated using Cox regression models, adjusting for age, sex, ethnicity, education, smoking status, family history of pancreatic cancer, body mass index, diabetes, and alcohol intake. We further evaluated associations among subgroups defined by age, sex, ethnicity, follow-up time, and known pancreatic cancer risk factors. For specific aim 2, we conducted a retrospective cohort study of 1,499,627 patients from Kaiser Permanente Southern California (KPSC) from 2006-2016. Diabetes patients were identified using glucose and hemoglobin A1c (HbA1c) measurements based on diagnostic criteria by the American Diabetes Association (ADA). We used Cox regression with time-varying exposures to assess the relationship between diabetes status/duration and pancreatic cancer. For individuals with incident

diabetes, we evaluated longitudinal changes in glucose, HbA1c and weight measurements leading up to the diabetes diagnosis between those with and without pancreatic cancer. We further performed Cox proportional hazards regression to examine the associations between the percent differences in glucose, HbA1c and weight and pancreatic cancer risk. All models included gender, race/ethnicity, smoking, BMI, alcohol use, family history of pancreatic cancer, history of pancreatitis, and education as covariates. For specific aim 3, we performed a retrospective cohort study of 162 pancreatic cancer patients from The Cancer Genome Atlas (TCGA). We evaluated methylation on CpG probes in proximity to seven genes: *IL10*, *IL6*, *IL8*, *TGFβ1*, *TGFβ2*, *TGFβ3*, and *TNF*. Methylation was assessed using the Illumina Infinium HumanMethylation450 (HM450) BeadChip methylation assay. We used Cox proportional hazards regression to evaluate the relationship between site- and region-specific DNA methylation and pancreatic cancer survival, adjusting for age, gender, stage and five independent surrogate variables associated with potential confounders measured with error (smoking, receipt of radiation, sample plate). We assessed the relationship between methylation at each given site/region and the expression of the gene in closest proximity using the Spearman's correlation coefficient.

Results: For specific aim 1, 1,455 incident cases of pancreatic cancer were identified among the white, African American, Latino, Japanese American and Native Hawaiian participants of the MEC with an average 16-year follow-up. AACs (relative risk/risk ratio [RR] 1.00, 95% CI 0.88-1.12) and antihistamines (RR 0.92, 95% CI 0.78-1.07) were not clearly associated with pancreatic cancer incidence. While these associations were also null for most subgroups, we did observe inverse associations of AACs (RR 0.74, 95% CI 0.56-0.98) and antihistamines (RR 0.66, 95% CI 0.45-0.96) among the oldest participants (70+). For specific aim 2, we identified 2,002 incident cases of pancreatic cancer among the white, African American, Latino and Asian

members of KPSC with 7.5 million person-years of follow-up. Compared to those without diabetes, individuals with incident diabetes and shorter diabetes durations (≤1 year: RR 6.91, 95% CI 5.76-8.30; >1 year: RR 1.96, 95% CI 1.62-2.38) had the highest risk of pancreatic cancer. Among incident diabetes patients, those with pancreatic cancer had steeper increases of glucose, HbA1c and greater weight loss during the time prior to diabetes. Racial/ethnic differences in metabolic changes by pancreatic cancer status were observed, with more pronounced differences in Asians and blacks for glucose, in blacks and whites for HbA1c, and in Asians and whites for weight (p-values for heterogeneity ≤0.01). For specific aim 3, we observed poorer survival for increased methylation in several CpG probes in *TGFβ1* (cg03313751: hazard ratio [HR] 1.62, 95% CI 1.14-2.31), *TGFβ2* (cg16658719: HR 1.58, 95% CI 1.21-2.06), *TGFβ3* (cg16292972: HR 1.45, 95% CI 1.01-2.09) and *IL6* (cg15703690: HR 1.03, 95% CI 1.00-1.05), specifically those near transcription start sites. Conversely, higher methylation in one CpG locus in *IL10* (cg14789529: HR 0.97, 95% CI 0.94-1.00) was associated with increased survival.

Conclusions: Our study provides additional evidence of the intricate relationships between immune dysregulation and pancreatic cancer risk and progression. The findings for atopic allergic conditions support the null association for pancreatic cancer observed in prior prospective cohorts. Moreover, increased pancreatic cancer risk for new-onset diabetes is observed among racial/ethnic minorities, while the metabolic profiles for incident diabetes patients appear to vary by pancreatic cancer status and race/ethnicity. Lastly, epigenetic alterations and regulation of cytokine-related gene expression may be involved in the progression of pancreatic cancer.

The dissertation of Brian Huang is approved.

Chun Chao

Catherine Ann Sugar

Alexandra M L Binder

Veronica Wendy Setiawan

Zuofeng Zhang, Committee Chair

University of California, Los Angeles

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The results of Aim 3 (Chapter 5) are in preparation for publication. Co-authors include Alexandra M. Binder, Catherine A. Sugar, Chun R. Chao, Veronica Wendy Setiawan and Zuo-Feng Zhang. BZH and ZFZ conceived and designed the study. BZH and AMB performed statistical analysis and drafted the manuscript. All authors helped interpret results and critically review the manuscript.

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VITA

EDUCATION

2014 M.P.H in Epidemiology & Biostatistics, Johns Hopkins Bloomberg School of Public Health

2010 B.S. in Biology, University of California, Los Angeles

PROFESSIONAL EMPLOYMENT

2015-Present Biostatistician, Kaiser Permanente Southern California, Department of Research & Evaluation

2014-2015 Analytical Programmer, Kaiser Permanente Southern California, Department of Research & Evaluation

2010-2013 Program Coordinator, UCLA Jonnson Comprehensive Cancer Center, UCLA Genetics Program & UCLA-LIVE**STRONG**™ Survivorship Center of Excellence

2009-2010 Research Associate, UCLA Jonnson Comprehensive Cancer Center, Division of Cancer Prevention and Control

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CHAPTER 1. BACKGROUND

1.1 Pancreatic Cancer

1.1.1 Overview

Pancreatic cancer is a malignancy in the exocrine and endocrine cells of the pancreas. Cancers of the exocrine cells are the most prevalent, with adenocarcinoma making up 95% of all exocrine cancers[1,2]. Less common exocrine tumors include adenosquamous, squamous cell, signet ring cell and undifferentiated carcinomas. Malignancies in the endocrine cells, called pancreatic neuroendocrine tumors (NETs), are fairly rare and comprise less than 5% of all pancreatic cancers[1].

1.1.2 Pancreatic Cancer Incidence & Mortality

Pancreatic cancer is the 12th most common cancer and 7th leading cancer-related death in the world[3]. According to GLOBOCAN estimates, there were approximately 458,918 new cases and 432,242 deaths in 2018. Pancreatic cancer is more frequently diagnosed in males (243,033 males cases vs. 215,885 female cases in 2018) and in developed regions, such as Western Europe, North America and Australia[4]. Due to inadequate screening, most cases are diagnosed at advanced stages where treatment options are scare and offer limited benefit[2]. As over 90% of cases die from the disease[5], patterns for mortality are similar to those for incidence across gender and geographic region[3]. Five-year survival rates differ by region and range from roughly 4.4% in Russia, to 10-15% in Canada, China, Australia and many European countries, to 23.6% in Kuwait from 2000-2014[6]. Survival rates have had minor increases (3-5%) over the past years in several countries[6,7].

In the United States, pancreatic cancer is the 10th most commonly diagnosed malignancy and 3rd leading cause of cancer death. The American Cancer Society estimates that there will be

56,770 new cases and 45,750 deaths from pancreatic cancer in 2019[8]. Furthermore, pancreatic cancer has the lowest survival of all cancers with an overall five-year survival rate of 9% from 2008-2014. Survival varies widely by stage at diagnosis, from 34% for localized tumors to 3% for distance stage tumors[8].

Incidence and death rates for pancreatic cancer in the United States have increased moderately in recent years. The American Cancer Society reports that the incidence rate increased by 0.9% per year for men and 1.0% per year for women from 2005-2014, while the mortality rate increased by 0.3% per year for men and had no change per year for women from 2006-2015[9]. Due to advancements in the screening and treatment for breast and colorectal cancers, pancreatic cancer is expected to become the second leading cause of cancer-related death by 2030[10]. Five-year survival rates have also improved slightly, from a rate of 4% in the late 1980's to the current rate of 9%[8,11]. Despite this, overall survival is still fairly dismal and more efforts are needed improve the outcomes of patients with pancreatic cancer. For instance, recent literature has suggested that genomic tumor sequencing could offer potential clinical benefits by identifying molecular subtypes of pancreatic cancer that are more responsive to certain therapies[12,13].

1.1.3 Pancreatic Cancer Risk Factors

Modifiable lifestyle risk factors for pancreatic cancer include smoking, obesity and heavy alcohol use[7,14,15]. Smoking is a major contributor and accounts for approximately 20-30% of all pancreatic cancer cases[14,16,17]. Current smokers have around a two-fold increased risk compared to never smokers and this susceptibility increases with the intensity and duration of smoking[14,16,18]. Additionally, cancer risk increases by 10% per 5-unit increase in body mass index, corresponding to a 20% elevated risk for obese individuals compared to normal weight

individuals[19,20]. Heavy alcohol drinking has also been reported to increase pancreatic cancer susceptibility by approximately 20%[21–23].

Pancreatic cancer is more commonly diagnosed among older individuals, males, African Americans and diabetics[14]. The average age of onset is 71 and about two-thirds of cases are over 65 years old. Higher pancreatic cancer rates in males and blacks may be explained by the greater prevalence of other risk factors (e.g. smoking, diabetes, obesity) in these groups[14]. Several meta-analyses have reported a doubled risk of pancreatic cancer among type 2 diabetics compared to non-diabetics[24,25]. Other non-modifiable risk factors include non-O blood type, *Helicobacter pylori* infection, chronic pancreatitis, family history, and inherited familial genetic mutations[15]. Chronic pancreatitis affects about <1% of the population and has been associated with a two- to six-times increase in pancreatic cancer susceptibility[15,26]. Furthermore, pancreatic cancer risk increases substantially with the number of relatives with past malignancy[5]. Inherited genetic syndromes, which constitute about 10% of all pancreatic cancers, include familial pancreatitis (PRSS1), Peutz-Jeghers syndrome (STK11), familial atypical multiple mole melanoma syndrome (p16), hereditary non-polyposis colorectal cancer (MLH1, MSH2) and hereditary breast and ovarian cancer syndrome (BRCA1/2, PALB2)[5,14].

1.2 Immune Dysregulation and Pancreatic Cancer

1.2.1 Overview

As in many other cancers, the immune system is involved in several mechanisms that may influence the development and progression of pancreatic cancer[27–30]. Chronic inflammation has been recognized as a key pathway in pancreatic tumorigenesis, where pro-inflammatory cytokines and transcription factors induce cell transformation via recurring cycles of DNA damage, inhibited apoptosis and cell proliferation[27]. Concurrently, the innate and adaptive

immune systems engage in active immunosurveillance to identify and eliminate damaged and dysregulated cells before they become malignant[28]. However, cancer cells that are less immunogenic can escape detection and subsequently promote further growth by modulating immune function and impairing anti-tumor processes[28–30]. Pancreatic cancers cells can evade the immune system by directly releasing anti-inflammatory cytokines, such as IL-10 and TGF-β, and expressing immune-inhibitory cell surface molecules such as PD-L1[29]. These mechanisms create an immunosuppressed microenvironment that favors tumor progression, characterized by increased regulatory cells and inactivated immune effector cells[29].

Given these intricate pathways, the dysregulation of the immune system can perhaps compromise its ability to recognize and control cancer progression. Immune overactivity, chronic low-grade inflammation, and epigenetic modification of immune function may thus be important indicators for pancreatic cancer risk and development. In particular, this dissertation will evaluate the influence of atopic allergic conditions, diabetes onset and duration, and methylation of cytokine-related genes on pancreatic cancer incidence and survival (see Figure 1 for conceptual model).

1.2.2 Atopic Allergic Conditions (AACs)

Atopic allergic conditions (AACs) are comprised of multiple syndromes including asthma, hay fever/rhinitis, skin allergies and food allergies. It affects over 50 million individuals in the United States per year, and nearly 30% of adults and 40% of children suffer from some type of allergic condition[31]. Family history, sensitization to aeroallergens, respiratory virus infections, and air pollutions are among the known risk factors for AACs[32].

Allergies have been theorized to increase cancer risk through chronic inflammation or reduce risk via enhanced immunosurveillance, with the exact mechanism appearing to vary by

malignancy type[33]. There has been support for the immunosurveillance pathway in regards to pancreatic cancer, as several retrospective studies have observed inverse associations for allergic conditions[34,35]. In a recent meta-analysis of fourteen case-control studies with a total of 5,550 cases, summary estimates revealed protective associations for asthma, nasal allergies and skin allergies with pancreatic cancer risk[34]. While most of the individual studies in the meta-analysis showed reduced risks for nasal allergies, there was less agreement across the studies for skin allergies and asthma[34]. Asthma has also been assessed in several registry-based retrospective cohorts from Finland and Sweden, which have yielded conflicting results[36–38].

In contrast, there has been a scarcity of prospective evidence regarding this relationship. To date, there have only been four prospective cohorts examining AACs and pancreatic cancer, all of which have had findings inconsistent with those of the case-control studies[39–42]. Three out of the four cohorts observed null associations between pancreatic cancer and conditions such as asthma, hay fever, and dermal reactions[39–41], while the fourth cohort study observed an increased risk for asthma but a null association for skin allergies[42]. These four cohorts, however, were conducted in mainly Caucasian populations. The two larger cohorts were also conducted among special populations of 34,000 Seventh-Day Adventists and 29,000 male smokers from Finland[40,42]. Moreover, none of these cohorts evaluated whether allergy-treating medications were related to pancreatic cancer incidence. Given the current literature, the influence of AACs and the treatment for such conditions on pancreatic cancer risk has not been prospectively investigated in a large heterogeneous population or within particular ethnic groups.

1.2.3 Type 2 Diabetes Onset

Type 2 diabetes is a metabolic disorder associated with chronic low-grade inflammation and immune imbalance[43,44]. Diabetics have been observed to have higher levels of inflammatory

responses due to the dysregulation of both innate and adaptive immune systems[43–46]. This chronic inflammatory environment may hence play a role in the development of pancreatic malignancy. Diabetes-related conditions such as insulin resistance and hyperglycemia may also influence the risk of pancreatic cancer. Prior literature has suggested that higher levels of insulin may support cancer cell proliferation through various signaling pathways[47,48]. In addition, hyperglycemia promotes pancreatic cancer growth by providing glucose for tumor cells[49,50]. Higher levels of glucose also activate the transcription factor NF-κB, which is involved in several inflammatory pathways and the disruption of normal cell cycle regulation[27].

Type 2 diabetes has been recognized as a risk factor for pancreatic cancer in epidemiologic studies[25]. However, diabetes may also be a manifestation of the pancreatic tumor itself, as supported by evidence showing that the prevalence of new-onset diabetes is higher among pancreatic cancer cases[51]. Furthermore, cases with recently diagnosed diabetes appear to have improved glucose metabolism and control following tumor resection[52,53]. Although still unclear, current literature suggests that individuals with recent-onset diabetes have a higher pancreatic cancer risk compared to those with long-standing diabetes[25,51,54].

To date, the relationship between recent-onset diabetes and pancreatic malignancy has been mainly explored in either case-control studies[55] or within cohorts of primarily Caucasian individuals[56–59]. Furthermore, a majority of the cohort studies consisted solely of diabetes patients and could only report a standardized incidence ratio because they lacked a non-diabetes comparison group[57–59]. Recently, in the prospective Multiethnic Cohort Study, African Americans and Latinos with recent-onset diabetes had elevated risks for pancreatic cancer compared to individuals with long-standing diabetes[60]. Other than these latest findings, however, research in ethnically diverse populations is still quite limited.

1.2.4 Methylation of Cytokine Genes

Pancreatic cancers are capable of modulating the immune response and establishing an immunosuppressive microenvironment that promotes tumor growth. In this immune-inhibitory state, increased levels of T regulatory cells and myeloid derived suppressor cells impair the antitumor activity of effector cells such as cytotoxic CD8+ T cells, natural killer cells, and dendritic cells[29]. In addition, helper CD4+ T cells transition from a Th1 phenotype, which facilitates tumor-eliminating processes, to a Th2 phenotype, which supports more tumor-tolerating immune responses[29,61,62]. Current literature suggests that pancreatic cancer patients with higher levels of T regulatory cells[63], myeloid derived suppressor cells[64] and Th2-type T helper cells[65] have worse prognoses, while those with higher concentrations of immune effector cells have increased survival[29,66,67].

These changes in immune function are influenced in part by the aberrant expression and production of regulatory cytokines. Pancreatic tumors cells have been found to directly secrete the anti-inflammatory cytokines IL-10 and TGF-β, which skew T helper cell differentiation towards the Th2 phenotype and suppress tumor-killing Th1 responses[62,68]. Circulating levels of pro-inflammatory cytokines (e.g. IL-6, IL-8, TNF-α) have also been observed to be elevated in pancreatic cancer patients compared to normal controls[69–72]. Other studies have further detected increased genetic and protein expression of both pro-inflammatory (e.g. IL-1β, IL-6, IL-8, TNF-α) and anti-inflammatory (e.g. IL-10, TGF-β, IL-11) cytokines by pancreatic cancer cells[70,73–75]. Given the complex interplay between cytokines and immune function, the expression and serum levels of various cytokines have been found to be predictive markers of pancreatic cancer progression in some small studies[70–72,74–80] (Table 1-1).

While serum and mRNA levels of cytokines have been evaluated in regard to survival, there is limited information as to whether prognosis is affected by the epigenetic regulation of

associated gene expression. DNA methylation is a form of epigenetic mark that involves the addition of a methyl group to cytosine bases at CpG dinucleotides. Aberrant DNA methylation patterns have been implicated in the development and progression of several cancers, perhaps by inducing genomic instability and altering gene expression to influence the tumor microenvironment[81–85]. Currently, research on DNA methylation and pancreatic cancer progression has been fairly nascent, and prior studies have been primarily epigenome-wide association studies (EWAS) or candidate gene studies that did not focus on genes related to the immune response[86–92]. Hence, it is unknown whether the immune dysfunction and poor prognosis observed in pancreatic cancer is attributed to the atypical methylation of cytokine-related genes. Identifying and understanding these relationships could potentially identify methylation biomarkers of survival and allow for the development of novel methylation-targeted therapies.

1.3 Gaps in Knowledge

Despite several retrospective epidemiologic studies investigating allergies and pancreatic cancer, there is limited prospective evidence of this relationship from a multiethnic population. Likewise, the association between type 2 diabetes onset and pancreatic cancer incidence has not been evaluated within a large, racially diverse cohort. Lastly, no prior study has examined the impact of the methylation of cytokine genes on pancreatic cancer survival.

CHAPTER 2. RESEARCH OBJECTIVES

2.1 Rationale and Objectives

The purpose of this dissertation is to further understand the role of atopic allergic conditions and type 2 diabetes onset on the risk of pancreatic cancer, and the impact of methylation of cytokine genes on pancreatic cancer survival. Understanding the relationships between these factors and pancreatic cancer might further elucidate the impact of immune dysregulation on pancreatic cancer tumorigenesis and progression. In addition, this research may help identify potential immunotherapy targets for the prevention, control and treatment of the disease.

2.2 Specific Aims and Hypotheses

Aim 1: To conduct a prospective analysis of atopic allergic conditions and the treatment of these conditions on pancreatic cancer risk in a racially diverse population

- **Hypothesis 1:** Compared to those without allergies, individuals with a history of allergies will have a decreased risk of pancreatic cancer because an overactive immune response may be more protective in eliminating cancer cells
- **Hypothesis 2:** Compared to those without allergies, individuals with allergies who receive antihistamine treatment will have an increased risk of pancreatic cancer because these drugs may suppress the immune system and hinder its ability to eliminate cancer cells

Aim 2: To investigate the association between type 2 diabetes onset and pancreatic cancer risk in a racially diverse patient-based cohort, and to establish a metabolic profile of diabetes patients who are at high risk of developing pancreatic cancer

- **Hypothesis 1:** Compared to those without diabetes, individuals with a history of type 2 diabetes will have an increased risk for pancreatic cancer due to the carcinogenetic pathways involved in diabetes such as chronic inflammation, insulin resistance, and hyperglycemia
- **Hypothesis 2:** Compared to those without diabetes, individuals with shorter diabetes durations will have an increased risk for pancreatic cancer because the new-onset diabetes may be a symptom of the pancreatic cancer tumor itself

Aim 3: To evaluate the relationship between the methylation of cytokine genes measured on pancreatic tumor tissues and overall survival

- **Hypothesis:** Individuals with tumor samples with epigenetic profiles that favor the expression of immune-inhibitory cytokines (e.g. IL-10, TGF-β) will have poorer survival compared to those without this epigenetic profile because immune-inhibitory cytokines could possibly create a pro-tumor microenvironment

See Figure 1-1 for conceptual framework of dissertation aims.

CHAPTER 3. SPECIFIC AIM 1 – ATOPIC ALLERGIC CONDITIONS

3.1 Methods

3.1.1 Study population

The Multiethnic Cohort Study was established in 1993-1996 to study cancer and chronic disease etiology among individuals living in California (mostly Los Angeles County) and Hawaii. It consists of roughly 215,000 participants ages 45-75 of five primary ethnic groups: white, African American, Latino, Japanese American and Native Hawaiian[93]. Upon enrollment, cohort members completed a 26-page baseline questionnaire, which included questions on demographics, lifestyle factors, personal medical conditions and family history of cancer.

Cohort members were excluded if they were not in the five main ethnic groups, had a previous history of pancreatic cancer prior to cohort entry, or were missing information on AAC status and pancreatic cancer risk factors (e.g. smoking, diabetes). The participants of the study were followed from the date of the baseline questionnaire to pancreatic cancer diagnosis, death, or the closure date of follow-up on December 31, 2012. Incident, invasive pancreatic cancer cases were identified by annual linkage with the statewide Surveillance, Epidemiology, and End Results (SEER) registries of Hawaii and California. Mortality information was obtained through linkage with states' death certificate files and the National Death Index.

3.1.2 Exposure assessment

Participants were asked to self-report on the baseline questionnaire whether a physician had ever informed them that they had "asthma, hay fever, skin allergy, food allergy or any other allergy," asked as a single combined exposure on the baseline questionnaire. In addition, participants were asked whether they had used any antihistamine medications ("allergy pills or

shots") for "at least two times per week for one month or longer" and the duration at which they used these medications.

History of AACs was assessed as a binary exposure. Antihistamine medication was analyzed as ever use and duration of use (none, ≤5 years, >5 years). We also combined these exposures to create an index of allergy severity (no AACs, AACs with no medication use, AACs with medication use).

3.1.3 Statistical analyses

Baseline characteristics were compared across individuals with and without AACs using a t-test for age and chi-square tests for all other variables. Pancreatic cancer incidence rates, truncated to ages 45-95, were computed within the MEC, age standardized by 5-year age groups to the United States Census 2000 standard population.

The influence of AACs and antihistamine use on pancreatic cancer incidence was evaluated using Cox proportional hazards regression models with time since baseline as the time metric. We used separate models to evaluate the associations between each of our exposures of interest (history of AACs, ever use of antihistamine, duration of antihistamine use, and allergy severity) and pancreatic cancer. We fit minimally adjusted models that included age at cohort entry, sex, and race/ethnicity (white, African American, Latino, Japanese American, Native Hawaiian) as strata variables, as well as fully adjusted models that also included smoking status (never, past, current), family history of pancreatic cancer, diabetes, education (≤12 years, some college/vocational, college graduate), alcohol intake (none, <24, 24-48, >48 g/day), and body mass index (BMI) (<25, 25-30, ≥30 kg/m^2) as covariates. We also ran models with continuous measurements for alcohol intake and BMI and pack-year information for smoking and observed

no change in the results; thus, only findings from the original models are presented. Individuals were censored at death or end of follow-up.

To assess effect modification of the associations of AACs or ever use of antihistamine medication on pancreatic cancer, we ran additional models among subgroups defined by age group (<50, 50-54, 55-59, 60-64, 65-69, 70+), sex, ethnicity, follow-up time since baseline (<5 years, ≥5 years), and known/potential pancreatic cancer risk factors (cigarette smoking, family history of pancreatic cancer, diabetes, alcohol use, BMI). For AACs, we also ran models stratified by antihistamine use (ever, none). We tested for heterogeneity by fitting a separate model with a cross-product term for the exposure (history of AACs or ever use of antihistamine medication) and the stratifying variable. The proportional hazards assumption was assessed using Schoenfeld residuals while model fit was evaluated using Martingale and deviance residuals[94]. All analyses were conducted using SAS 9.3 (Cary, NC) and reported P values are two-sided.

3.2 Results

After exclusions, the present study consisted of 187,226 total individuals (females N=101,845, males N=85,381). The mean age at cohort entry was 59.9 (standard deviation 8.8). Japanese American (28.7%), whites (25.0%), and Latinos (22.2%) were the most represented ethnicity groups, followed by African Americans (16.8%) and Native Hawaiians (7.3%). During an average follow-up period of 16.2 years, 1,455 incident cases were identified among participants at risk.

Roughly one quarter of individuals reported a history of AACs (N=49,696, 26.5%). Age-standardized pancreatic cancer incidence rates (left truncated at age 45) were lower in those with AACs (44.8 cases per 100,000) than in those without AACs (50.2 cases per 100,000) (Table 3-1). Demographic characteristics and risk factors differed across individuals with and without

AACs. Those who reported having AACs tended to be younger, female, and white and were more likely to have a family history of pancreatic cancer and more years of education. Additionally, these individuals were less commonly diabetics, current smokers, or heavy alcohol drinkers (Table 1). Antihistamine medication use was reported in 28,413 (15.2%) participants. Among these individuals, 15,405 (54.2%) used antihistamines for five or less years. In regard to allergy severity, 15.0% of participants reported having AACs without antihistamine use and 10.7% reported a history of both AACs and medication use (Table 3-1).

In our models minimally adjusted for age, sex and race, we detected no significant associations between AACs, antihistamine use, or allergy severity with pancreatic cancer (Table 3-2). However, we found a borderline protective association for those who had used antihistamines for five or less years (RR 0.81, 95% CI 0.65-1.00) compared to those who did not use any medications. All of the aforementioned associations were quite similar and non-significant after including the remaining covariates in our fully adjusted models. Again, we observed a borderline reduced risk for individuals with five or less years of antihistamine use (RR 0.82, 95% CI 0.66-1.01) compared to those without any history of medication use (Table 3-2).

The null associations for AACs and ever use of antihistamines were present across all stratified analyses, except within the oldest individuals (age 70+) in the cohort (Tables 3-3 & 3-4). Among individuals who were 70 or older at cohort entry, those who had a history of AACs had a 26% reduced risk (RR 0.74, 95% CI 0.56-0.98) while those who used antihistamines had a 34% reduced risk (RR 0.66, 95% CI 0.45-0.96) of pancreatic cancer. In addition, AACs had a borderline protective association among those who were followed for less than five years (RR 0.76, 95% CI 0.56-1.04). There were no associations of AACs and antihistamines within any of

the other subgroups stratified by sex, ethnicity, BMI, family history of pancreatic cancer, smoking, diabetes, and alcohol use (Table 3-3 & 3-4).

3.3 Discussion

In this prospective cohort study, we investigated the influence of atopic allergic conditions and antihistamine medication use on pancreatic cancer risk in a population of white, African American, Latino, Japanese American and Native Hawaiian individuals. After adjusting for covariates, we observed no clear association between any prior history of AACs, antihistamine use, or allergy severity with the incidence of pancreatic cancer. The null associations for AACs and antihistamine medication were consistent across subgroups defined by sex, ethnicity, follow-up time, BMI, family history, smoking status, diabetes and alcohol use. However, we did observe protective associations among those who were aged 70+ at cohort entry. While these findings may have resulted from censoring due to death, further evaluations of AACs and antihistamines on pancreatic cancer risk among older individuals might be worthwhile.

Four previous prospective cohorts have studied the association of AACs and pancreatic cancer. In these studies, the number of pancreatic cancer cases ranged between 4 and 172 and the resulting RR's ranged from 0.59 to 2.16[39–41]. Thus, the null findings in these cohorts may have been attributed to insufficient statistical power. Though the largest number of cases came from the 29,000 Finnish male smokers from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, the results from this study cannot be easily extrapolated to the general population given the specificity of the study participants[42]. These cohort members were also at a higher baseline risk for pancreatic cancer given their shared smoking history[95]. Our results in the MEC agree with the null results of these past studies and, with nearly 1,500 cases from an ethnically diverse

population, provide more convincing evidence of a null association between overall allergies and pancreatic cancer.

On the other hand, our findings conflict with the protective associations found in several case-control studies. This discrepancy may be due to differences in the participant selection across prospective and retrospective study designs. For instance, many of the case-control studies excluded a large proportion of cases due to death, refusal and non-response. Among the seven case-control studies that reported a decreased cancer risk for any allergic condition, nearly all were unable to recruit over half of the entire population of cases[96–102]. These exclusions could have introduced selection bias if the association of AACs and cancer risk was different across those enrolled and not enrolled in the studies. This is further supported by the fact that the strongest associations were observed in studies that recruited the lowest number of cases[96,97]. Though prospective cohorts are susceptible to selection bias due to loss of follow-up, our cohort was able to monitor the outcomes of all of our participants by passive linkage to the statewide SEER registry and National Death Index.

Based on the existing literature, it appears that the influence of AACs on pancreatic risk may differ according to the type of allergy. Past case-control studies have showed fairly consistent inverse associations with hay fever and other nasal allergies[34,96–99,101–103], but only a few studies have showed decreased risks for asthma[34,100,103] and skin allergies[99,100,103]. While most studies had null findings for skin allergies and asthma, one retrospective cohort from Sweden found an increased pancreatic cancer risk among hospitalized asthma patients[38], suggesting that disease severity may also play a role in carcinogenesis. For several studies that used a composite assessment of allergies, the protective overall associations seemed to be driven by the individual effects of hay fever and other nasal allergies[96–99,101,102]. Since we do not know the breakdown and prevalence of each allergic condition in the Multiethnic Cohort, our null

result could have been attributed to a condition that is not as strongly associated with pancreatic cancer. As hay fever represents a modest percentage of all atopic allergic conditions at roughly 30%[104,105], it is plausible that we could have missed a strong association in this subset.

The mechanism in which allergies may impact cancer risk is still not well understood. AACs have been theorized to either decrease risk through heightened immunosurveillance or increase risk through chronic inflammation[33]. Although the protective associations from previous studies provide evidence for the immunosurveillance pathway, other studies have found no relationship between IgE levels, a biologic marker for allergic response, and pancreatic cancer risk[106,107]. Medical treatment of allergies may also modify the underlying mechanism, but there has been limited information regarding allergy medications and pancreatic cancer. Aside from our current analysis that detected no association of antihistamines, one other case-control study found protective associations among those who reported receiving medical treatment for allergies[100]. Another case-control study suggested that medication use may confound the association of allergies with pancreatic cancer, but did not conduct a formal analysis of this relationship[98]. In our study, we attempted to tease apart the interaction between allergies and medications with our allergy severity index, but did not observe any significant associations with this measurement.

Other important factors in determining pancreatic cancer risk could possibly include the timing and duration of allergic conditions. In the recent PanGenEU case-control study, researchers found that participants who had post-childhood onset or ≥17 years of asthma had a reduced risk for pancreatic cancer[34]. Likewise, the Pancreatic Cancer Case-Control Consortium observed a stronger protective effect among individuals with late onset of allergic conditions compared to those with early onset[108]. We addressed timing of AACs in our cohort by examining duration of antihistamine use and performing subgroup analyses by follow-up time, since those

who had used medications for shorter lengths or completed the baseline questionnaire within five years may have had a more recent diagnosis of allergies. Hence, the borderline protective associations that we detected for the shorter periods of both antihistamine use and follow-up time may support the trend of a reduced risk among individuals with later onset allergies. In general, case-control studies would be more likely than prospective studies to catch more recent diagnoses of AACs since exposure assessment was done after identifying all cases and controls.

One of the major strengths of this study is the large and heterogeneous sample, which allowed us to evaluate the relationship between AACs and pancreatic cancer in many different subgroups. In comparison to the previous four prospective cohorts, the present results are based on the greatest number of pancreatic cancer cases and are more generalizable to a heterogeneous population. We were also able to examine both medication use and an allergy severity index, which was not done in the past prospective cohorts. Since we collected epidemiologic data prior to disease diagnosis and linked to cancer registries with virtually complete case-ascertainment, our study is less susceptible to the recall and selection bias that may exist in case-control studies. However, we could have missed some pancreatic cases if the participant moved out of state and was not captured in the California or Hawaii tumor registries. Our assessment of AACs was also self-reported and combined into a non-specific single exposure, so we could not evaluate the influence of individual conditions or the duration of disease. Furthermore, we did not have any biological measurements (e.g. IgE) or information on the timing of AACs, limiting our ability to elucidate the detailed pathways in which allergies may impact risk.

To our knowledge, this is the first study to examine the relationship between AACs and antihistamines with pancreatic cancer in a diverse and well-powered prospective setting. Our results are consistent with that of past prospective studies and provide additional support of a null association for overall AACs. Future prospective studies should aim to conduct a more

comprehensive assessment of specific AACs and allergy-treating medications on pancreatic cancer risk.

CHAPTER 4. SPECIFIC AIM 2 – TYPE 2 DIABETES ONSET

4.1 Methods

4.1.1 Study Population

We conducted a retrospective cohort study of patients at-risk for pancreatic cancer from KPSC, an integrated health system comprising of 14 medical centers and over 200 medical offices throughout Southern California. KPSC's diverse member population of over 4 million individuals is representative of the Southern California region, consisting of 45% whites, 23% Hispanics, 15% African Americans, 9% Asians and 7% other ethnicities[109]. All patient data, including information on outpatient and inpatient hospital visits, diagnosis codes, pharmacy prescriptions and laboratory tests, are stored in the electronic health record and regional data warehouse.

Patients were eligible for the study if they met the following inclusion criteria during 2006-2016: aged 45-90 years, at least two years of continuous membership in KPSC, and available information for body mass index (BMI), smoking status, and alcohol use. The two-year membership requirement was used to ensure that an individual had been in the KPSC health system long enough to establish an accurate medical history. Otherwise eligible patients were further excluded if they did not have a glucose (fasting, random or oral glucose tolerance) or hemoglobin A1c (HbA1c) measurement (N=67,486), were not in the four major race/ethnicity groups (Asian, non-Hispanic black, Hispanic, non-Hispanic white) (N=80,801), or had a prior diagnosis of pancreatic cancer (N=523) (Figure 1). The date of cohort entry was the January 1st of the first year after the patient met all inclusion criteria (*e.g.* if a patient met the final inclusion criterion in December 2006, the date of cohort entry would be January 1, 2007).

This study was approved by the KPSC Institutional Review Board (IRB).

4.1.2 Exposure Assessment

Individuals were identified as having diabetes if they had any of the following measurements based on the American Diabetes Association criteria: HbA1c ≥6.5%, fasting glucose ≥126 mg/dL, random glucose ≥200 mg/dL or two-hour oral glucose tolerance test ≥200 mg/dL. The date of the earliest lab test within the diabetes range was treated as the diabetes diagnosis date. Diabetes status (prevalent, incident, vs. none) was defined based on the date of diabetes diagnosis in relation to cohort entry. Individuals were classified as prevalent cases of diabetes if their diabetes diagnosis was before or on the date of cohort entry and were categorized as incident cases of diabetes if the diagnosis was after cohort entry. For incident diabetes patients, we evaluated diabetes duration as incident diabetes ≤1 year and incident diabetes >1 year using the time elapsed since diabetes diagnosis.

4.1.3 Assessment of Other Covariates

Covariate data were obtained from most recent measurement or record prior to cohort entry. BMI was grouped into <25, 25-30, ≥30 kg/m^2, smoking was categorized as never, former, and current smoker, and alcohol use was treated as a dichotomous variable (yes vs. no). We used International Classification of Diseases (ICD) codes to identify patients with pancreatitis (ICD-9: 577.0, 577.1; ICD-10: K85.x, K86.0, K86.1). Family history of pancreatic cancer (any relative) was attained from the patient history files and assessed as a dichotomous variable (yes vs. no). Information on education was obtained from the geocoding database[109] and was categorized as completion of high school or less, some college, and college graduate.

4.1.4 Outcome

Our primary outcome of interest was time from cohort entry to pancreatic cancer diagnosis. Patients in the final study cohort were censored at the first of the following: death, end of membership or end of study on December 31, 2016. Pancreatic cancer cases (ICD-O-3 codes C25.0-C29.9) were identified from the internal KPSC cancer registry, which collects tumor characteristics such as date of diagnosis, tumor size, grade, and histology on all patients diagnosed with cancer and/or treated at KPSC since 1988. The KPSC registry reports to the Surveillance, Epidemiology and End Result (SEER) program and meets its high reporting standards[110]. Mortality information was obtained from inpatient records, the internal cancer registry and the California state death index.

4.1.5 Statistical Analyses

Baseline characteristics were compared across diabetes status using ANOVA for age and chi-square tests for all other variables. Pancreatic cancer incidence rates, age-standardized to the United States 2000 standard population and truncated to age 45+, were calculated for each diabetes group.

The relationship between diabetes and pancreatic cancer incidence was analyzed using Cox proportional hazards regression. Both diabetes status (prevalent, incident vs. none) and duration (incident ≤1 year, incident >1 year, vs. none) were treated as time-varying exposures, which was assessed at each pancreatic cancer event for all members in the risk set. All models included age at cohort entry, gender, race/ethnicity, smoking, BMI, alcohol use, family history of pancreatic cancer, history of pancreatitis, and education as covariates.

Additional analyses were conducted within subgroups defined by gender, race/ethnicity, BMI, smoking, alcohol use and pancreatitis. Heterogeneity was evaluated using models with an

additional interaction term for the exposure (diabetes status or duration) and subgroup variable of interest performed in the full sample.

Schoenfeld residuals were used to confirm no violation of the proportional hazards assumption for each covariate, while Martingale and deviance residuals were used to evaluate model fit. All analyses were conducted using SAS 9.3 (Cary, NC).

4.1.6 Mediation Analysis

To further understand the mechanisms in which diabetes may influence pancreatic cancer risk, we also examined whether diabetes mediated the associations for other pancreatic cancer risk factors. We performed a causal mediation analysis to examine whether diabetes acted as a mediator for smoking (former, current) and adiposity (overweight, obesity), as these factors may include diabetes in their causal mechanisms for pancreatic cancer. For each factor, we calculated the total effect, controlled direct effect not through diabetes, natural indirect effect through diabetes, and proportion mediated using the mediation analysis SAS macro developed for survival data[111,112]. This analysis was only performed among non- and incident diabetes patients to ensure that the smoking and adiposity risk factors preceded the onset of diabetes.

4.1.7 Metabolic Profiling of Incident Diabetes Patients

To investigate which patients are at highest risk for pancreatic cancer, we evaluated metabolic profiles of incident diabetes patients at different timepoints of diabetes progression. Specifically, we examined fasting glucose, HbA1c and weight measurements at the time of diabetes diagnosis (closest record within 30 days before/after diagnosis) and during the period prior to diagnosis (closest record within 6 months to 2 years before diagnosis). We also calculated the percent difference and monthly rate of change between these two time windows

for each characteristic among individuals who had measurements in both periods (N=50,118 for glucose, N=23,936 for HbA1c, N=92,846 for weight).

Metabolic characteristics were compared across incident diabetes patients with and without pancreatic cancer using Mann-Whitney U tests, as all non-weight associated measurements had right skewed distributions. As a sensitivity analysis, we also performed t-tests for the normally distributed weight variables and observed similar results as the Mann-Whitney U tests. We further performed Cox proportional hazards regression to examine the association between clinically meaningful percent differences (glucose: per 10% unit increase; HbA1c: per 1% unit increase; weight: per 3% unit decrease) and monthly rates of change (glucose: per mg/dL/month increase; HbA1c: per 0.1%/month increase; weight: per kg/month decrease) and pancreatic cancer risk. In these models, individuals were followed from diabetes diagnosis to pancreatic cancer diagnosis, death, end of membership or end of the study. All models included age at diabetes diagnosis, gender, race/ethnicity, smoking and BMI as covariates, with smoking and BMI information obtained from the closest value prior to diabetes diagnosis.

In order to assess whether these relationships differed by race/ethnicity, we performed subgroup analyses for the percent differences of glucose, HbA1c and weight. Within each subgroup, variations in the percent difference between patients with and without pancreatic cancer were evaluated using Mann-Whitney U tests. Overall heterogeneity by race/ethnicity was assessed using models of the percent difference regressed on pancreatic cancer status, race/ethnicity and an interaction term for pancreatic cancer and race/ethnicity. We used a linear regression for weight, which was fairly normally distributed, and a generalized linear model with a gamma distribution and log link for glucose and HbA1c due to right skewed distributions. We also performed separate Cox models to examine the relationships between percent difference or rate of change and pancreatic cancer incidence within each subgroup. We tested for

heterogeneity using a separate model with an additional interaction term for percent difference/rate of change and race/ethnicity.

4.1.8 Sensitivity Analyses

To assess the accuracy of the identification of incident diabetes, we re-ran all analyses dropping incident diabetes patients who did not have a prior metabolic test in the normal range. This did not exclude many individuals, as 97% of incident diabetes patients had a prior normal metabolic measurement. In order to address the potential effects of diabetes treatment, we also re-ran the metabolic profile analyses dropping individuals with metabolic measurements taken within the 30 days after diabetes diagnosis (N=15,589 for glucose, N=3,052 for HbA1c, N=25,337 for weight). Results were unchanged in both situations and thus we only present findings from the original models.

4.2 Results

The final study cohort consisted of 1,499,627 patients at-risk for pancreatic cancer. The study population was on average 57.9 years old (standard deviation 10.9) and consisted of 55% females. Whites comprised nearly half of the cohort (45.7%), followed by Hispanics (32.5%), Asians (11.1%) and blacks (10.7%). From nearly 7.5 million person-years of follow-up, 2,002 persons developed incident pancreatic cancer, translating to age-adjusted incidence rate of 30.7 cases per 100,000 person-years.

Among the entire cohort, about 110,699 developed incident diabetes (7.4%), 332,939 had prevalent diabetes (22.2%) and 1,055,996 never had diabetes (70.4%). Age-adjusted incidence rates for pancreatic cancer were highest in patients with incident diabetes (43.7 cases per 100,000 person-years), followed by patients with prevalent diabetes (41.2 cases per 100,000 person-

years) and lowest in patients without diabetes (23.8 cases per 100,000 person-years). Prevalent and incident diabetes patients were more likely to be older, male, non-white, former smokers, and non-alcohol users. These individuals also tended to have higher BMI, lower education, and a prior diagnosis of pancreatitis (Table 4-1). Within the individual race/ethnicity groups, blacks (26.5%), Hispanics (25.2%) and Asians (24.6%) had the highest proportion of prevalent diabetes while blacks (9.2%) and Asians (8.9%) had the greatest proportion of incident diabetes.

4.2.1 Diabetes Status/Duration and Pancreatic Cancer Risk

Pancreatic cancer risk was elevated three times for patients with incident diabetes (RR 3.17, 95% CI 2.75-3.65) and roughly two times for prevalent diabetes (RR 1.85, 95% CI 1.67-2.05) compared to individuals without diabetes. When evaluating diabetes duration, we found that patients with incident diabetes with ≤1 year and >1 year of disease duration had almost seven times (RR 6.91, 95% CI 5.76-8.30) and two times (RR 1.96, 95% CI 1.62-2.38) the risk of pancreatic cancer, respectively, compared to individuals without diabetes. Similar patterns of associations for both diabetes status and duration were present across all race/ethnicity subpopulations (Table 4-2), as well as within subgroups of gender, BMI, smoking, and alcohol (Tables 4-3 & 4-4).

4.2.2 Mediation Analysis

In the mediation analysis, we observed that former and current smokers had a 38% (total effect RR 1.38, 95% CI 1.26-1.50) and 89% (total effect RR 1.89, 95% CI 1.60-2.25) higher risk of pancreatic cancer, respectively, compared to non-smokers. There was little mediation by diabetes and the controlled direct effect was similar for both former (RR 1.40, 95% CI 1.28-1.54)

and current (RR 1.97, 95% CI 1.79-2.17) smoking. Diabetes mediated 3.2% of the effect for former smoking and 2.4% of the effect for current smoking.

Moreover, we detected an 11% (total effect RR 1.11, 95% CI 1.02-1.20) increased risk of pancreatic cancer for overweight and a 25% (total effect RR 1.25, 95% CI 1.07-1.45) increased risk for obesity, compared to individuals with normal BMI. There was substantial mediation by diabetes for both overweight (proportion mediated 32.6%) and obesity (proportion mediated 38.5%). For overweight, the controlled direct effect (RR 1.08, 95% CI 0.99-1.17) was about double of the indirect effect (RR 1.03, 95% CI 1.02-1.04) through diabetes. This pattern was similar for obesity, where the controlled direct effect (RR 1.16, 95% CI 1.06-1.26) was twice as large as the natural indirect effect (RR 1.08, 95% CI 1.05-1.12).

4.2.3 Metabolic Profiling of Incident Diabetes Patients

Among the 110,699 incident diabetes patients, 52.6% of incident diabetes patients had at least two fasting glucose measurements, 21.2% had at least two HbA1c measurements and 89.5% had at least two weight measurements. Individuals who had at least two measurements tended to be older, female, non-smokers, and have lower BMI.

Compared to those without pancreatic cancer, individuals who developed pancreatic cancer (N=306, 0.3%) had higher fasting glucose values at diabetes diagnosis (median 135.0 vs. 131.0 mg/dL, $p<0.0001$), but not prior to diagnosis (106.0 vs. 106.0 mg/dL, $p=0.87$) (Figure 4-2). When assessing changes between the two time windows, pancreatic cancer patients had a larger percent increase (median 27.4 vs. 19.8%, $p<0.0001$) and monthly rate of change (2.4 vs. 1.6 mg/dL/month, $p<0.0001$) in their fasting glucose measurements. For HbA1c, pancreatic cancer patients had higher levels at diabetes diagnosis (median 6.8 vs. 6.6%, $p<0.0001$), but slightly lower levels in the period prior to diagnosis (6.1 vs. 6.2%, $p<0.01$) (Figure 4-2). The

percent difference (median 9.4 vs. 5.3%, p<0.0001) and monthly rate of change (0.05 vs. 0.03%/month, p<0.001) between these two time periods were nearly double for individuals with pancreatic cancer. Weight was lower among pancreatic cancer cases compared to non-cases at diabetes diagnosis (median 79.8 vs. 85.3 kg, p<0.0001) and in the window prior to diagnosis (83.0 vs. 85.4 kg, p<0.01) (Figure 4-2). Additionally, there were greater decreases in the percent difference (median -2.0 vs. 0.0%, p<0.0001) and monthly rate of change (-0.2 vs. 0.0 kg/month, p<0.0001) in weight among individuals who developed pancreatic cancer compared to those who did not.

For all of the metabolic characteristics, the relationship between percent change over time and pancreatic cancer status appeared to vary by race/ethnicity. Pancreatic cancer patients had more pronounced percent increases in fasting glucose compared to non-cases among blacks (42.9 vs. 19.6%, p<0.001) and Asians (median 22.5 vs. 13.4%, p<0.01), and less so among whites (27.9 vs. 22.9%, p<0.01), but there was no significant difference among Hispanics (20.6 vs. 19.3%, p=0.44) (Figure 4-3, p-heterogeneity=0.01). Additionally, the percent differences in HbA1c was doubled for cases compared to non-cases among whites (median 11.5 vs. 5.2%, p<0.001) and blacks (12.9 vs. 5.0%, p<0.01), but was not significant for other race/ethnicity groups (Figure 4-3, p-heterogeneity<0.0001). Finally, the difference in the percent weight lost between cases and non-cases was significant among whites (-3.7 vs. 0.0%, p<0.0001) and Asians (-2.0 vs. 0.2%, p<0.01), but not among blacks (-0.5 vs 0.0%, p=0.08) or Hispanics (0.0 vs. 0.1%, p=0.08) (Figure 4-3, p-heterogeneity<0.001).

For pancreatic cancer risk, each 10% unit increase in the percent difference of fasting glucose was associated with a 4% greater risk (RR 1.04, 95% CI 1.03-1.05), while each unit increase in the rate of change was associated with a 6% elevated risk (RR 1.06, 95% CI 1.04-1.07). These associations were similar across race/ethnicity, but were stronger among Asians

(percent difference: RR 1.12, 95% CI 1.06-1.17, p-heterogeneity=0.02; rate of change: RR 1.13, 95% CI 1.07-1.19, p-heterogeneity=0.06; Table 4-5). Moreover, each unit increase in the percent difference in HbA1c was associated with a 2% elevated risk (RR 1.02, 95% CI 1.02-1.03) and each 0.1%/month increase in the rate of change had a 45% increased risk (RR 1.45, 95% CI 1.26-1.66). For weight, each 3% unit decrease in the percent difference had a 24% increased risk (RR 1.24, 95% CI 1.18-1.31) and each unit decrease in the rate of change had a 62% increase (RR 1.62, 95% CI 1.44-1.82) in pancreatic cancer risk. The impact of the percent difference in weight on pancreatic cancer risk was more pronounced among whites (RR 1.32, 95% CI 1.24-1.40) and Asians (RR 1.22, 95% 1.03-1.45) than among Hispanics (RR 1.14, 95% 1.01-1.29) and blacks (RR 1.07, 95% CI 0.92-1.26, p-heterogeneity=0.02) (Table 4-5).

4.3 Discussion

In this study, we evaluated the influence of diabetes status, diabetes duration and metabolic profiles on pancreatic cancer risk within a large and diverse population-based cohort. We observed that patients with incident and prevalent diabetes had three and two times the risk, respectively, of pancreatic cancer compared with patients without diabetes. In addition, this risk was elevated nearly seven times for incident diabetes patients with ≤1 year of disease duration and about two times for incident diabetes patients with >1 year disease duration. Among incident diabetes patients, those who went on to develop pancreatic cancer had greater increases in fasting glucose, HbA1c and weight loss in the time leading up to diabetes diagnosis. The longitudinal changes in metabolic characteristics between cases and non-cases also appeared to differ by race/ethnicity.

Prior epidemiologic studies have found that diabetes is associated with a two times higher risk of pancreatic cancer, with higher risks observed for those with shorter durations of

diabetes[25,54]. Our main findings are generally consistent with past research, as both incident and prevalent diabetes patients had around a two to three times greater risk compared to non-diabetes patients. The elevated risk among incident diabetes patients was driven primarily by the seven-fold increased risk for those who had diabetes for ≤1 year, while those with longer disease durations had risks resembling that of prevalent diabetes patients. These results add support for the hypothesis that pancreatic cancer patients with diabetes for shorter periods may actually have a distinct form of diabetes that is induced by the pancreatic tumor[51,52].

Importantly, we observed that these patterns of association were similar across all race/ethnicity subgroups. Most of the prior literature on diabetes duration has been focused on white populations and only a sparse number of studies have examined this relationship among minorities[60,113,114]. Despite this, our results are congruent with previous research showing that shorter durations of diabetes are also associated with higher pancreatic cancer risk among non-whites. To our knowledge, this is the first population-based cohort study to evaluate multiple race/ethnicity groups together with sufficient pancreatic cancer cases within each race/ethnicity to power analyses to detect a range of associations.

In our metabolic profiling of incident diabetes patients, we found that pancreatic cancer cases had similar levels of fasting glucose and HbA1c in the period before diabetes, but higher levels at diabetes diagnosis, compared to non-cases. However, weight was significantly lower for pancreatic cancer patients in both time windows, indicating that changes in weight may occur earlier in the pathogenesis of pancreatic cancer. These patterns are fairly similar to findings from earlier studies of new-onset diabetes patients, which found lower weight and higher blood glucose and HbA1c at diabetes diagnosis among those who ultimately developed pancreatic cancer[115,116].

Moreover, pancreatic cancer patients had higher percent increases of glucose, HbA1c and weight loss in the time leading up to diabetes diagnosis. Of multiple metabolic changes, percent differences in fasting glucose seemed to show the most variability by race/ethnicity; Asians and blacks had the biggest deviations between cases and non-cases, while whites had smaller variations and Hispanics had no significant difference. While there appeared to be dissimilarities for HbA1c by race/ethnicity, these results are based on a smaller group of patients, as there were few pancreatic cancer cases with at least two HbA1c measurements. Weight loss had the least fluctuations across race/ethnicity, with percent decreases of 1-4% for cases and very little change among non-cases. This indicates that weight loss is a more consistent biomarker of pancreatic carcinogenesis across race/ethnicity[5]. Of note, none of the percent differences in glucose, HbA1c or weight between cases and non-cases were statistically significant among Hispanics, suggesting that pancreatic cancer may have less of an influence on these metabolic markers in this population.

Higher percent differences and rates of change for fasting glucose, HbA1c and weight were all significantly associated with pancreatic cancer in our multivariate modeling. These metabolic changes have also been associated with pancreatic cancer in past studies of new-onset diabetes[117–119] and have been identified as significant parameters in recent risk prediction models[115,116]. In particular, the Enriching New-onset Diabetes for Pancreatic Cancer model from the Mayo Clinic reported that increases in blood glucose and weight loss by diabetes diagnosis were predictive markers of pancreatic cancer risk[116]. Additionally, the model developed in The Health Improvement Network in the United Kingdom found that higher levels of HbA1c and larger decreases in BMI at diabetes diagnosis were also significant predictors of pancreatic malignancy[115]. As these previous studies were all conducted in mainly white populations, our study is the first to show that these metabolic changes can also be used to determine risk in

heterogeneous populations with different lifestyles and genetic susceptibilities. The general consistency in findings for higher risk of pancreatic cancer associated with recent-onset diabetes across multiple racial/ethnic groups suggests that prediction models developed in predominantly white individuals[115,116] would apply broadly to minority populations in the U.S.

The major strengths of this study include the diverse patient population and the largest number of pancreatic cancer cases among all past cohort studies, which make our findings more robust and generalizable. Establishing a cohort from members of an integrated health system further assured that individuals had similar healthcare access and were less likely to be lost to follow-up. We also had more reliable and complete data by using the prospectively collected electronic health records. In particular, using glucose and HbA1c tests for both exclusion criteria and case ascertainment controlled for healthcare utilization and improved the accuracy of identifying diabetes patients, respectively. However, only a proportion of patients had more than one glucose or HbA1c measurement, so our findings for metabolic changes are based on a subset of patients that have more clinic visits, which could include a spectrum of patients from healthy individuals compliant with annual physicals to individuals with greater disease burden that necessitates more frequent patient-provider encounters. In addition, the varying lengths of time between the two metabolic measurements across patients could have influenced the results comparing percent differences between cases and non-cases. Nonetheless, a majority of the patients had less than one year between their two measurements and the results for the percent differences were consistent with those for the rates of change for all metabolic markers. Lastly, we did not have much information on systemic insulin levels, another potential important metabolic biomarker[120], as this lab test is not frequently ordered by KPSC providers. Ultimately, data from electronic health records are not as detailed and complete as epidemiologic questionnaires for certain exposures, such as smoking and alcohol use.

In this population-based cohort study, we illustrate that the elevated pancreatic cancer risk associated with new-onset diabetes is also present among multiple racial/ethnic minorities. Additionally, the metabolic profiles of these incident diabetes patients appear to vary across race/ethnicity, suggesting that certain biomarkers could have more clinical relevance or utility for specific populations. These findings offer more insight into the complex diabetes-pancreatic cancer relationship and may help guide decisions towards tailoring prevention techniques and methods for identifying patients most appropriate for screening.

CHAPTER 5. SPECIFIC AIM 3 – METHYLATION OF CYTOKINE GENES

5.1 Methods

5.1.1 Study Population

We performed a retrospective cohort study of pancreatic cancer patients from The Cancer Genome Atlas (TCGA). TCGA is a large-scale collaboration between the National Cancer Institute (NCI) and National Human Research Institute (NHGRI) that provides complete genomic sequencing and epigenetic data on 11,000 patients across 33 different tumor types. It involves the participation of twenty institutions across the United States and Canada and includes clinical data and comprehensive genomic profiling of both tumor and matched normal tissues[121]. For this study, we included all patients with a primary pancreatic cancer tumor sample. Since this patient sample was primarily white (88%), we excluded all non-white individuals from the analysis. All de-identified data was downloaded from TCGA using the *TCGAbiolinks* package in R[122].

5.1.2 Methylation Values

DNA methylation profiles of TCGA pancreatic cancer samples were obtained using the Illumina Infinium HumanMethylation450 (HM450) BeadChip methylation assay[123,124]. This assay measures the methylated and unmethylated signal intensities of over 450,000 CpG dinucleotide sites across the genome, which are enriched for regulatory elements and promoter regions[125]. Raw methylation intensities downloaded from TCGA were pre-processed using normal-exponential out-of-band (noob) background correction with dye-bias normalization and converted to beta-values with the *minfi* package[123,126]. Beta-values were calculated from the corrected methylated (m_s) and unmethylated (m_u) signal intensities using the formula

$\beta=m_s/(m_s+m_u)$ and range from 0 to 1, with larger values indicating higher levels of methylation[123].

We filtered CpG probes by dropping those that overlapped with single nucleotide polymorphisms (SNPs) with minor allele frequency of 5% based on the CEU population[127]. These SNPs can interfere with probe binding, as well as influence methylation signal intensities since the HM450 assay can erroneously detect the common C to T polymorphism as a change in methylation[125,128]. We also dropped loci that had poor hybridization using the updated characterization file for the HM450 assay developed by Zhou et al[129].

As a sensitivity analysis, we performed additional pre-processing and calculated beta-values using the newly developed *SeSAMe* pipeline, which uses the P-value with out-of band array hybridization (pOOBAH) method to further adjust for poorly performing probes with failed hybridization[130].

5.1.3 CpG Probe Selection

We conducted a candidate gene approach, focusing on CpG loci in proximity to genes coding for cytokines that have been associated with pancreatic cancer survival in previous studies: *IL10*, *IL6*, *IL8*, *TGFβ1*, *TGFβ2*, *TGFβ3*, and *TNF*. Using the annotation file from the HM450 assay, we identified 113 total CpG loci across these seven genes. One probe in *TGFB2* was dropped due to failed hybridization and one probe in *TGFB3* was dropped due to SNP overlap, leaving a total of 111 CpG loci (6 for *IL10*, 11 for *IL6*, 2 for *IL8*, 25 for *TGFβ1*, 23 for *TGFβ2*, 16 for *TGFβ3* and 28 for *TNF*).

5.1.4 Assessment of Genetic Regulation and Expression

Genetic regulatory elements associated with the selected CpG loci were determined using the core 15-state model of chromatin states for the pancreas tissue epigenome developed by the NIH Roadmap Epigenomics Mapping Consortium[131]. The core 15-state model was created with ChromHMM, which establishes chromatin state signatures using multivariate Hidden Markov Models[132] and indicates whether the genetic region is associated with regulatory elements, such as active transcription start sites (TSS), weak transcription, enhancers, and heterochromatin[131]. Chromatin states were mapped to the CpG loci using the *GenomicRanges* package[133].

To evaluate whether the methylation sites were associated with changes in gene expression, we also downloaded Level 3 mRNA expression data from TCGA. Raw expression reads were evaluated with RESM and standardized using the upper-quartile normalization approach[134].

5.1.5 Assessment of Other Variables

Data on smoking, alcohol, tumor characteristics (e.g. stage) and treatment (e.g. radiation therapy), death, and last day of follow-up were obtained from the TCGA clinical files.

5.1.6 Statistical Analyses

We evaluated methylation at three levels of analysis: at the level of the individual CpG locus, at the level of each chromatin state per gene, and at the level of the entire gene. For the chromatin state- and gene-level analyses, we used the average methylation across all CpG loci in the given region as the exposure. For all units of analysis, the relationship between methylation and expression of its respective gene was assessed by calculating the Spearman's correlation coefficient (ρ). We used Cox proportional hazards regression to assess the relationship between

methylation and pancreatic cancer survival. Individuals were followed from time of diagnosis and censored at death or date of last contact. There was one tied event time, which was handled by calculating the exact likelihood. Methylation beta-values were rescaled to model each 0.01 increase in the mean beta value.

We used independent surrogate variable analysis (ISVA) to identify potential batch effects and measurement error in confounders that may bias results[128,135]. In this step, the residual variation in DNA methylation across the array that was not associated with time to death or last contact was first decomposed into independent components. Next, independent surrogate variables (ISVs) were constructed from the independent components that were significantly associated (using an alpha level of 0.05) with our confounders of interest: age (continuous), gender (male vs. female), stage (I, II, III/IV), smoking (never, former, current), receipt of radiation therapy (yes vs. no), and sample plate. Since we planned to adjust for age, gender, and stage in all fully adjusted models, we selected the ISVs that were significantly associated with any of the remaining potential confounders (smoking, receipt of radiation, or sample plate) as covariates in our models. ISVA identified seven total independent surrogate variables, five of which were included in our final models.

For each unit of analysis (CpG locus, chromatin state, or gene), we ran one model minimally adjusted for age and gender, and a fully adjusted model with age, gender, stage and the five ISVs as covariates. To evaluate the impact of probes with failed hybridization, we repeated all analyses using the beta-values pre-processed by the *SeSAMe* pipeline. As there were no differences in the results for two beta-value datasets, only the results from the noob-processed dataset are presented.

For the chromatin state- and gene-level analyses, we performed additional subgroup analyses stratified by gender and smoking (ever vs. never). Heterogeneity was assessed using a separate model with a product term for methylation and the stratifying variable.

Schoenfeld residuals were used to confirm no violation of the proportional hazards assumption. Martingale residuals, deviance residuals, and delta-beta values were reviewed to examine model fit. Wald tests with an alpha level of 0.05 were used to determine significance. All analysis was performed in R version 3.5.1.

5.2 Results

Our sample included 162 pancreatic cancer cases with primary tumor samples in the TCGA database (Table 5-1). The cohort consisted of 70 females (43.2%) and 92 males (56.8%) and the mean age of pancreatic cancer diagnosis was 65.5 years (standard deviation 10.7 years). Over 90% of patients had tumors of early stage (Stage I/II). There were 57 (35.2%) deaths by the end of follow up (Table 5-1). Samples were spread across twelve different plates in the methylation assay.

The 111 CpG probes of interest were associated with a variety of chromatin states in pancreas tissue. Flanking active TSS (27.9%) were the most common, followed by quiescent/low (26.1%), weak transcription (19.8%), active TSS (17.1%), enhancers (7.2%), strong transcription (0.9%) and weak repressed polycomb (0.9%).

Increased DNA methylation at several loci in *TGFβ1, TGFβ2* and *TGFβ3* was significantly associated with poor pancreatic cancer survival in the models fully adjusted for age, gender, stage and the five surrogate variables. Each 0.01 increase in DNA methylation beta-value at cg03313751 and cg24767336, both located in proximity to the *TGFβ1* TSS, was associated with a 62% (HR 1.62, 95% CI 1.14-2.31) and 46% (HR 1.46, 95% CI 1.06-2.02) elevated hazard

of pancreatic cancer death, respectively (Table 5-2, Figure 5-1). Methylation at these sites also had a weak to moderate negative correlation with *TGFβ1* expression (cg03313751: Spearman's ρ=-0.295, p<0.001; cg24767336: ρ=-0.223, p=0.005). A 0.01 increase in beta-value was associated with a borderline increased hazard of death for cg11037750 (HR 1.02, 95% CI 1.00-1.05) and cg09926389 (HR 1.02, 95% CI 1.00-1.03) within the *TGFβ1* gene body. Methylation at both of these CpG loci had a moderate positive correlation with *TGFβ1* expression (cg11037750: ρ=0.506, p<0.001; cg09926389: ρ=0.438, p<0.001).

For cg16658719 and cg16361301, both located near the *TGFβ2* TSS, each 0.01 increase in beta-value was associated with a 58% (HR 1.58, 95% CI 1.21-2.06) and 19% (HR 1.19, 95% CI 1.00-1.41) higher hazard of mortality, respectively. However, methylation was not correlated with *TGFβ2* expression at either locus (Table 5-2, Figure 5-2). Increased methylation at two additional loci within the *TGFβ2* gene body (cg20698667, cg16967578) were associated with a slight (2-5%) increased hazard of mortality (Table 5-2, Figure 5-2). In addition, methylation at cg20698667 had a weak negative correlation with *TGFβ2* expression (ρ=-0.174, p=0.030).

For *TGFβ3*, each 0.01 increase in the methylation beta-value at cg16292972, located near the *TGFβ3* TSS, was associated with a 45% elevated hazard of mortality (HR 1.45, 95% CI 1.01-2.09). However, methylation at this probe was not correlated with *TGFβ3* expression (ρ=0.066, p=0.414, Figure 5-3). Higher methylation at three other probes on *TGFβ3* (cg13121428, cg24696715, cg18298494) was associated with improved survival in the models minimally adjusted for age and gender, but were no longer significant after adjusting for all covariates (Table 5-2). All of these loci had a moderate inverse correlation with expression (cg13121428: ρ=-0.415, p<0.001; cg24696715: ρ=-0.466, p<0.001; cg18298494: ρ=-0.282, p<0.001).

In *TNF*, increased methylation at seven CpG sites, all associated with either weak transcription or quiescent/low chromatin states, were associated with a modest increased hazard

of death (3-5%) in the models minimally adjusted for age and gender (Table 5-2). All of these CpG loci were no longer significant after adjusting for the remaining covariates. Only cg09637172, located in the gene body, had a borderline increased hazard of death in the fully adjusted models (HR 1.03, 95% CI 1.00-1.07). Methylation at cg09637172 also had a moderate negative correlation with *TNF* expression (ρ=-0.397, p<0.001).

Elevated methylation in two CpG loci located in proximity to the *IL6* TSS were associated with mortality in the fully adjusted models. Each 0.01 increase in beta-value was associated with a borderline 2% higher hazard of death in cg00087425 (HR 1.02, 95% CI 1.00-1.04) and a 3% higher hazard of mortality in cg15703690 (HR 1.03, 95% CI 1.00-1.05). There was no correlation between methylation and *IL6* expression for either of these CpG loci (Table 5-2, Figure 5-4).

Conversely, higher methylation in cg14789529, located near the *IL10* three prime untranslated region (3' UTR), was associated with a decreased hazard of death (HR 0.97, 95% CI 0.94-1.00). There was also a weak inverse correlation between methylation at this CpG locus and *IL10* expression (ρ=-0.253, p=<0.001, Figure 5-5). For the remaining five *IL10* probes, all associated with quiescent/low chromatin states, methylation was not associated with mortality. In addition, none of the probes on *IL8* were associated with pancreatic cancer survival (Table 5-3).

When evaluating the average methylation levels across chromatin states, we observed higher hazards of death for higher methylation levels in quiescent/low chromatin states in *IL6* (HR 1.04, 95% CI 1.00-1.07), weak transcription in *TGFβ1* (HR 1.06, 95% CI 1.01-1.10), and weak transcription in *TNF* (HR 1.07, 95% 1.02, 1.13) in the minimally adjusted models. However, none of these associations remained significant after adjusting for all covariates. For average methylation across entire genes, higher methylation in *TGFβ1* was associated with an elevated hazard of death in the minimally adjusted model (HR 1.16, 95% 1.01-1.33), but not in

the fully adjusted model (HR 1.11, 95% 0.93-1.32). Higher average methylation in *TGFβ1* had a moderate positive correlation with expression (ρ=0.330, $p<0.001$) (Table 5-4).

In the subgroup analyses, higher methylation in *IL6* was associated with an increased hazard of death among never smokers (HR 1.14, 95% 1.01-1.29), but not among ever smokers (HR 0.97, 95% CI 0.88-1.07, p-interaction=0.05). This association was driven mainly by the methylation across the three CpG loci in the enhancer region of *IL6*, which was associated with a greater hazard of death in never smokers (HR 1.17, 95% CI 1.02-1.34) and a borderline decreased hazard of death in ever smokers (HR 0.88, 95% CI 0.77-1.00, p-interaction=0.001). There was no other significant interaction by smoking status for methylation in any of the other genes and chromatin states. In addition, we did not observe any heterogeneity by gender for any chromatin state- or gene-level analyses.

5.3 Discussion

In this study, we examined the associations between DNA methylation in proximity to cytokine-related genes and survival among pancreatic cancer patients from The Cancer Genome Atlas. Increased methylation in several CpG probes in *TGFβ1*, *TGFβ2* and *TGFβ3* was significantly associated with lower survival, with the highest hazard of death found among CpG loci located near transcription start sites. Elevated methylation in two CpG loci in *IL6* was associated with a modest increase in pancreatic cancer mortality rates while higher methylation in one CpG locus in *IL10* appeared to have a protective association.

TGF-β is an immunosuppressive cytokine that has been found to be directly secreted by pancreatic cancer tumor cells[62]. Past literature has shown that higher serum concentrations of TGF-β are associated with lower pancreatic cancer survival[74,78,136]. In our study, we observed that higher methylation in multiple loci across *TGFβ1*, *TGFβ2* and *TGFβ3* was associated with

increased hazards of mortality. DNA methylation in TGF-β genes and pancreatic cancer survival have been evaluated in two previous studies, which actually found longer survival among individuals with greater methylation in *TGFβ2*[91,92]. Both of these studies, however, had patient samples and methods that were dissimilar from the present analysis. In the first study, which used a subset of TCGA pancreatic tumor samples, the authors evaluated overall *TGFβ2* gene methylation and separated individuals into short (<1 year) and long (>2 year) survival groups[92]. In the other study, the researchers found that higher methylation in one CpG site in *TGFβ2* was associated with improved survival, but the results were not fully adjusted and were only based on a small sample of eleven pancreatic cancer tumors[91]. Given these differences in study design, future research with more consistent methods are needed to validate the current findings.

Among the *TGFβ1*, *TGFβ2* and *TGFβ3* CpG sites that were associated with mortality in our study, the strongest associations were observed among loci located near transcription start sites. However, the methylation of the CpG loci in proximity to the transcription start sites had weak to moderate correlation with expression in their respective genes. One possible explanation for this scenario could be that methylation at these particular CpG sites may not account for all epigenetic regulation of gene expression. Additionally, there has been limited literature on the association between TGF-β gene expression and pancreatic cancer mortality. The only previous study examining this relationship, also using TCGA data, found enhanced survival among patients with *TGFβ1* overexpression[79].

TNF-α is a pro-inflammatory cytokine that has been observed to be involved in the progression and invasiveness of pancreatic tumors[70,137]. Past literature has shown that pancreatic cancer patients have higher levels of both *TNF* expression and serum TNF-α concentrations compared to controls[69,138]. In our study, higher methylation in a few CpG loci in *TNF* was associated with shorter survival in the minimally adjusted models, but not in the fully adjusted

models. Previous DNA methylation studies on *TNF* are sparse and have focused on genes in the TNF receptor superfamily, which observed higher methylation in *TNFRSF1A* in the leukocyte DNA of pancreatic cancer patients[139] and lower methylation for *TNFRSF10C* in pancreatic cancer cell lines[140]. Research on *TNF* expression and pancreatic cancer mortality has also been somewhat inconsistent. While two prior studies found no relationship between *TNF* expression and mortality[71,141], another study found that increased expression was associated with decreased survival[75]. To our knowledge, this is the first study to evaluate DNA methylation in *TNF* and survival in pancreatic cancer patients.

IL-6 is a pleiotropic cytokine that is involved in several pathways in pancreatic cancer tumorigenesis[142,143]. Many prior studies have found poorer survival among pancreatic cancer patients with higher serum levels of IL-6[71,74,77,80]. In our analysis, we detected modest increased risks for mortality with increased methylation at two CpG loci in *IL6*. The relationship between DNA methylation in *IL6* and pancreatic cancer has not been well evaluated in current literature. While a previous study observed lower leukocyte DNA methylation in *IL6* among pancreatic cancer patients[139], no prior study has evaluated DNA methylation in this gene in the context of pancreatic cancer survival. In addition, both of these CpG sites were located near the *IL6* TSS, but there was no significant correlation between methylation and *IL6* expression. It is possible that DNA methylation in this region does not have a strong influence on genetic expression.

IL-10 is an anti-inflammatory cytokine that is released by pancreatic cancer cells and contributes to the development of a tumor-tolerating microenvironment[70,137]. Current evidence has suggested that higher concentrations of IL-10 is associated with poorer survival in pancreatic cancer patients[71,77]. We found that increased methylation in one CpG site in *IL10*, which was not correlated with expression, had a protective association with mortality. Though there has been no other literature on *IL10* DNA methylation and survival, previous studies have found lower *IL10*

methylation in leukocyte DNA[139] and increased *IL10* expression[74] in the tumor tissue among pancreatic cancer cases. In addition, *IL10* has been shown to be hypomethylated in other malignancies, such as colon, kidney, stomach, lung, breast cancer[144].

A major strength of this study is that it is one of the larger cohorts to evaluate DNA methylation in proximity to cytokine genes and pancreatic cancer survival using a candidate gene approach. Past research on this topic has been evaluated primarily in epigenome-wide association studies with much smaller samples size. We also incorporated chromatin states and expression in our analyses, which enhanced the interpretability of our results. However, our sample size was not sufficiently powered to perform subgroup analyses, so we do not know if the observed associations differ by certain patient characteristics. Generalizability of our results may also be an issue since the patient population was comprised of only white individuals with early-stage tumors. However, the age and sex distributions of the cohort are fairly similar to observed rates among pancreatic cancer patients in the United States[145]. Furthermore, the pancreatic tissue samples may likely be within a mixture of other cells that could have impacted some of the associations. As the pancreatic cancer tumor microenvironment involves the interaction of many different cell types, we did not adjust for cellular heterogeneity because it could have perhaps overcorrected and removed true biologic signals.

Each cytokine gene in this study was selected specifically because increased expression or serum concentrations of the cytokine was associated with pancreatic cancer in prior literature. Thus, we treated each gene as a unique hypothesis and did not perform correction for multiple testing. However, if we were to apply the Benjamini and Hochberg procedure to control the false discovery rate at a q-level of 0.05 in each gene[146], only cg16658719 in *TGFB2* would still be considered significant. On the other hand, several loci in the minimally adjusted models would still meet the corrected threshold for significance, indicating a potential loss of statistical power

when adjusting for additional covariates. Ultimately, our observed findings are still helpful for describing associations and generating hypotheses on the relationship between the immune system and pancreatic carcinogenesis.

In this study of pancreatic cancer patients from TCGA, we report reduced survival for higher methylation in CpG sites across *TGFB1*, *TGFB2*, *TGFB3*, and *IL6* and improved survival for higher methylation in one CpG locus in *IL10*. Our findings suggest that the interactions between cytokines and pancreatic cancer progression may potentially be influenced by epigenetic modifications. As epigenetics research on cytokine-related genes and pancreatic cancer is still quite sparse, future studies should evaluate these associations in larger samples to further elucidate the immune-associated mechanisms behind pancreatic cancer development and mortality.

CHAPTER 6. CONCLUSIONS AND PUBLIC HEALTH IMPLICATIONS

In this study, we investigated the relationships between several immune- or inflammation-related exposures and pancreatic cancer risk and survival. In Aim 1 using data from the prospective Multiethnic Cohort, we conducted the first study examining the association between AACs and antihistamines with pancreatic cancer in a diverse and well-powered prospective setting. Our results are consistent with that of past prospective studies and provide additional support of a null association for overall AACs. In Aim 2 utilizing hospital-based records from Kaiser Permanente Southern California, we found that the elevated pancreatic cancer risk associated with new-onset diabetes is also present among multiple racial/ethnic minorities. Additionally, the metabolic profiles of these incident diabetes patients appear to vary across race/ethnicity. Finally, in Aim 3 evaluating pancreatic cancer patients from The Cancer Genome Atlas, we detected reduced survival for higher methylation in CpG sites across *TGFB1*, *TGFB2*, *TGFB3, IL6* and enhanced survival for higher methylation in one CpG locus in *IL10*.

These findings provide further evidence of the complex interaction of the immune system and pancreatic cancer. While overall allergies may not be a reliable indicator for risk, utilizing markers of metabolic disorder could potentially be more effective at identifying high risk individuals. Specifically, the interethnic variations in metabolic changes prior to pancreatic cancer suggest that certain biomarkers could have more clinical relevance or utility for specific populations. Lastly, the outcomes from our methylation analysis indicate that the epigenetic alterations of immune activity may be involved in pancreatic carcinogenesis and survival.

Elucidating these immune-related mechanisms may support not only the tailoring of prevention and screening techniques, but also the enhancement of current immunotherapy-based therapies. By further understanding the associations between allergies and diabetes on pancreatic

cancer risk, clinicians can better define patient populations that are most appropriate for screening. In particular, our results for diabetes onset and metabolic markers may have significant implications for clinical practice by providing further opportunities for early detection. Moreover, our epigenetic findings may promote the development of targeted treatments with new methylation- and/or immune-based markers. Together, these results can be incorporated into the refinement of risk and survival prediction models that will allow patients to better understand their disease risk and prognoses, ultimately helping to control and reduce the burden of this lethal malignancy.

TABLES

Table 1-1. Cytokines and their relationship with pancreatic cancer survival

Cytokine	Function[70,71]	Association with survival*
Anti-inflammatory		
IL-10	• Inhibit Th1 responses, promote Th2 responses	Decreased survival[71,77]
TGF-β	• Inhibit Th1 responses, promote Th2 responses	Decreased survival[74,75,78] Increased survival[79]
Pro-inflammatory		
IL-6	• Stimulates angiogenesis and tumor vascularization • Interacts with IL-10 and TGF-β to inhibit dendritic cell proliferation	Decreased survival[71,74]
IL-8	• Promotion of angiogenesis • Activates MAPK pathway which stimulates cell growth • Promotes tumor aggressiveness and invasiveness	Decreased survival[72] No association[71]
TNF-α	• Promotes cancer proliferation • Increases tumor invasiveness	Decreased survival[72,75] Increased survival[80]

*For higher concentrations or expression of cytokine

Table 3-1. Baseline characteristics of Multiethnic Cohort Study participants from 1993-2012, stratified by atopic allergic conditions (AAC) status

	Total (N=187,226)		No AACs (N=137,530)		AACs (N=49,696)		
Characteristic	N	%	N	%	N	%	p[1]
Pancreatic cancer cases	1,455	0.8	1,094	0.8	361	0.7	
Follow-up time (years)[2]	16.2	(4.7)	16.1	(4.8)	16.5	(4.5)	
Pancreatic cancer incidence rate[3]	49.0		50.2		44.8		
Age at baseline (years)[2]	59.9	(8.8)	60.2	(8.8)	58.8	(8.9)	<0.0001
Age group at baseline (years)							<0.0001
<50	31,454	16.8	21,439	15.6	10,015	20.2	
50-54	28,050	15.0	19,853	14.4	8,197	16.5	
55-59	29,875	16.0	21,830	15.9	8,045	16.2	
60-64	32,245	17.2	24,068	17.5	8,177	16.5	
65-69	32,885	17.6	25,093	18.2	7,792	15.7	
70+	32,717	17.5	25,247	18.4	7,470	15.0	
Sex							<0.0001
Male	85,381	45.6	67,962	49.4	17,419	35.1	
Female	10,1845	54.4	69,568	50.6	32,277	64.9	
Ethnicity							<0.0001
White	46,858	25.0	32,245	23.4	14,613	29.4	
African American	31,500	16.8	23,252	16.9	8,248	16.6	
Latino	41,547	22.2	32,876	23.9	8,671	17.4	
Japanese American	53,746	28.7	39,484	28.7	14,262	28.7	
Native Hawaiian	13,575	7.3	9,673	7.0	3,902	7.9	
Family history of pancreatic cancer	3,193	1.7	2,197	1.6	996	2.0	<0.0001
Diabetes	21,997	11.7	16,773	12.2	5,224	10.5	<0.0001
Body mass index (kg/m^2)							<0.0001
<25	77,887	41.6	56,562	41.1	21,325	42.9	
25-30	71,887	38.4	54,141	39.4	17,746	35.7	
≥30	37,452	20.0	26,827	19.5	10,625	21.4	
Smoking status							<0.0001
Never	82,113	43.9	59,630	43.4	22,483	45.2	
Past	75,100	40.1	54,685	39.8	20,415	41.1	
Current	30,013	16.0	23,215	16.9	6,798	13.7	
Education							<0.0001
≤12 years	81,972	43.8	64,784	47.1	17,188	34.6	
Some college/vocational	55,406	29.6	39,211	28.5	16,195	32.6	
College graduate	49,848	26.6	33,535	24.4	16,313	32.8	
Alcohol intake (g/day)[4]							<0.0001
None	95,609	51.1	69,742	50.7	25,867	52.1	
<24 g	70,227	37.5	51,509	37.5	18,718	37.7	

24-48 g	13,351	7.1	10,069	7.3	3,282	6.6	
>48 g	8,039	4.3	6,210	4.5	1,829	3.7	
Ever use of antihistamines	28,413	15.2	8,381	6.1	20,032	40.3	<0.0001
Allergy severity							<0.0001
None	137,530	73.5	137,530	100.0	0	0.0	
AACs with no medication use	28,089	15.0	0	0.0	28,089	56.5	
AACs with medication use	20,032	10.7	0	0.0	20,032	40.3	

Abbreviations: AAC, atopic allergic condition; SD, standard deviation
[1]From a t-test for age and chi-square test for all other variables
[2]Mean (SD)
[3]Incidence rate per 100,000 person-years, age-standardized to US Census 2000 standard population, left truncated at age 45
[4]Intake during the year prior to cohort entry

Table 3-2. Associations between various allergy-related exposures and pancreatic cancer

Exposure	N	Cases	Minimally adjusted RR (95% CI)[1]	Fully adjusted RR (95% CI)[2]
Atopic allergic conditions (AACs)				
No	137,530	1,094	1 (ref)	1 (ref)
Yes	49,696	361	0.97 (0.86-1.10)	1.00 (0.88-1.12)
Ever use of antihistamines[3]				
No	149,760	1,191	1 (ref)	1 (ref)
Yes	28,413	190	0.90 (0.77-1.05)	0.92 (0.78-1.07)
Duration of antihistamine use[4]				
None	149,760	1,191	1 (ref)	1 (ref)
≤5 years of medication use	15,405	92	0.81 (0.65-1.00)	0.82 (0.66-1.01)
>5 years of medication use	9,538	76	1.09 (0.86-1.37)	1.12 (0.89-1.42)
p_{trend}[5]			0.71	0.94
Allergy severity				
None	137,530	1,094	1 (ref)	1 (ref)
AACs with no medication use	28,089	208	0.99 (0.85-1.15)	1.01 (0.87-1.17)
AACs with medication use	20,032	142	0.96 (0.81-1.15)	0.99 (0.83-1.19)
p_{trend}[5]			0.67	0.98

[1]Adjusted for age, sex, ethnicity
[2]Adjusted for age, sex, ethnicity, education, smoking status, family history of pancreatic cancer, education, BMI, diabetes, and alcohol intake.
[3]Information on antihistamine use missing for 9,053 participants (4.8% of cohort)
[4]Length of antihistamine use missing for 3,470 participants (12.2% of medication users)
[5]From a model treating exposure as a continuous variable

Table 3-3. Association between atopic allergic conditions and pancreatic cancer among subgroups of cohort

Subgroup	Non-AAC Cases	AAC Cases	RR (95% CI)[1]	p
All	1094	361	1.00 (0.88-1.12)	0.94
Age group at baseline				0.20[2]
<50	69	35	1.15 (0.76-1.74)	0.50
50-54	86	34	1.03 (0.69-1.54)	0.88
55-59	146	66	1.29 (0.96-1.74)	0.09
60-64	185	63	0.99 (0.74-1.32)	0.94
65-69	314	100	1.00 (0.80-1.26)	0.98
70+	294	63	0.74 (0.56-0.98)	0.03
Sex				0.91[2]
Male	555	133	1.01 (0.83-1.22)	0.95
Female	539	228	0.99 (0.84-1.15)	0.85
Ethnicity				0.52[2]
Whites	209	83	0.95 (0.73-1.23)	0.70
African Americans	216	68	0.92 (0.70-1.21)	0.54
Latinos	200	58	1.16 (0.86-1.56)	0.33
Japanese Americans	376	126	1.06 (0.86-1.30)	0.58
Native Hawaiians	93	26	0.78 (0.50-1.22)	0.28
Follow-up time (years)				0.27[2]
<5	211	54	0.76 (0.56-1.04)	0.08
≥5	883	307	1.04 (0.91-1.18)	0.59
Family history of pancreatic cancer				0.13[2]
No	1055	351	1.01 (0.90-1.15)	0.85
Yes	39	10	0.61 (0.30-1.23)	0.17
Diabetes				0.74[2]
No	929	310	0.99 (0.87-1.13)	0.85
Yes	165	51	1.04 (0.75-1.43)	0.82
Body mass index (kg/m^2)				0.19[2]
<25	453	134	0.88 (0.72-1.07)	0.19
25-30	426	139	1.06 (0.87-1.28)	0.58
≥30	215	88	1.13 (0.88-1.46)	0.33
Smoking status				0.49[2]

Never	461	168	1.07 (0.89-1.28)	0.49
Past	424	133	0.89 (0.73-1.09)	0.25
Current	209	60	1.06 (0.79-1.42)	0.70
Alcohol intake (g/day)[3]				0.67[2]
None	579	202	1.03 (0.87-1.21)	0.75
<24 g	388	125	0.98 (0.80-1.20)	0.82
24-48 g	78	25	1.11 (0.70-1.75)	0.67
>48 g	49	9	0.62 (0.30-1.28)	0.19
Ever use of antihistamines[4]				0.18[2]
No	983	208	0.99 (0.85-1.15)	0.84
Yes	48	142	1.30 (0.94-1.82)	0.12

[1]Adjusted for age, sex, ethnicity, education, smoking status, family history of pancreatic cancer, education, BMI, diabetes, and alcohol intake. Among subgroups, models are not adjusted for the subgroup variable
[2]P-value for heterogeneity
[3]Intake during the year prior to cohort entry
[4]Information on antihistamine use missing for 9,053 participants (4.8% of cohort)

Table 3-4. Association between antihistamine medication use and pancreatic cancer among subgroups of cohort

Subgroup	Cases with no antihistamine use	Cases with antihistamine use	RR (95% CI)[1]	p
All	1191	190	0.92 (0.78-1.07)	0.26
Age group at baseline				0.36[2]
<50	80	18	1.11 (0.66-1.87)	0.69
50-54	103	16	0.82 (0.48-1.40)	0.47
55-59	167	34	1.04 (0.72-1.51)	0.84
60-64	206	34	0.91 (0.63-1.32)	0.62
65-69	334	58	1.03 (0.78-1.37)	0.84
70+	301	30	0.66 (0.45-0.96)	0.03
Sex				0.09[2]
Male	578	76	1.08 (0.85-1.38)	0.52
Female	613	114	0.82 (0.67-1.00)	0.05
Ethnicity				0.28[2]
Whites	244	42	0.78 (0.56-1.09)	0.15
African Americans	221	37	0.76 (0.54-1.08)	0.13
Latinos	206	33	0.89 (0.62-1.29)	0.54
Japanese Americans	421	66	1.15 (0.89-1.50)	0.29
Native Hawaiians	99	12	0.95 (0.52-1.75)	0.88
Follow-up time (years)				0.73[2]
<5	220	28	0.83 (0.55-1.23)	0.35
≥5	971	162	0.94 (0.79-1.11)	0.46
Family history of pancreatic cancer				0.30[2]
No	1149	185	0.93 (0.79-1.09)	0.35
Yes	42	5	0.62 (0.24-1.58)	0.32
Diabetes				0.11[2]
No	1019	158	0.87 (0.73-1.03)	0.10
Yes	172	32	1.24 (0.84-1.81)	0.23
Body mass index (kg/m^2)				0.72[2]
<25	488	79	0.97 (0.76-1.23)	0.79
25-30	466	73	0.91 (0.71-1.17)	0.46
≥30	237	38	0.83 (0.59-1.18)	0.30

Smoking status				0.81[2]
Never	503	89	0.97 (0.77-1.22)	0.79
Past	464	71	0.86 (0.67-1.11)	0.24
Current	224	30	0.90 (0.61-1.22)	0.60
Alcohol intake (g/day)[3]				0.79[2]
None	634	97	0.88 (0.71-1.09)	0.23
<24 g	421	76	1.00 (0.78-1.27)	0.97
24-48 g	87	10	0.77 (0.40-1.49)	0.44
>48 g	49	7	0.93 (0.42-2.07)	0.86

[1]Adjusted for age, sex, ethnicity, education, smoking status, family history of pancreatic cancer, education, BMI, diabetes, and alcohol intake. Among subgroups, models are not adjusted for the subgroup variable
[2]P-value for heterogeneity
[3]Intake during the year prior to cohort entry

Table 4-1. Baseline characteristics of Kaiser Permanente Southern California patients from 2006-2016, by diabetes status

	Total (N=1,499,627)		No diabetes (N=1,055,996)		Incident diabetes (N=110,699)		Prevalent diabetes (N=332,932)	
Characteristic	N	%	N	%	N	%	N	%
Pancreatic cancer cases	2,002	0.1	937	0.1	306	0.3	759	0.2
Person-years, total	7,462,072.5		5,060,344.5		726,055.0		1,675,673.1	
Follow-up time (years), mean (SD)	5.0 (3.0)		4.8 (2.9)		6.6 (2.5)		5.0 (3.0)	
Time to pancreatic cancer (years), mean (SD)	3.8 (2.5)		3.6 (2.5)		4.3 (2.4)		3.7 (2.5)	
Incidence rate, age-adjusted[1]	30.7		23.8		43.7		41.2	
Age, mean (SD)	57.9 (10.9)		56.5 (10.5)		59.5 (10.8)		61.8 (11.2)	
Age group								
45-50	433,225	28.9	353,854	33.5	23,417	21.2	55,954	16.8
50-54	249,754	16.7	187,661	17.8	19,124	17.3	42,969	12.9
55-59	227,267	15.2	158,721	15.0	18,244	16.5	50,302	15.1
60-64	191,088	12.7	125,114	11.9	15,673	14.2	50,301	15.1
65-69	153,534	10.2	93,519	8.9	12,945	11.7	47,070	14.1
≥70	244,759	16.3	137,127	13.0	21,296	19.2	86,336	25.9
Gender								
Female	827,460	55.2	608,694	57.6	59,145	53.4	159,621	47.9
Male	672,167	44.8	447,302	42.4	51,554	46.6	173,311	52.1
Race/ethnicity								
Asian	166,395	11.1	110,579	10.5	14,836	13.4	40,980	12.3
Black	160,228	10.7	103,112	9.8	14,730	13.3	42,386	12.7
Hispanic	487,185	32.5	327,726	31.0	36,892	33.3	122,567	36.8
White	685,819	45.7	514,579	48.7	44,241	40.0	126,999	38.2
BMI kg/m^2, mean (SD)	29.2 (6.2)		28.3 (5.7)		31.2 (6.5)		31.2 (6.8)	
BMI kg/m^2 category								
<25	379,339	25.3	308,552	29.2	16,887	15.3	53,900	16.2

25-30	555,925	37.1	410,837	38.9	36,280	32.8	108,808	32.7
≥30	564,363	37.6	336,607	31.9	57,532	52.0	170,224	51.1
Smoking status								
Never	967,251	64.5	703,859	66.7	67,582	61.1	195,810	58.8
Past	392,214	26.2	253,045	24.0	30,909	27.9	108,260	32.5
Current	140,162	9.4	99,092	9.4	12,208	11.0	28,862	8.7
Alcohol user								
No	864,634	57.7	570,286	54.0	67,683	61.1	226,665	68.1
Yes	634,993	42.3	485,710	46.0	43,016	38.9	106,267	31.9
Education[2]								
High school or less	586,579	39.1	388,368	36.8	47,785	43.2	150,426	45.2
Some college	523,205	34.9	370,140	35.1	40,980	37.0	112,085	33.7
College graduate	318,594	21.2	243,112	23.0	19,828	17.9	55,654	16.7
Missing	71,249	4.8	54,376	5.2	2,106	1.9	14,767	4.4
Family history of pancreatic cancer	16,066	1.1	12,068	1.1	1,063	1.0	2,935	0.9
Pancreatitis	18,418	1.2	8,510	0.8	1,403	1.3	8,505	2.6

[1]Incidence rate per 100,000 person-years, age-standardized to US Census 2000 standard population, truncated to ages 45+
[2]Based on population geocoding files and not specific to individual patient
All tests comparing distribution of risk factors across diabetes status had p-values <0.0001

Table 4-2. Association between diabetes status and pancreatic cancer, among the entire cohort and within race/ethnicity subgroups

	Total (N=1,499,627)		Asian (N=166,395)		Black (N=160,228)		Hispanic (N=487,185)		White (N=685,819)		
Exposure	N Cases/ Person-years	RR (95% CI)[1]	N Cases/ Person-years	RR (95% CI)[1]	N Cases/ Person-years	RR (95% CI)[1]	N Cases/ Person-years	RR (95% CI)[1]	N Cases/ Person-years	RR (95% CI)[1]	p[2]
Diabetes status											0.25
None	937/ 5,060,344.5	1 (ref)	67/ 519,170.1	1 (ref)	117/ 528,713.0	1 (ref)	116/ 1,439,741.3	1 (ref)	587/ 2,572,720.0	1 (ref)	
Incident	306/ 726,055.0	3.17 (2.75-3.65)	34/ 98,859.0	3.21 (2.04-5.03)	37/ 100,617.6	2.82 (1.91-4.14)	82/ 235,261.2	4.12 (3.10-5.47)	153/ 291,317.3	2.90 (2.37-3.53)	
Prevalent	759/ 1,675,673.1	1.85 (1.67-2.05)	73/ 211,612.1	1.72 (1.22-2.43)	116/ 230,597.3	1.74 (1.33-2.28)	213/ 579,355.2	2.19 (1.77-2.71)	357/ 654,108.5	1.77 (1.54-2.03)	
Diabetes duration											0.09
None	937/ 5,060,344.5	1 (ref)	67/ 519,170.1	1 (ref)	117/ 528,713.0	1 (ref)	166/ 1,439,741.3	1 (ref)	587/ 2,572,720.0	1 (ref)	
Incident ≤1 yr	175/ 102,711.5	6.91 (5.76-8.30)	19/ 13,729.9	7.48 (4.17-13.41)	20/ 13,540.9	6.01 (3.68-9.82)	38/ 34,371.0	7.50 (5.09-11.06)	98/ 41,069.8	6.89 (5.37-8.83)	
Incident >1 yr	131/ 623,343.4	1.96 (1.62-2.38)	15/ 85,129.1	2.25 (1.24-4.10)	17/ 87,076.7	1.65 (0.97-2.80)	44/ 200,890.2	2.73 (1.91-3.89)	55/ 250,247.5	1.65 (1.24-2.19)	

NOTE: Incident diabetes status and duration treated as time-varying exposures

[1]From a Cox proportional hazards regression with age at cohort entry, gender, race, smoking, BMI, alcohol, pancreatitis, family history of pancreatic cancer and education as covariates

[2]P-values for heterogeneity across race/ethnicity

Table 4-3. Association between diabetes status and pancreatic cancer, among specific subgroups

	No diabetes			Incident diabetes			Prevalent diabetes			
Subgroup	N Cases	Person-years	RR (95% CI)[1]	N Cases	Person-years	RR (95% CI)[1]	N Cases	Person-years	RR (95% CI)[1]	p[2]
Gender										0.75
Females	521	2,993,149.9	1 (ref)	171	396,488.0	3.27 (2.70-3.96)	355	2,067,194.6	1.85 (1.61-2.14)	
Males	416	2,067,194.6	1 (ref)	135	329,567.0	3.05 (2.48-3.76)	404	849,473.1	1.85 (1.60-2.14)	
BMI (kg/m^2)										0.40
<25	303	1,516,627.4	1 (ref)	55	108,002.9	2.89 (2.10-3.99)	142	262,633.0	1.76 (1.42-2.17)	
25-30	373	1,985,713.6	1 (ref)	125	241,533.6	3.62 (2.91-4.51)	284	554,451.0	1.96 (1.66-2.30)	
≥30	261	1,558,003.5	1 (ref)	126	376,518.5	2.88 (2.29-3.61)	333	858,589.1	1.79 (1.51-2.11)	
Smoking status										0.02
Never	501	3,377,796.1	1 (ref)	172	447,694.9	3.35 (2.77-4.06)	428	992,526.0	2.13 (1.86-2.44)	
Past	313	1,232,906.0	1 (ref)	97	200,397.7	3.02 (2.36-3.85)	251	546,545.1	1.50 (1.26-1.79)	
Current	123	449,642.3	1 (ref)	37	77,962.4	2.81 (1.87-4.22)	80	136,602.0	1.75 (1.29-2.36)	
Alcohol user										0.86
No	509	2,745,676.0	1 (ref)	185	444,507.4	3.11 (2.59-3.73)	534	1,146,417.6	1.85 (1.63-2.11)	
Yes	428	2,314,668.5	1 (ref)	121	281,547.6	3.30 (2.63-4.13)	225	529,255.4	1.85 (1.56-2.19)	
Pancreatitis										0.10
No	912	5,017,861.9	1 (ref)	295	717,016.4	3.20 (2.77-3.69)	728	1,633,082.8	1.89 (1.70-2.09)	
Yes	25	42,482.6	1 (ref)	11	9,038.6	2.06 (0.83-5.09)	31	42,590.3	1.03 (0.61-1.74)	

Note: Incident diabetes treated as a time-varying exposure

[1]From a Cox proportional hazards regression with age at cohort entry, gender, race, smoking, BMI, alcohol, pancreatitis, family history of pancreatic cancer and education as covariates

[2]P-values for heterogeneity across particular subgroup

Table 4-4. Association between incident diabetes duration and pancreatic cancer, among specific subgroups

	No diabetes			Incident diabetes ≤1 year			Incident diabetes >1 year			
Subgroup	N Cases	Person-years	RR (95% CI)[1]	N Cases	Person-years	RR (95% CI)[1]	N Cases	Person-years	RR (95% CI)[1]	p[2]
Gender										0.09
Female	521	2,993,149.9	1 (ref)	91	54,946.7	6.42 (4.94-8.33)	80	341,541.2	2.29 (1.79-2.93)	
Male	416	2,067,194.6	1 (ref)	84	47,764.8	7.42 (5.74-9.58)	51	281,802.2	1.60 (1.18-2.17)	
BMI (kg/m^2)										0.35
<25	303	1,516,627.4	1 (ref)	36	18,627.6	6.51 (4.34-9.76)	19	89,375.3	1.61 (1.00-2.59)	
25-30	373	1,985,713.6	1 (ref)	73	35,958.6	8.32 (6.29-11.00)	52	205,575.0	2.15 (1.60-2.91)	
≥30	261	1,558,003.5	1 (ref)	66	48,125.3	5.91 (4.39-7.97)	60	328,393.2	1.97 (1.46-2.65)	
Smoking status										0.63
Never	501	3,377,796.1	1 (ref)	101	62,999.4	7.67 (5.99-9.81)	71	384,695.5	2.02 (1.55-2.62)	
Past	313	1,232,906.0	1 (ref)	52	27,922.0	6.07 (4.39-8.38)	45	172,475.7	2.03 (1.47-2.81)	
Current	123	449,642.3	1 (ref)	22	11,790.2	6.33 (3.83-10.46)	15	66,172.2	1.57 (0.88-2.81)	
Alcohol user										0.52
No	509	2,745,676.0	1 (ref)	101	61,959.7	6.46 (5.08-8.21)	84	382,547.7	2.03 (1.59-2.59)	
Yes	428	2,314,668.5	1 (ref)	74	40,751.8	7.67 (5.79-10.16)	47	240,795.8	1.85 (1.35-2.54)	

Note: Incident diabetes duration treated as time-varying exposure

[1]From a Cox proportional hazards regression with age at cohort entry, gender, race, smoking, BMI, alcohol, pancreatitis, family history of pancreatic cancer and education as covariates

[2]P-values for heterogeneity across particular subgroup

Table 4-5. Association between percent difference and rate of change in glucose, HbA1c and weight and pancreatic cancer, among incident diabetics in the entire cohort and within race/ethnicity subgroups

	Total	Asian	Black	Hispanic	White	
Parameter	RR (95% CI)[1]	RR (95% CI)[1]	RR (95% CI)[1]	RR (95% CI)[1]	RR (95% CI)[1]	p[2]
Glucose						
Percent difference (per 10% unit increase)	1.04 (1.03-1.05)	1.12 (1.06-1.17)	1.06 (1.02-1.09)	1.05 (0.99-1.10)	1.04 (1.02-1.05)	0.02
Rate of change (per mg/dL/month increase)	1.06 (1.04-1.07)	1.13 (1.07-1.19)	1.07 (1.03-1.11)	1.05 (0.97-1.13)	1.05 (1.03-1.07)	0.06
HbA1c						
Percent difference (per 1% unit increase)	1.02 (1.02-1.03)	1.02 (0.92-1.14)	1.02 (0.99-1.04)	1.01 (0.97-1.04)	1.03 (1.02-1.04)	0.41
Rate of change (per 0.1%/month increase)	1.45 (1.26-1.66)	2.06 (0.34-12.66)	1.32 (0.89-1.96)	1.23 (0.76-2.00)	1.60 (1.37-1.88)	0.39
Weight						
Percent difference (per 3% unit decrease)	1.24 (1.18-1.31)	1.22 (1.03-1.45)	1.07 (0.92-1.26)	1.14 (1.01-1.29)	1.32 (1.24-1.40)	0.02
Rate of change (per kg/month decrease)	1.62 (1.44-1.82)	1.78 (1.02-3.11)	1.17 (0.75-1.83)	1.54 (1.15-2.08)	1.74 (1.51-2.00)	0.34

[1]From a Cox proportional hazards regression with age at diabetes diagnosis, gender, race, smoking, and BMI as covariates. BMI was not included as a covariate for the models assessing changes in weight
[2]P-values for heterogeneity across race/ethnicity

Table 5-1. Baseline characteristics of 162 patients with primary tumor samples in TCGA, by vital status

	Total (N=162)		Alive (N=105)		Dead (N=57)		
Characteristic	N	%	N	%	N	%	p[1]
Follow-up time (mos), median (IQR)	7.7 (3.1, 16.8)		6.0 (0.9, 12.6)		14.3 (5.1, 20.6)		0.002
Age, mean (SD)	65.5 (10.7)		64.7 (10.8)		67.1 (10.6)		0.176
Gender							0.170
Female	70	43.2	50	47.6	20	35.1	
Male	92	56.8	55	52.4	37	64.9	
Stage							0.108
I	20	12.3	17	16.2	3	5.3	
II	130	80.2	79	75.2	51	89.5	
III/IV	9	5.6	6	5.7	3	5.3	
Missing	3	1.9	3	2.9	0	0.0	
Smoking status							0.811
Never	57	35.2	32	30.5	25	43.9	
Former	58	35.8	36	34.3	22	38.6	
Current	17	10.5	10	9.5	7	12.3	
Missing	30	18.5	27	25.7	3	5.3	
Received radiation							0.030
No	93	57.4	46	43.8	47	82.5	
Yes	28	17.3	21	20.0	7	12.3	
Missing	41	25.3	38	36.2	3	5.3	

[1]Mann-Whitney U test for follow-up time, t-test for age, chi-square test for all other variables

Table 5-2. Hazard ratios and 95% confidence intervals for the top associations between methylation in CpG loci and pancreatic cancer survival

							Expression		Minimally adjusted[1]		Fully adjusted[2]	
CpG site	Chr	Position	UCSC Gene Name	UCSC Gene Group	Chromatin state	Beta value, median (IQR)	Spearman's correlation coefficient	p	HR (95% CI)	p	HR (95% CI)	p
IL10												
cg14789529	chr1	206941464	IL10	3'UTR	Weak transcription	0.75 (0.68, 0.81)	-0.253	0.001	0.96 (0.93, 0.98)	0.001	0.97 (0.94, 1.00)	0.027
cg10978799	chr1	206945924	IL10	TSS200	Quiescent/Low	0.67 (0.56, 0.76)	-0.446	0.000	0.98 (0.97, 1.00)	0.063	0.99 (0.97, 1.01)	0.271
TGFB2												
cg16658719	chr1	218518963	TGFB2	TSS1500	Active TSS	0.04 (0.03, 0.04)	0.035	0.662	1.34 (1.08, 1.66)	0.007	1.58 (1.21, 2.06)	0.001
cg16361301	chr1	218519549	TGFB2	1stExon;5'UTR	Active TSS	0.05 (0.03, 0.06)	0.043	0.596	1.18 (1.01, 1.39)	0.039	1.19 (1.00, 1.41)	0.046
cg06899755	chr1	218520325	TGFB2	1stExon	Active TSS	0.08 (0.06, 0.10)	-0.291	0.000	0.96 (0.92, 1.01)	0.092	0.98 (0.92, 1.03)	0.385
cg01558923	chr1	218520959	TGFB2	Body	Flanking Active TSS	0.07 (0.05, 0.11)	-0.241	0.002	0.98 (0.95, 1.00)	0.080	1.00 (0.97, 1.03)	0.860
cg20698667	chr1	218523325	TGFB2	Body	Weak transcription	0.64 (0.55, 0.75)	-0.174	0.030	1.00 (0.99, 1.02)	0.871	1.02 (1.00, 1.05)	0.049
cg07810039	chr1	218524558	TGFB2	Body	Weak transcription	0.44 (0.31, 0.60)	-0.441	0.000	0.98 (0.97, 0.99)	0.002	0.99 (0.97, 1.00)	0.091
cg25132662	chr1	218537543	TGFB2	Body	Enhancers	0.34 (0.26, 0.43)	0.143	0.074	1.02 (1.00, 1.04)	0.097	1.01 (0.99, 1.04)	0.298
cg10484211	chr1	218547935	TGFB2	Body	Weak transcription	0.36 (0.26, 0.46)	-0.193	0.016	0.98 (0.97, 1.00)	0.075	0.98 (0.96, 1.00)	0.115
cg16967578	chr1	218575437	TGFB2	Body	Weak transcription	0.82 (0.75, 0.88)	0.141	0.078	1.05 (1.01, 1.08)	0.005	1.05 (1.01, 1.10)	0.020
TNF												
cg14910524	chr6	31541948	TNF	3'UTR;TSS1500	Quiescent/Low	0.81 (0.78, 0.83)	-0.270	0.001	1.05 (1.00, 1.09)	0.034	1.02 (0.97, 1.08)	0.403
cg27531490	chr6	31542459	TNF	TSS1500	Quiescent/Low	0.54 (0.48, 0.60)	0.193	0.015	1.02 (1.00, 1.05)	0.078	1.01 (0.98, 1.04)	0.349
cg24452282	chr6	31542740	TNF	TSS1500	Quiescent/Low	0.62 (0.54, 0.69)	-0.256	0.001	1.03 (1.01, 1.05)	0.008	1.01 (0.98, 1.04)	0.485
cg21370522	chr6	31543219	TNF	TSS200	Quiescent/Low	0.36 (0.28, 0.45)	-0.352	0.000	0.98 (0.96, 1.00)	0.020	1.00 (0.97, 1.02)	0.730
cg20477259	chr6	31544960	TNF	Body	Quiescent/Low	0.55 (0.47, 0.63)	-0.207	0.009	1.04 (1.01, 1.07)	0.010	1.03 (0.99, 1.06)	0.168
cg09637172	chr6	31545252	TNF	Body	Weak transcription	0.87 (0.78, 0.92)	-0.397	0.000	1.05 (1.01, 1.08)	0.004	1.03 (1.00, 1.07)	0.091
cg05952498	chr6	31545257	TNF	Body	Weak transcription	0.75 (0.67, 0.82)	-0.337	0.000	1.04 (1.01, 1.07)	0.003	1.03 (0.99, 1.06)	0.144
cg26736341	chr6	31545342	TNF	3'UTR	Weak transcription	0.58 (0.52, 0.64)	-0.152	0.057	1.02 (1.00, 1.05)	0.085	1.01 (0.97, 1.04)	0.709
cg04472685	chr6	31545473	TNF	3'UTR	Weak transcription	0.77 (0.69, 0.81)	-0.358	0.000	1.05 (1.01, 1.09)	0.005	1.04 (0.99, 1.10)	0.105
cg19124225	chr6	31545836	TNF	3'UTR	Weak transcription	0.85 (0.77, 0.89)	0.016	0.842	1.05 (1.01, 1.08)	0.010	1.03 (0.99, 1.08)	0.127
IL6												
cg10140158	chr7	22765750	IL6	TSS1500	Enhancers	0.82 (0.73, 0.86)	0.107	0.183	1.02 (1.00, 1.04)	0.095	1.01 (0.99, 1.04)	0.345
cg26061582	chr7	22766209	IL6	TSS1500	Enhancers	0.05 (0.04, 0.07)	0.050	0.537	0.91 (0.83, 1.00)	0.057	0.94 (0.85, 1.05)	0.281
cg00087425	chr7	22766829	IL6	1stExon;5'UTR	Flanking Active TSS	0.16 (0.10, 0.30)	-0.127	0.112	1.00 (0.99, 1.02)	0.834	1.02 (1.00, 1.04)	0.084
cg15703690	chr7	22766992	IL6	Body	Flanking Active	0.16 (0.12, 0.21)	-0.072	0.371	1.01 (0.99, 1.03)	0.189	1.03 (1.00, 1.05)	0.024

					TSS							
cg07998387	chr7	22767571	IL6	Body	Quiescent/Low	0.63 (0.56, 0.68)	0.052	0.521	1.02 (1.00, 1.04)	0.028	1.01 (0.99, 1.04)	0.423
TGFB3												
cg13121428	chr14	76446161	TGFB3	Body	Active TSS	0.79 (0.73, 0.84)	-0.415	0.000	0.97 (0.94, 1.00)	0.030	0.98 (0.95, 1.02)	0.316
cg24696715	chr14	76446681	TGFB3	Body	Flanking Active TSS	0.56 (0.51, 0.63)	-0.466	0.000	0.96 (0.93, 0.99)	0.016	0.98 (0.95, 1.02)	0.275
cg18298494	chr14	76447327	TGFB3	1stExon;5'UTR	Flanking Active TSS	0.57 (0.49, 0.63)	-0.282	0.000	0.98 (0.95, 1.00)	0.043	0.99 (0.97, 1.02)	0.565
cg08615333	chr14	76447413	TGFB3	1stExon;5'UTR	Flanking Active TSS	0.63 (0.53, 0.71)	-0.167	0.036	0.98 (0.97, 1.00)	0.054	0.99 (0.97, 1.01)	0.516
cg03395898	chr14	76448011	TGFB3	1stExon;5'UTR	Active TSS	0.17 (0.12, 0.22)	0.256	0.001	0.97 (0.94, 1.00)	0.093	0.99 (0.95, 1.03)	0.580
cg16292972	chr14	76448509	TGFB3	TSS1500	Active TSS	0.04 (0.03, 0.04)	0.066	0.414	1.18 (0.88, 1.59)	0.265	1.45 (1.01, 2.09)	0.044
TGFB1												
cg04614483	chr19	41836950	TGFB1	3'UTR	Weak transcription	0.49 (0.41, 0.55)	0.519	0.000	1.03 (1.01, 1.05)	0.016	1.01 (0.98, 1.04)	0.478
cg11037750	chr19	41837015	TGFB1	Body	Weak transcription	0.72 (0.63, 0.77)	0.506	0.000	1.04 (1.01, 1.06)	0.002	1.02 (1.00, 1.05)	0.087
cg09926389	chr19	41837123	TGFB1	Body	Weak transcription	0.76 (0.62, 0.88)	0.438	0.000	1.03 (1.01, 1.04)	0.000	1.02 (1.00, 1.03)	0.053
cg15735736	chr19	41854950	TGFB1	Body	Weak transcription	0.41 (0.33, 0.49)	-0.092	0.251	0.98 (0.96, 1.00)	0.019	0.99 (0.97, 1.01)	0.376
cg15726807	chr19	41857198	TGFB1	Body	Flanking Active TSS	0.09 (0.07, 0.12)	-0.255	0.001	0.96 (0.92, 1.00)	0.079	1.01 (0.95, 1.08)	0.649
cg21198010	chr19	41857456	TGFB1	Body	Flanking Active TSS	0.03 (0.03, 0.04)	-0.057	0.481	1.09 (0.78, 1.52)	0.630	1.37 (0.95, 1.96)	0.091
cg03313751	chr19	41857626	TGFB1	Body	Flanking Active TSS	0.03 (0.03, 0.04)	-0.295	0.000	1.64 (1.17, 2.30)	0.004	1.62 (1.14, 2.31)	0.007
cg00389176	chr19	41859014	TGFB1	1stExon;5'UTR	Flanking Active TSS	0.06 (0.05, 0.06)	0.012	0.878	1.23 (0.98, 1.53)	0.074	1.07 (0.79, 1.45)	0.670
cg24767336	chr19	41860095	TGFB1	TSS1500	Flanking Active TSS	0.03 (0.02, 0.03)	-0.223	0.005	1.15 (0.92, 1.44)	0.211	1.46 (1.06, 2.02)	0.020

[1]Adjusted for age and sex
[2]Adjusted for age, sex, stage and five surrogate variables

Table 5-3. Hazard ratios and 95% confidence intervals for the associations between methylation in CpG loci and pancreatic cancer survival

							Expression		Minimally adjusted[1]		Fully adjusted[2]	
CpG site	Chr	Position	UCSC Gene Name	UCSC Gene Group	Chromatin state	Beta value, median (IQR)	Spearman's correlation coefficient	p	HR (95% CI)	p	HR (95% CI)	p
IL10												
cg14789529	chr1	206941464	IL10	3'UTR	Weak transcription	0.75 (0.68, 0.81)	-0.253	0.001	0.96 (0.93, 0.98)	0.001	0.97 (0.94, 1.00)	0.027
cg15096505	chr1	206943566	IL10	Body	Quiescent/Low	0.84 (0.80, 0.88)	-0.310	0.000	1.01 (0.98, 1.04)	0.627	0.99 (0.95, 1.02)	0.450
cg17067005	chr1	206945346	IL10	Body	Quiescent/Low	0.93 (0.92, 0.94)	0.101	0.207	1.11 (0.94, 1.31)	0.230	1.00 (0.90, 1.12)	0.953
cg10978799	chr1	206945924	IL10	TSS200	Quiescent/Low	0.67 (0.56, 0.76)	-0.446	0.000	0.98 (0.97, 1.00)	0.063	0.99 (0.97, 1.01)	0.271
cg17744604	chr1	206946166	IL10	TSS1500	Quiescent/Low	0.82 (0.75, 0.87)	-0.573	0.000	0.98 (0.95, 1.01)	0.159	0.99 (0.96, 1.03)	0.580
cg14180511	chr1	206946187	IL10	TSS1500	Quiescent/Low	0.78 (0.71, 0.83)	-0.516	0.000	0.98 (0.96, 1.01)	0.286	1.00 (0.97, 1.04)	0.885
TGFB2												
cg21387604	chr1	218518468	TGFB2	TSS1500	Flanking Active TSS	0.11 (0.07, 0.16)	-0.083	0.304	0.99 (0.95, 1.04)	0.722	0.98 (0.93, 1.03)	0.437
cg16899280	chr1	218518579	TGFB2	TSS1500	Flanking Active TSS	0.04 (0.03, 0.04)	-0.125	0.118	1.02 (0.82, 1.28)	0.841	1.00 (0.76, 1.33)	0.980
cg26343258	chr1	218518675	TGFB2	TSS1500	Flanking Active TSS	0.07 (0.06, 0.08)	-0.053	0.513	1.07 (0.95, 1.20)	0.250	1.04 (0.91, 1.18)	0.591
cg16658719	chr1	218518963	TGFB2	TSS1500	Active TSS	0.04 (0.03, 0.04)	0.035	0.662	1.34 (1.08, 1.66)	0.007	1.58 (1.21, 2.06)	0.001
cg25851842	chr1	218519232	TGFB2	TSS200	Active TSS	0.02 (0.02, 0.02)	0.064	0.425	1.01 (0.97, 1.05)	0.666	1.00 (0.97, 1.04)	0.943
cg16361301	chr1	218519549	TGFB2	1stExon;5'UTR	Active TSS	0.05 (0.03, 0.06)	0.043	0.596	1.18 (1.01, 1.39)	0.039	1.19 (1.00, 1.41)	0.046
cg08746138	chr1	218519552	TGFB2	1stExon;5'UTR	Active TSS	0.05 (0.04, 0.05)	-0.044	0.584	0.92 (0.66, 1.28)	0.635	0.98 (0.69, 1.40)	0.917
cg12461345	chr1	218519565	TGFB2	1stExon;5'UTR	Active TSS	0.03 (0.02, 0.03)	0.077	0.336	1.47 (0.82, 2.63)	0.192	1.20 (0.62, 2.33)	0.582
cg09167119	chr1	218519576	TGFB2	1stExon;5'UTR	Active TSS	0.03 (0.03, 0.03)	-0.051	0.526	0.76 (0.48, 1.22)	0.261	0.93 (0.49, 1.77)	0.827
cg11976166	chr1	218520090	TGFB2	1stExon	Active TSS	0.09 (0.08, 0.12)	-0.244	0.002	1.00 (0.96, 1.04)	0.973	1.03 (0.98, 1.08)	0.287
cg06899755	chr1	218520325	TGFB2	1stExon	Active TSS	0.08 (0.06, 0.10)	-0.291	0.000	0.96 (0.92, 1.01)	0.092	0.98 (0.92, 1.03)	0.385
cg13285637	chr1	218520435	TGFB2	Body	Flanking Active TSS	0.07 (0.06, 0.08)	-0.037	0.644	0.96 (0.88, 1.05)	0.389	0.95 (0.85, 1.07)	0.415
cg22021178	chr1	218520468	TGFB2	Body	Flanking Active TSS	0.02 (0.02, 0.02)	-0.254	0.001	0.81 (0.63, 1.04)	0.104	0.89 (0.73, 1.10)	0.282
cg27508144	chr1	218520789	TGFB2	Body	Flanking Active TSS	0.07 (0.05, 0.11)	-0.355	0.000	0.99 (0.97, 1.01)	0.341	1.00 (0.97, 1.04)	0.845
cg17934824	chr1	218520792	TGFB2	Body	Flanking Active TSS	0.02 (0.02, 0.03)	-0.315	0.000	0.99 (0.97, 1.01)	0.488	1.01 (0.98, 1.04)	0.405
cg01558923	chr1	218520959	TGFB2	Body	Flanking Active TSS	0.07 (0.05, 0.11)	-0.241	0.002	0.98 (0.95, 1.00)	0.080	1.00 (0.97, 1.03)	0.860
cg20698667	chr1	218523325	TGFB2	Body	Weak transcription	0.64 (0.55, 0.75)	-0.174	0.030	1.00 (0.99, 1.02)	0.871	1.02 (1.00, 1.05)	0.049
cg07810039	chr1	218524558	TGFB2	Body	Weak transcription	0.44 (0.31, 0.60)	-0.441	0.000	0.98 (0.97, 0.99)	0.002	0.99 (0.97, 1.00)	0.091
cg25132662	chr1	218537543	TGFB2	Body	Enhancers	0.34 (0.26, 0.43)	0.143	0.074	1.02 (1.00, 1.04)	0.097	1.01 (0.99, 1.04)	0.298
cg10484211	chr1	218547935	TGFB2	Body	Weak transcription	0.36 (0.26, 0.46)	-0.193	0.016	0.98 (0.97, 1.00)	0.075	0.98 (0.96, 1.00)	0.115
cg18876728	chr1	218557095	TGFB2	Body	Weak transcription	0.80 (0.65, 0.86)	-0.173	0.030	1.00 (0.99, 1.02)	0.793	1.00 (0.98, 1.02)	0.733
cg16967578	chr1	218575437	TGFB2	Body	Weak transcription	0.82 (0.75, 0.88)	0.141	0.078	1.05 (1.01, 1.08)	0.005	1.05 (1.01, 1.10)	0.020
cg20991819	chr1	218617769	TGFB2	3'UTR	Quiescent/Low	0.72 (0.65, 0.80)	0.311	0.000	1.01 (0.99, 1.03)	0.187	1.01 (0.99, 1.04)	0.386
IL8												
cg16468729	chr4	74606465	IL8	Body	Quiescent/Low	0.13 (0.10, 0.16)	0.155	0.053	1.03 (0.97, 1.09)	0.363	1.01 (0.93, 1.10)	0.828
cg04392234	chr4	74608458	IL8	3'UTR	Quiescent/Low	0.84 (0.77, 0.89)	-0.054	0.502	0.98 (0.95, 1.02)	0.281	0.97 (0.92, 1.03)	0.310
TNF												
cg14910524	chr6	31541948	TNF	3'UTR;TSS1500	Quiescent/Low	0.81 (0.78, 0.83)	-0.270	0.001	1.05 (1.00, 1.09)	0.034	1.02 (0.97, 1.08)	0.403

cg27531490	chr6	31542459	TNF	TSS1500	Quiescent/Low	0.54 (0.48, 0.60)	0.193	0.015	1.02 (1.00, 1.05)	0.078	1.01 (0.98, 1.04)	0.349
cg24452282	chr6	31542740	TNF	TSS1500	Quiescent/Low	0.62 (0.54, 0.69)	-0.256	0.001	1.03 (1.01, 1.05)	0.008	1.01 (0.98, 1.04)	0.485
cg11484872	chr6	31543169	TNF	TSS200	Quiescent/Low	0.69 (0.60, 0.75)	-0.340	0.000	1.00 (0.98, 1.03)	0.795	1.00 (0.97, 1.02)	0.739
cg21370522	chr6	31543219	TNF	TSS200	Quiescent/Low	0.36 (0.28, 0.45)	-0.352	0.000	0.98 (0.96, 1.00)	0.020	1.00 (0.97, 1.02)	0.730
cg01569083	chr6	31543289	TNF	TSS200	Quiescent/Low	0.38 (0.30, 0.46)	-0.178	0.025	0.99 (0.97, 1.02)	0.622	0.99 (0.97, 1.02)	0.656
cg03037030	chr6	31543300	TNF	TSS200	Quiescent/Low	0.26 (0.20, 0.35)	-0.166	0.038	1.00 (0.97, 1.02)	0.854	1.00 (0.97, 1.02)	0.958
cg12681001	chr6	31543540	TNF	1stExon	Quiescent/Low	0.29 (0.23, 0.36)	-0.178	0.026	0.99 (0.97, 1.02)	0.532	1.00 (0.97, 1.03)	0.959
cg21222743	chr6	31543545	TNF	1stExon	Quiescent/Low	0.29 (0.23, 0.35)	-0.258	0.001	0.99 (0.97, 1.02)	0.690	1.00 (0.97, 1.03)	0.957
cg10717214	chr6	31543557	TNF	1stExon	Quiescent/Low	0.32 (0.27, 0.37)	-0.193	0.015	1.01 (0.98, 1.04)	0.723	1.00 (0.96, 1.04)	0.983
cg04425624	chr6	31543565	TNF	1stExon	Quiescent/Low	0.44 (0.39, 0.51)	-0.229	0.004	1.01 (0.98, 1.03)	0.537	1.00 (0.97, 1.03)	0.856
cg21467614	chr6	31543638	TNF	1stExon	Quiescent/Low	0.50 (0.43, 0.58)	-0.316	0.000	1.01 (0.99, 1.03)	0.391	1.00 (0.98, 1.02)	0.928
cg08553327	chr6	31543647	TNF	1stExon	Quiescent/Low	0.59 (0.50, 0.65)	-0.314	0.000	0.99 (0.97, 1.02)	0.489	0.99 (0.96, 1.02)	0.371
cg26729380	chr6	31543655	TNF	1stExon	Quiescent/Low	0.50 (0.43, 0.57)	-0.345	0.000	1.00 (0.97, 1.03)	0.938	1.00 (0.97, 1.03)	0.947
cg10650821	chr6	31543686	TNF	1stExon	Quiescent/Low	0.42 (0.36, 0.49)	-0.294	0.000	1.02 (1.00, 1.05)	0.112	1.00 (0.97, 1.03)	0.942
cg17741993	chr6	31544694	TNF	Body	Quiescent/Low	0.80 (0.70, 0.87)	-0.409	0.000	1.00 (0.98, 1.03)	0.723	1.01 (0.99, 1.04)	0.325
cg01360627	chr6	31544931	TNF	Body	Quiescent/Low	0.81 (0.71, 0.88)	-0.393	0.000	1.02 (0.99, 1.04)	0.203	1.02 (0.99, 1.05)	0.261
cg23384708	chr6	31544934	TNF	Body	Quiescent/Low	0.67 (0.60, 0.73)	-0.405	0.000	1.00 (0.97, 1.04)	0.763	1.00 (0.97, 1.04)	0.839
cg20477259	chr6	31544960	TNF	Body	Quiescent/Low	0.55 (0.47, 0.63)	-0.207	0.009	1.04 (1.01, 1.07)	0.010	1.03 (0.99, 1.06)	0.168
cg09637172	chr6	31545252	TNF	Body	Weak transcription	0.87 (0.78, 0.92)	-0.397	0.000	1.05 (1.01, 1.08)	0.004	1.03 (1.00, 1.07)	0.091
cg05952498	chr6	31545257	TNF	Body	Weak transcription	0.75 (0.67, 0.82)	-0.337	0.000	1.04 (1.01, 1.07)	0.003	1.03 (0.99, 1.06)	0.144
cg15989608	chr6	31545321	TNF	3'UTR	Weak transcription	0.71 (0.65, 0.77)	-0.371	0.000	1.02 (0.99, 1.05)	0.145	1.01 (0.97, 1.05)	0.575
cg26736341	chr6	31545342	TNF	3'UTR	Weak transcription	0.58 (0.52, 0.64)	-0.152	0.057	1.02 (1.00, 1.05)	0.085	1.01 (0.97, 1.04)	0.709
cg04472685	chr6	31545473	TNF	3'UTR	Weak transcription	0.77 (0.69, 0.81)	-0.358	0.000	1.05 (1.01, 1.09)	0.005	1.04 (0.99, 1.10)	0.105
cg19124225	chr6	31545836	TNF	3'UTR	Weak transcription	0.85 (0.77, 0.89)	0.016	0.842	1.05 (1.01, 1.08)	0.010	1.03 (0.99, 1.08)	0.127
cg02137984	chr6	31545898	TNF	3'UTR	Weak transcription	0.88 (0.84, 0.91)	-0.457	0.000	1.04 (0.99, 1.09)	0.129	1.04 (0.98, 1.11)	0.156
cg06825478	chr6	31546067	TNF	3'UTR	Weak transcription	0.82 (0.77, 0.88)	-0.348	0.000	1.02 (0.98, 1.05)	0.309	1.01 (0.97, 1.05)	0.783
cg17755321	chr6	31546085	TNF	3'UTR	Weak transcription	0.90 (0.86, 0.92)	-0.389	0.000	0.99 (0.94, 1.04)	0.686	1.00 (0.95, 1.07)	0.896
IL6												
cg17067544	chr7	22765321	IL6	TSS1500	Weak Repressed PolyComb	0.50 (0.39, 0.62)	0.281	0.000	0.99 (0.98, 1.01)	0.217	0.99 (0.97, 1.01)	0.288
cg10140158	chr7	22765750	IL6	TSS1500	Enhancers	0.82 (0.73, 0.86)	0.107	0.183	1.02 (1.00, 1.04)	0.095	1.01 (0.99, 1.04)	0.345
cg01770232	chr7	22766155	IL6	TSS1500	Enhancers	0.17 (0.14, 0.21)	0.190	0.017	1.01 (0.97, 1.06)	0.525	0.99 (0.94, 1.04)	0.637
cg26061582	chr7	22766209	IL6	TSS1500	Enhancers	0.05 (0.04, 0.07)	0.050	0.537	0.91 (0.83, 1.00)	0.057	0.94 (0.85, 1.05)	0.281
cg05472934	chr7	22766657	IL6	TSS200	Enhancers	0.05 (0.04, 0.06)	0.212	0.008	0.96 (0.89, 1.03)	0.251	0.96 (0.88, 1.05)	0.405
cg00087425	chr7	22766829	IL6	1stExon;5'UTR	Flanking Active TSS	0.16 (0.10, 0.30)	-0.127	0.112	1.00 (0.99, 1.02)	0.834	1.02 (1.00, 1.04)	0.084
cg15703690	chr7	22766992	IL6	Body	Flanking Active TSS	0.16 (0.12, 0.21)	-0.072	0.371	1.01 (0.99, 1.03)	0.189	1.03 (1.00, 1.05)	0.024
cg13104385	chr7	22767384	IL6	Body	Flanking Active TSS	0.61 (0.54, 0.67)	-0.256	0.001	1.01 (0.98, 1.03)	0.608	1.00 (0.97, 1.03)	0.915
cg05265849	chr7	22767390	IL6	Body	Flanking Active TSS	0.54 (0.44, 0.63)	-0.338	0.000	1.01 (0.99, 1.02)	0.457	0.99 (0.97, 1.01)	0.544
cg07998387	chr7	22767571	IL6	Body	Quiescent/Low	0.63 (0.56, 0.68)	0.052	0.521	1.02 (1.00, 1.04)	0.028	1.01 (0.99, 1.04)	0.423
cg02335517	chr7	22768438	IL6	Body	Quiescent/Low	0.93 (0.92, 0.94)	0.012	0.877	1.02 (0.97, 1.07)	0.490	0.98 (0.92, 1.04)	0.500
TGFB3												
cg20096775	chr14	76425468	TGFB3	3'UTR	Strong transcription	0.81 (0.79, 0.83)	-0.263	0.001	0.98 (0.90, 1.06)	0.558	1.01 (0.92, 1.11)	0.795
cg10782206	chr14	76445988	TGFB3	Body	Active TSS	0.75 (0.65, 0.82)	-0.229	0.004	0.99 (0.98, 1.01)	0.539	1.00 (0.97, 1.03)	0.984
cg06958766	chr14	76446087	TGFB3	Body	Active TSS	0.69 (0.55, 0.78)	-0.163	0.041	1.01 (1.00, 1.03)	0.115	1.01 (0.99, 1.03)	0.469
cg13121428	chr14	76446161	TGFB3	Body	Active TSS	0.79 (0.73, 0.84)	-0.415	0.000	0.97 (0.94, 1.00)	0.030	0.98 (0.95, 1.02)	0.316

cg02057912	chr14	76446433	TGFB3	Body	Active TSS	0.48 (0.43, 0.55)	-0.199	0.012	1.01 (0.99, 1.03)	0.250	1.00 (0.98, 1.03)	0.884
cg24696715	chr14	76446681	TGFB3	Body	Flanking Active TSS	0.56 (0.51, 0.63)	-0.466	0.000	0.96 (0.93, 0.99)	0.016	0.98 (0.95, 1.02)	0.275
cg18298494	chr14	76447327	TGFB3	1stExon;5'UTR	Flanking Active TSS	0.57 (0.49, 0.63)	-0.282	0.000	0.98 (0.95, 1.00)	0.043	0.99 (0.97, 1.02)	0.565
cg08615333	chr14	76447413	TGFB3	1stExon;5'UTR	Flanking Active TSS	0.63 (0.53, 0.71)	-0.167	0.036	0.98 (0.97, 1.00)	0.054	0.99 (0.97, 1.01)	0.516
cg17928876	chr14	76447431	TGFB3	1stExon;5'UTR	Flanking Active TSS	0.56 (0.49, 0.64)	-0.026	0.751	0.99 (0.97, 1.01)	0.514	1.00 (0.98, 1.02)	0.691
cg13643339	chr14	76447949	TGFB3	1stExon;5'UTR	Active TSS	0.03 (0.02, 0.03)	0.074	0.360	1.08 (0.86, 1.35)	0.507	1.08 (0.83, 1.39)	0.568
cg03395898	chr14	76448011	TGFB3	1stExon;5'UTR	Active TSS	0.17 (0.12, 0.22)	0.256	0.001	0.97 (0.94, 1.00)	0.093	0.99 (0.95, 1.03)	0.580
cg16187883	chr14	76448191	TGFB3	TSS200	Active TSS	0.14 (0.11, 0.17)	0.454	0.000	1.02 (0.95, 1.09)	0.559	1.00 (0.93, 1.08)	0.905
cg16292972	chr14	76448509	TGFB3	TSS1500	Active TSS	0.04 (0.03, 0.04)	0.066	0.414	1.18 (0.88, 1.59)	0.265	1.45 (1.01, 2.09)	0.044
cg05864191	chr14	76448933	TGFB3	TSS1500	Active TSS	0.03 (0.02, 0.03)	-0.052	0.517	0.95 (0.66, 1.37)	0.785	0.87 (0.54, 1.41)	0.574
cg16411863	chr14	76448962	TGFB3	TSS1500	Active TSS	0.02 (0.02, 0.03)	-0.059	0.464	1.15 (0.84, 1.57)	0.388	1.60 (0.80, 3.21)	0.187
cg10502508	chr14	76449243	TGFB3	TSS1500	Flanking Active TSS	0.04 (0.03, 0.05)	0.113	0.158	0.92 (0.72, 1.16)	0.462	0.90 (0.69, 1.17)	0.441
TGFB1												
cg04614483	chr19	41836950	TGFB1	3'UTR	Weak transcription	0.49 (0.41, 0.55)	0.519	0.000	1.03 (1.01, 1.05)	0.016	1.01 (0.98, 1.04)	0.478
cg11037750	chr19	41837015	TGFB1	Body	Weak transcription	0.72 (0.63, 0.77)	0.506	0.000	1.04 (1.01, 1.06)	0.002	1.02 (1.00, 1.05)	0.087
cg09926389	chr19	41837123	TGFB1	Body	Weak transcription	0.76 (0.62, 0.88)	0.438	0.000	1.03 (1.01, 1.04)	0.000	1.02 (1.00, 1.03)	0.053
cg03630756	chr19	41839506	TGFB1	Body	Weak transcription	0.93 (0.92, 0.94)	0.054	0.500	1.06 (0.94, 1.20)	0.355	0.85 (0.68, 1.06)	0.144
cg20748073	chr19	41842629	TGFB1	Body	Weak transcription	0.88 (0.86, 0.89)	0.022	0.782	0.98 (0.92, 1.04)	0.481	0.98 (0.91, 1.06)	0.650
cg27540367	chr19	41848134	TGFB1	Body	Enhancers	0.58 (0.54, 0.64)	-0.248	0.002	0.98 (0.95, 1.02)	0.377	0.99 (0.95, 1.03)	0.543
cg15735736	chr19	41854950	TGFB1	Body	Weak transcription	0.41 (0.33, 0.49)	-0.092	0.251	0.98 (0.96, 1.00)	0.019	0.99 (0.97, 1.01)	0.376
cg15726807	chr19	41857198	TGFB1	Body	Flanking Active TSS	0.09 (0.07, 0.12)	-0.255	0.001	0.96 (0.92, 1.00)	0.079	1.01 (0.95, 1.08)	0.649
cg13342441	chr19	41857250	TGFB1	Body	Flanking Active TSS	0.04 (0.04, 0.05)	-0.348	0.000	0.87 (0.74, 1.03)	0.107	0.92 (0.78, 1.10)	0.364
cg21198010	chr19	41857456	TGFB1	Body	Flanking Active TSS	0.03 (0.03, 0.04)	-0.057	0.481	1.09 (0.78, 1.52)	0.630	1.37 (0.95, 1.96)	0.091
cg19580847	chr19	41857554	TGFB1	Body	Flanking Active TSS	0.03 (0.03, 0.04)	0.127	0.112	0.96 (0.79, 1.16)	0.650	0.97 (0.83, 1.14)	0.748
cg03313751	chr19	41857626	TGFB1	Body	Flanking Active TSS	0.03 (0.03, 0.04)	-0.295	0.000	1.64 (1.17, 2.30)	0.004	1.62 (1.14, 2.31)	0.007
cg18236665	chr19	41858816	TGFB1	1stExon	Flanking Active TSS	0.03 (0.03, 0.04)	-0.073	0.361	1.02 (0.81, 1.29)	0.846	1.00 (0.78, 1.28)	0.994
cg00389176	chr19	41859014	TGFB1	1stExon;5'UTR	Flanking Active TSS	0.06 (0.05, 0.06)	0.012	0.878	1.23 (0.98, 1.53)	0.074	1.07 (0.79, 1.45)	0.670
cg01107031	chr19	41859159	TGFB1	1stExon;5'UTR	Flanking Active TSS	0.03 (0.03, 0.04)	-0.190	0.017	0.96 (0.91, 1.02)	0.237	0.97 (0.90, 1.04)	0.411
cg13464744	chr19	41859304	TGFB1	1stExon;5'UTR	Flanking Active TSS	0.03 (0.03, 0.04)	-0.050	0.531	1.18 (0.81, 1.72)	0.376	0.95 (0.61, 1.46)	0.804
cg11714801	chr19	41859555	TGFB1	1stExon;5'UTR	Active TSS	0.07 (0.06, 0.09)	0.100	0.211	1.02 (0.97, 1.08)	0.381	1.02 (0.92, 1.14)	0.674
cg05637188	chr19	41860004	TGFB1	TSS200	Flanking Active TSS	0.02 (0.02, 0.02)	0.030	0.707	0.93 (0.28, 3.07)	0.902	0.92 (0.26, 3.21)	0.896
cg04547554	chr19	41860013	TGFB1	TSS200	Flanking Active TSS	0.01 (0.01, 0.02)	-0.209	0.009	0.82 (0.33, 2.04)	0.664	2.14 (0.59, 7.74)	0.247
cg23275502	chr19	41860019	TGFB1	TSS200	Flanking Active TSS	0.01 (0.01, 0.02)	-0.179	0.025	1.02 (0.53, 1.96)	0.945	1.76 (0.59, 5.24)	0.313
cg20410381	chr19	41860082	TGFB1	TSS1500	Flanking Active TSS	0.05 (0.04, 0.05)	-0.036	0.652	1.02 (0.86, 1.22)	0.812	1.13 (0.90, 1.42)	0.306
cg24767336	chr19	41860095	TGFB1	TSS1500	Flanking Active TSS	0.03 (0.02, 0.03)	-0.223	0.005	1.15 (0.92, 1.44)	0.211	1.46 (1.06, 2.02)	0.020
cg09489285	chr19	41860454	TGFB1	3'UTR;TSS1500	Enhancers	0.90 (0.88, 0.92)	-0.052	0.520	1.03 (0.97, 1.09)	0.382	1.01 (0.94, 1.10)	0.726
cg16883145	chr19	41860619	TGFB1	Body;TSS1500	Enhancers	0.85 (0.83, 0.87)	0.035	0.666	0.99 (0.92, 1.05)	0.696	0.97 (0.92, 1.03)	0.379
cg25975823	chr19	41860824	TGFB1	Body;TSS1500	Weak transcription	0.82 (0.80, 0.84)	0.148	0.064	1.02 (0.96, 1.09)	0.482	1.01 (0.94, 1.08)	0.844

[1]Adjusted for age and sex
[2]Adjusted for age, sex, stage and five surrogate variables

Table 5-4. Hazard ratios and 95% confidence intervals for the associations between average methylation across CpG loci in specific genomic regions and pancreatic cancer survival

			Expression		Minimally adjusted[1]		Fully adjusted[2]	
Genomic region	N CpG loci	Beta value, median (IQR)	Spearman's correlation coefficient	p	HR (95% CI)	p	HR (95% CI)	p
IL10								
Entire gene	6	0.79 (0.75, 0.83)	-0.474	<0.001	0.97 (0.93, 1.00)	0.069	0.98 (0.93, 1.02)	0.254
Weak transcription	1	0.75 (0.68, 0.81)	-0.253	0.001	0.96 (0.93, 0.98)	0.001	0.97 (0.94, 1.00)	0.027
Quiescent/Low	5	0.81 (0.76, 0.85)	-0.495	<0.001	0.98 (0.94, 1.01)	0.218	0.98 (0.94, 1.03)	0.448
TGFB2								
Entire gene	23	0.22 (0.20, 0.23)	-0.320	<0.001	0.96 (0.89, 1.04)	0.33	1.01 (0.91, 1.11)	0.91
Active TSS	8	0.05 (0.04, 0.06)	-0.280	<0.001	0.97 (0.84, 1.11)	0.631	1.05 (0.89, 1.23)	0.579
Flanking Active TSS	8	0.06 (0.05, 0.08)	-0.351	<0.001	0.96 (0.90, 1.02)	0.206	1.00 (0.93, 1.07)	0.900
Weak transcription	5	0.60 (0.56, 0.65)	-0.382	<0.001	0.98 (0.94, 1.01)	0.157	0.99 (0.95, 1.03)	0.633
Enhancers	1	0.34 (0.26, 0.43)	0.143	0.074	1.02 (1.00, 1.04)	0.097	1.01 (0.99, 1.04)	0.298
Quiescent/Low	1	0.72 (0.65, 0.80)	0.311	<0.001	1.01 (0.99, 1.03)	0.187	1.01 (0.99, 1.04)	0.386
IL8[3]								
Entire gene	2	0.48 (0.46, 0.50)	-0.008	0.926	0.97 (0.90, 1.05)	0.495	0.96 (0.88, 1.06)	0.466
TNF								
Entire gene	28	0.60 (0.57, 0.64)	-0.424	<0.001	1.04 (1.00, 1.10)	0.074	1.02 (0.96, 1.09)	0.455
Weak transcription	9	0.79 (0.74, 0.82)	-0.373	<0.001	1.07 (1.02, 1.13)	0.006	1.05 (0.99, 1.12)	0.117
Quiescent/Low	19	0.52 (0.48, 0.56)	-0.400	<0.001	1.02 (0.98, 1.06)	0.394	1.01 (0.96, 1.06)	0.755

IL6								
Entire gene	11	0.43 (0.39, 0.45)	-0.035	0.662	1.02 (0.98, 1.06)	0.404	1.02 (0.96, 1.07)	0.593
Flanking Active TSS	4	0.37 (0.32, 0.44)	-0.338	<0.001	1.01 (0.99, 1.04)	0.333	1.02 (0.99, 1.05)	0.246
Enhancers	4	0.27 (0.25, 0.30)	0.183	0.021	1.01 (0.96, 1.06)	0.787	1.01 (0.94, 1.07)	0.861
Weak Repressed PolyComb	1	0.50 (0.39, 0.62)	0.281	<0.001	0.99 (0.98, 1.01)	0.217	0.99 (0.97, 1.01)	0.288
Quiescent/Low	2	0.78 (0.74, 0.81)	0.050	0.534	1.04 (1.00, 1.07)	0.045	1.01 (0.97, 1.06)	0.583
TGFB3								
Entire gene	16	0.39 (0.36, 0.42)	-0.223	0.005	0.97 (0.93, 1.02)	0.309	0.99 (0.94, 1.05)	0.747
Active TSS	10	0.31 (0.29, 0.34)	-0.171	0.032	1.00 (0.95, 1.06)	0.999	1.00 (0.94, 1.07)	0.934
Flanking Active TSS	5	0.47 (0.41, 0.53)	-0.232	0.003	0.97 (0.94, 1.00)	0.055	0.99 (0.96, 1.02)	0.484
Strong transcription	1	0.81 (0.79, 0.83)	-0.263	0.001	0.98 (0.90, 1.06)	0.558	1.01 (0.92, 1.11)	0.795
TGFB1								
Entire gene	25	0.32 (0.31, 0.33)	0.330	<0.001	1.16 (1.01, 1.33)	0.040	1.11 (0.93, 1.32)	0.260
Active TSS	1	0.07 (0.06, 0.09)	0.100	0.211	1.02 (0.97, 1.08)	0.381	1.02 (0.92, 1.14)	0.674
Flanking Active TSS	14	0.04 (0.03, 0.04)	-0.380	<0.001	0.84 (0.65, 1.08)	0.174	1.02 (0.74, 1.42)	0.902
Weak transcription	7	0.71 (0.67, 0.75)	0.477	<0.001	1.06 (1.01, 1.10)	0.010	1.03 (0.98, 1.08)	0.236
Enhancers	3	0.78 (0.75, 0.80)	-0.173	0.030	0.99 (0.91, 1.07)	0.741	0.96 (0.87, 1.06)	0.443

[1]Adjusted for age and sex
[2]Adjusted for age, sex, stage and five surrogate variables
[3]Both CpG loci for *IL8* were associated with the Quiescent/Low chromatin state

FIGURES

Figure 1-1. Conceptual framework of dissertation aims

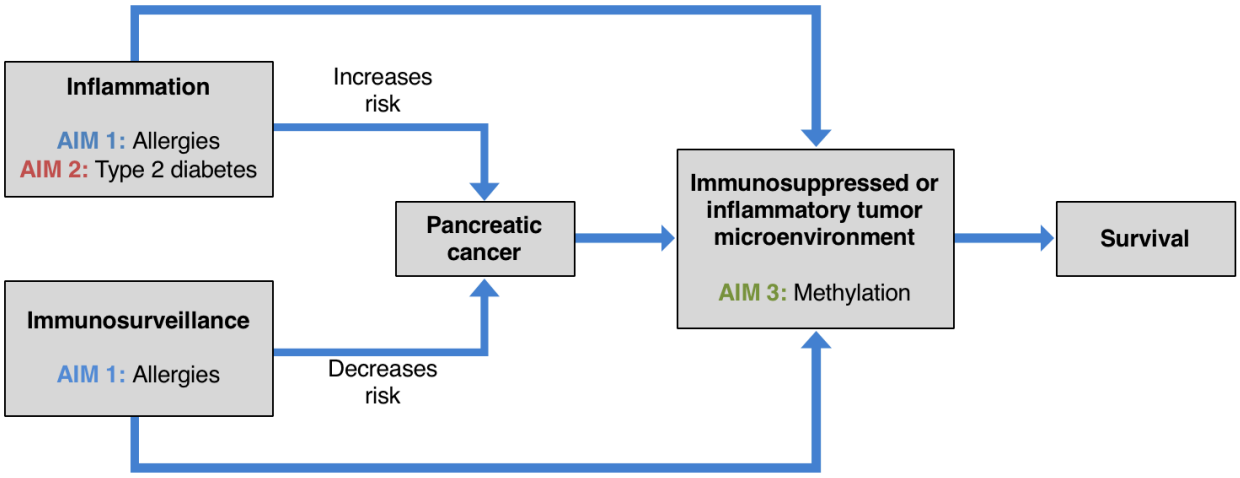


Figure 4-1. Flowchart of Kaiser Permanente Southern California study cohort

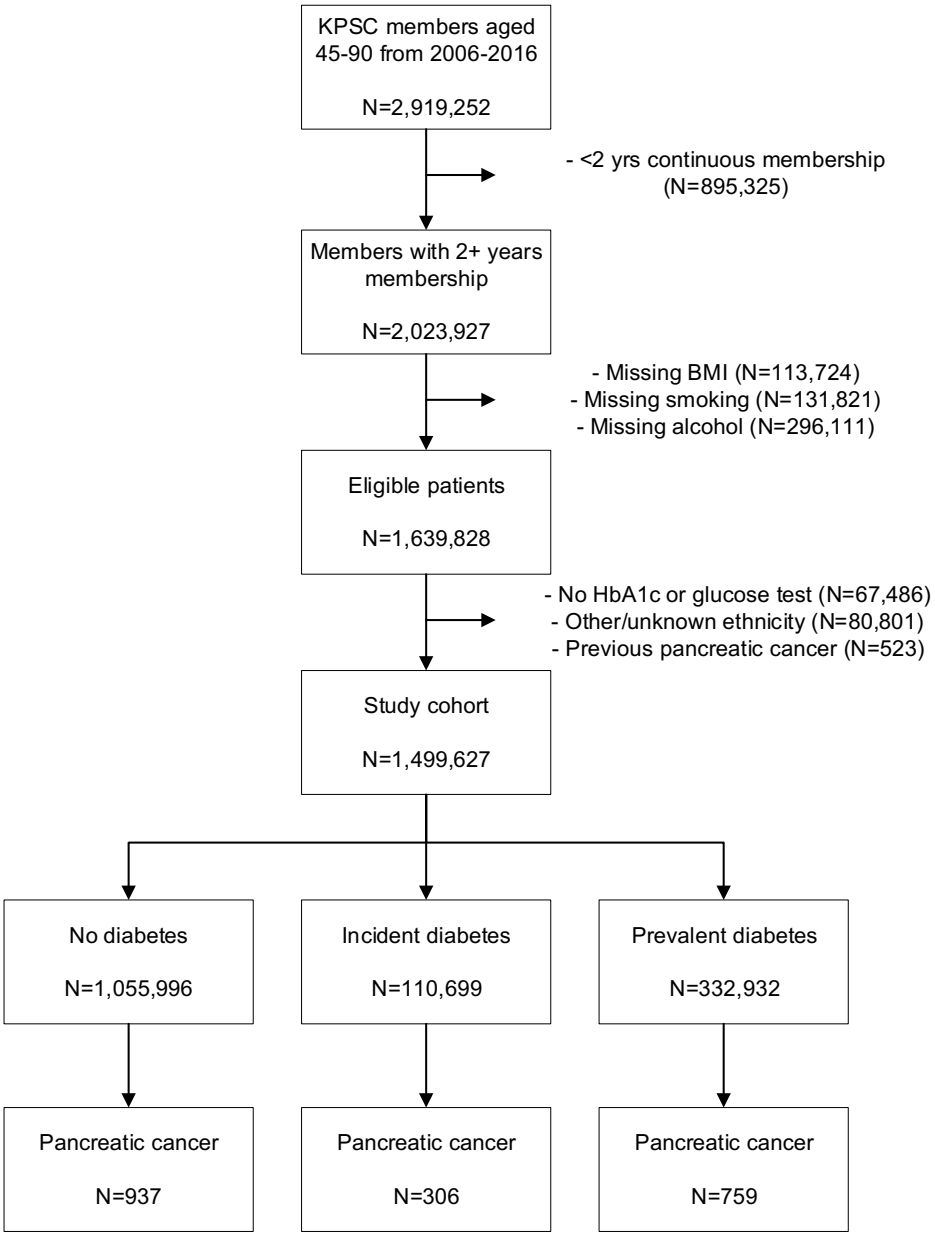


Figure 4-2. Boxplots of (a) fasting glucose, (b) HbA1c and (c) weight values before and at diabetes diagnosis, across pancreatic cancer status

Within boxplot, solid diamond indicates mean value, solid line indicates median.

P-values indicate results from a Mann-Whitney U test comparing distributions between individuals with and without pancreatic cancer in given time window.

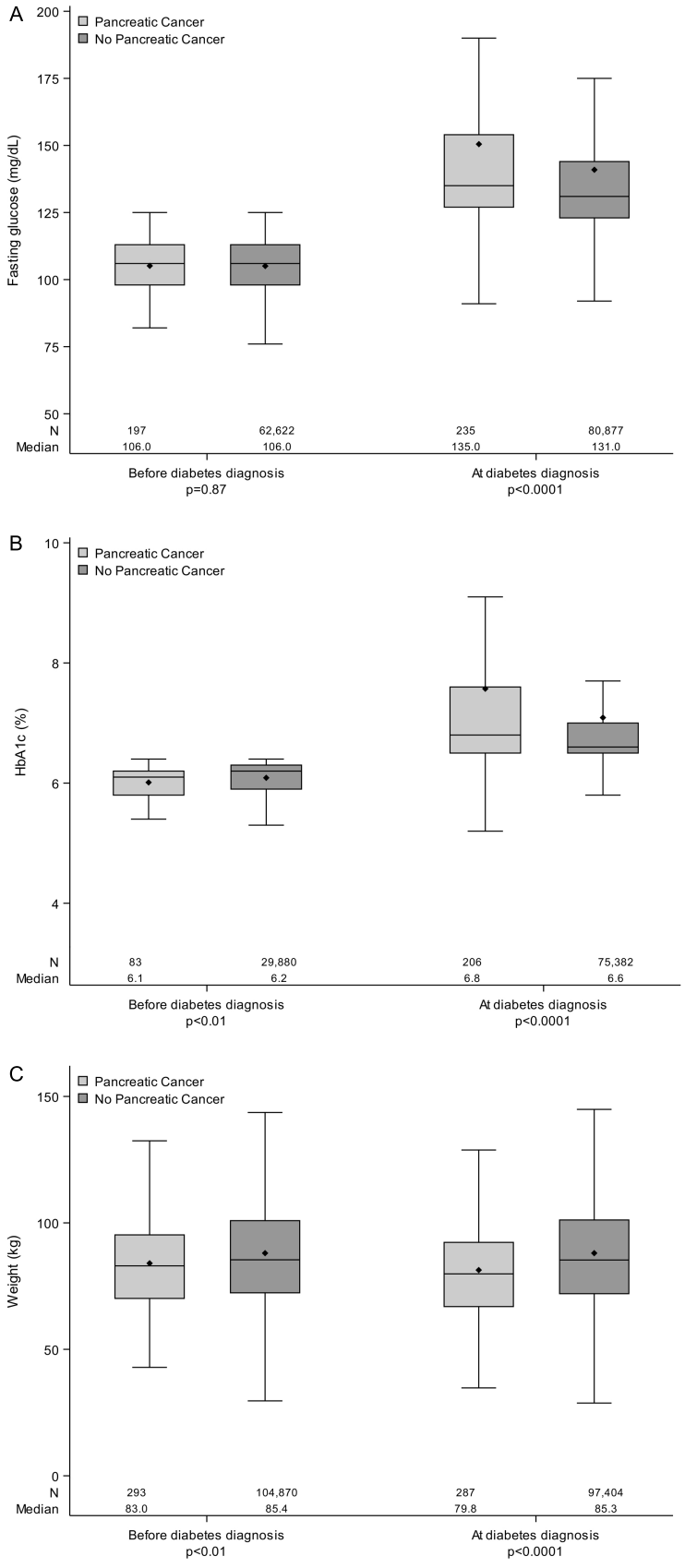


Figure 4-3. Boxplots of the percent differences in (a) fasting glucose, (b) HbA1c and (c) weight between the time before and at diabetes diagnosis, across pancreatic cancer status and race/ethnicity

Within boxplot, solid diamond indicates mean value, solid line indicates median.

P-values under subgroup indicate results from a Mann-Whitney U test comparing distributions between individuals with and without pancreatic cancer in given subgroup.

P-heterogeneity indicates overall heterogeneity for mean percent difference across pancreatic cancer status and race/ethnicity.

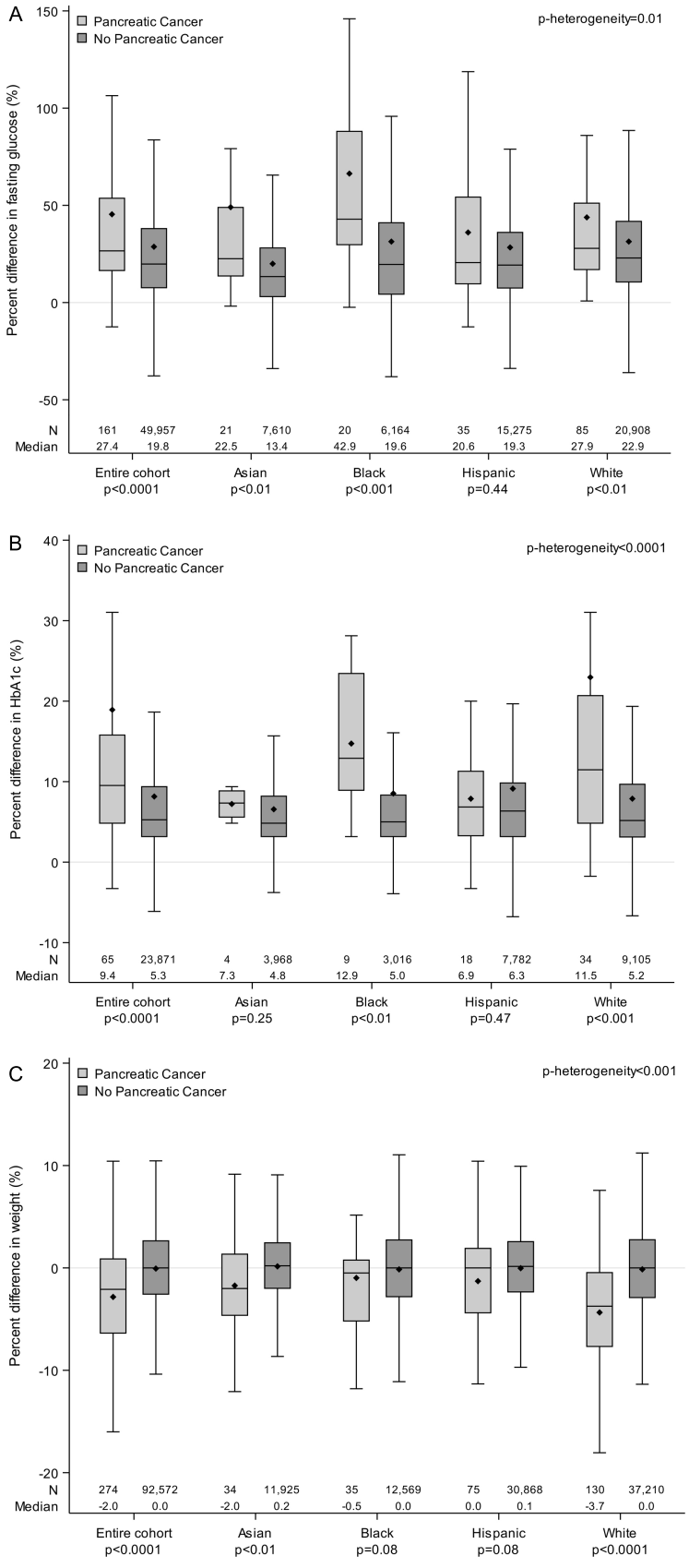


Figure 5-1. Genomic information, Spearman correlation coefficients for expression, and hazard ratios and 95% confidence intervals for selected CpG loci in *TGFβ1*.

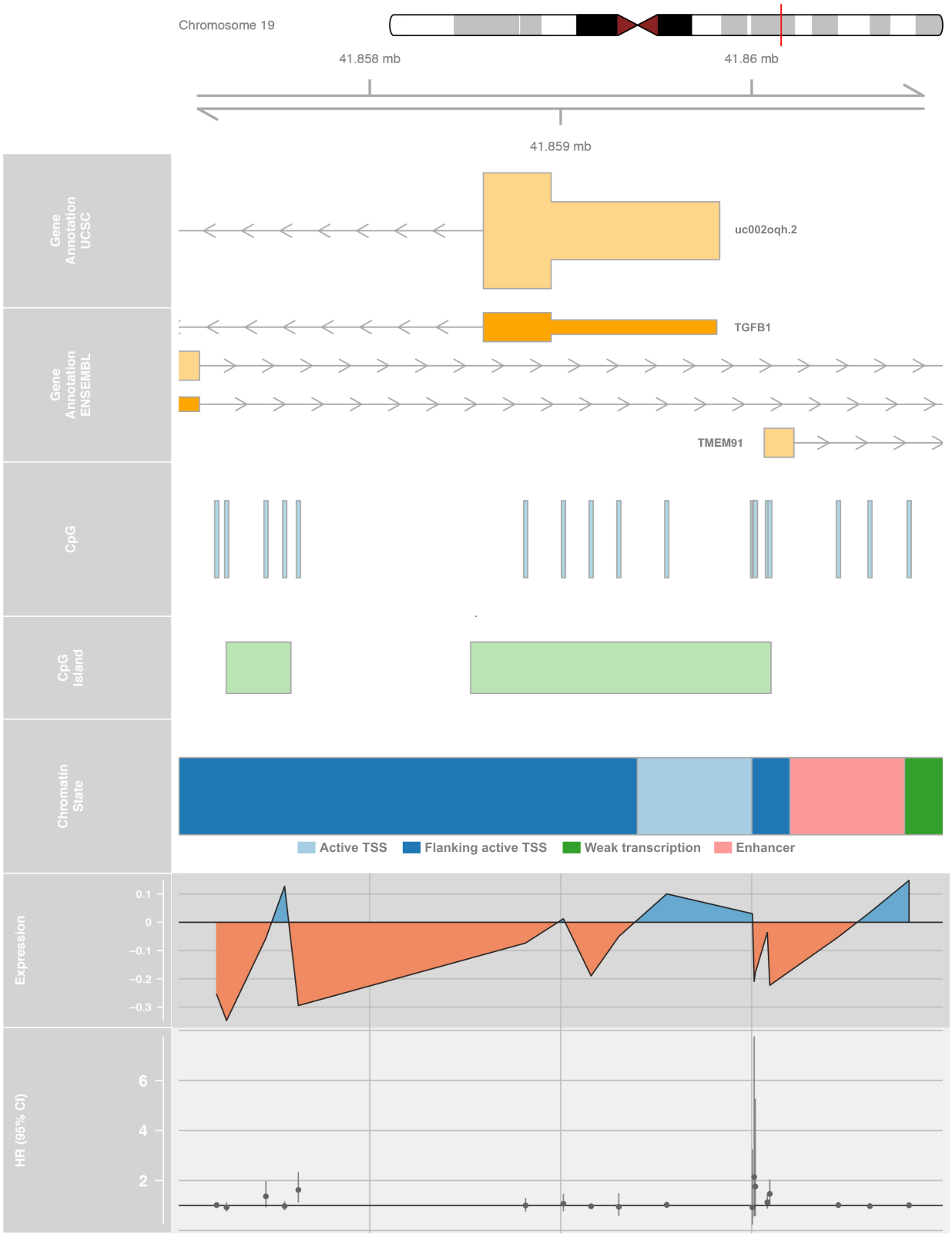


Figure 5-2. Genomic information, Spearman correlation coefficients for expression, and hazard ratios and 95% confidence intervals for selected CpG loci in *TGFβ2*.

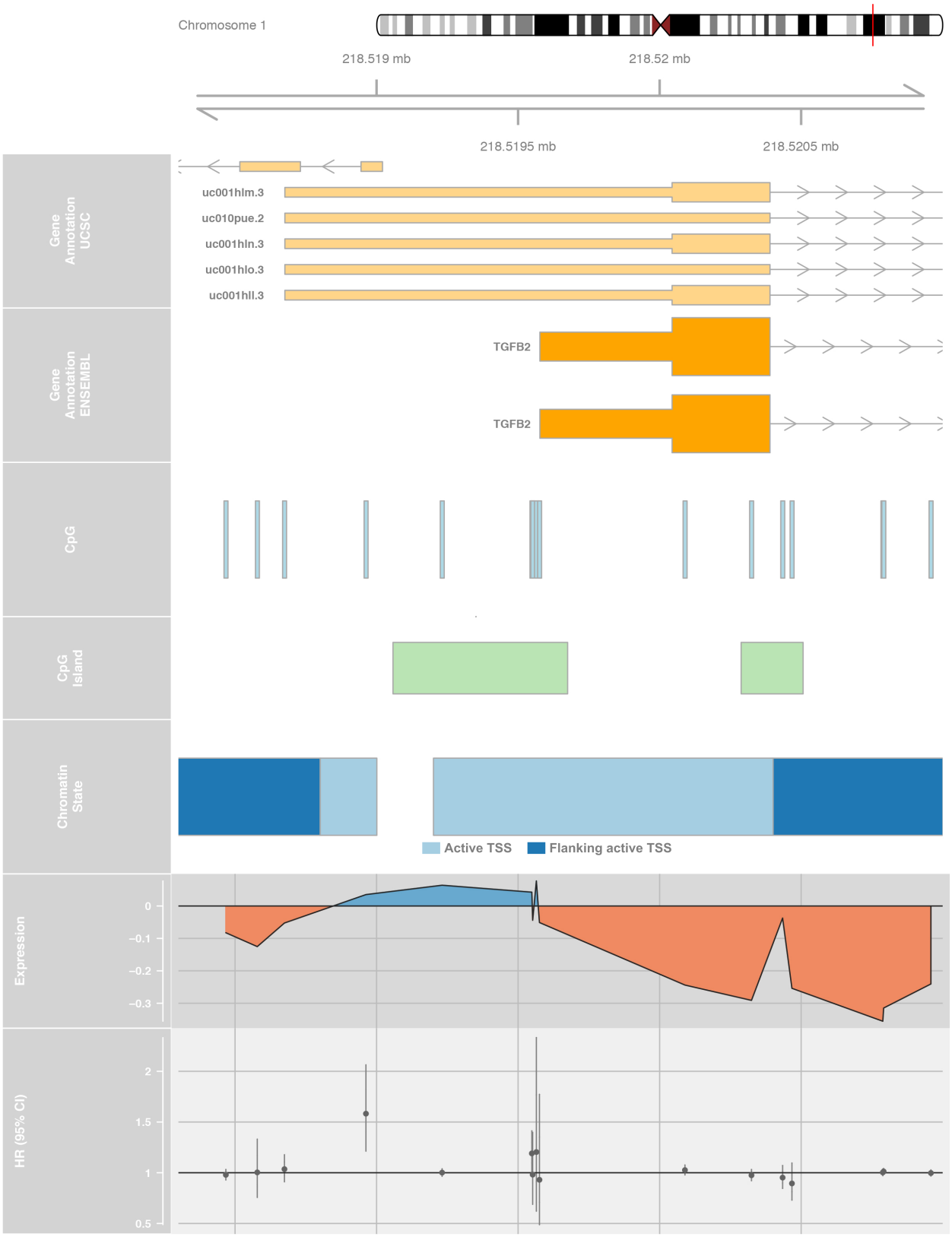


Figure 5-3. Genomic information, Spearman correlation coefficients for expression, and hazard ratios and 95% confidence intervals for selected CpG loci in *TGFβ3*.

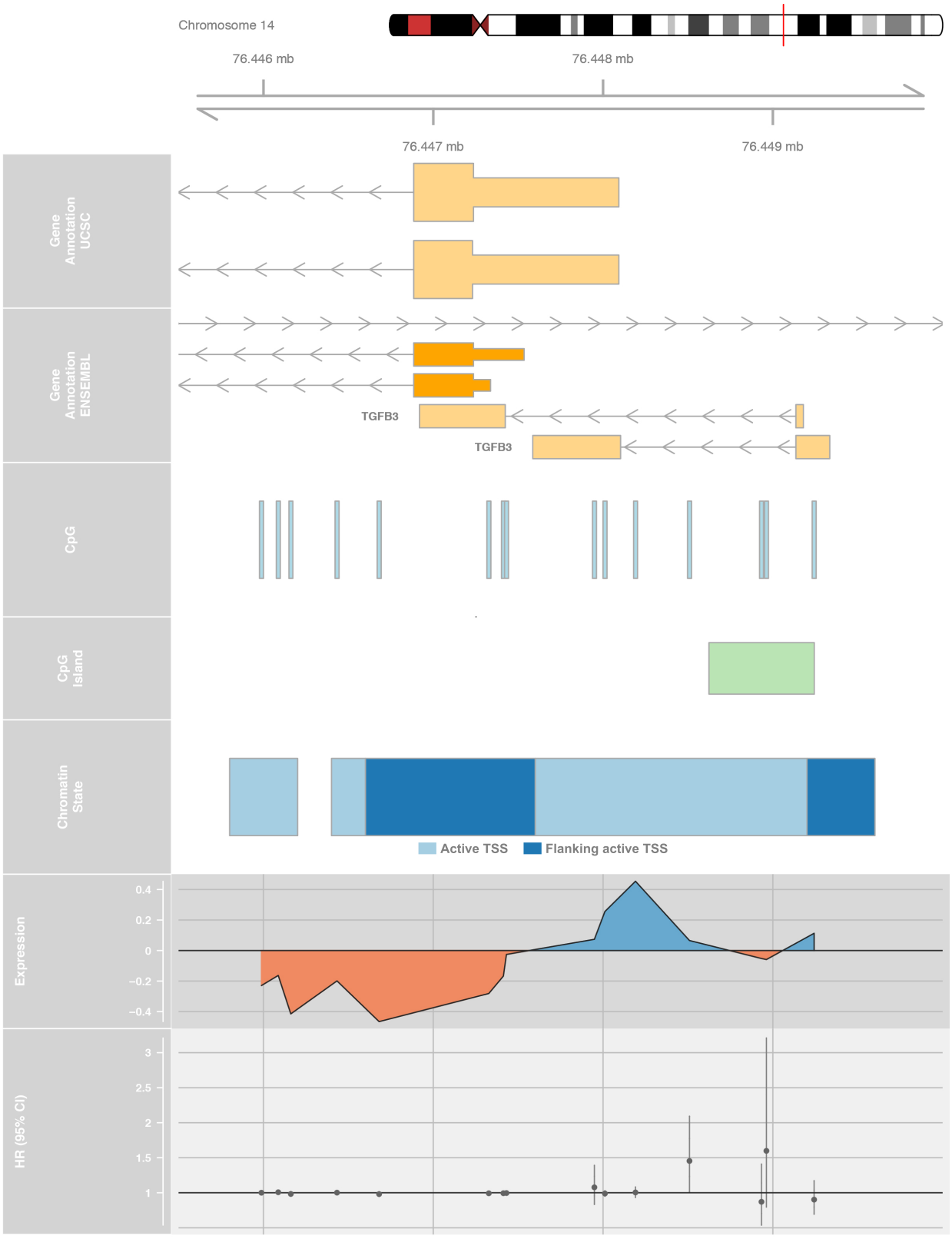


Figure 5-4. Genomic information, Spearman correlation coefficients for expression, and hazard ratios and 95% confidence intervals for selected CpG loci in *IL6*.

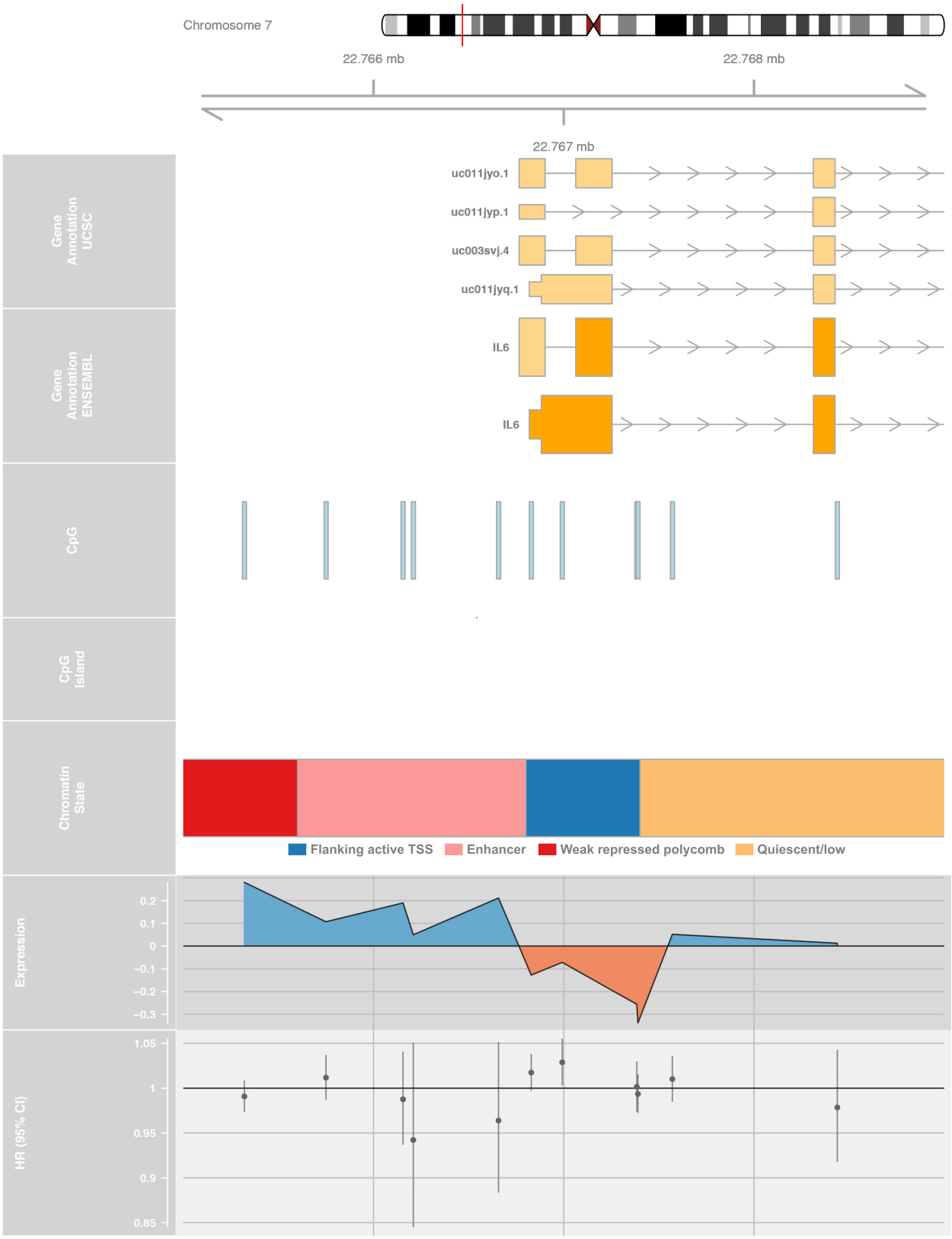
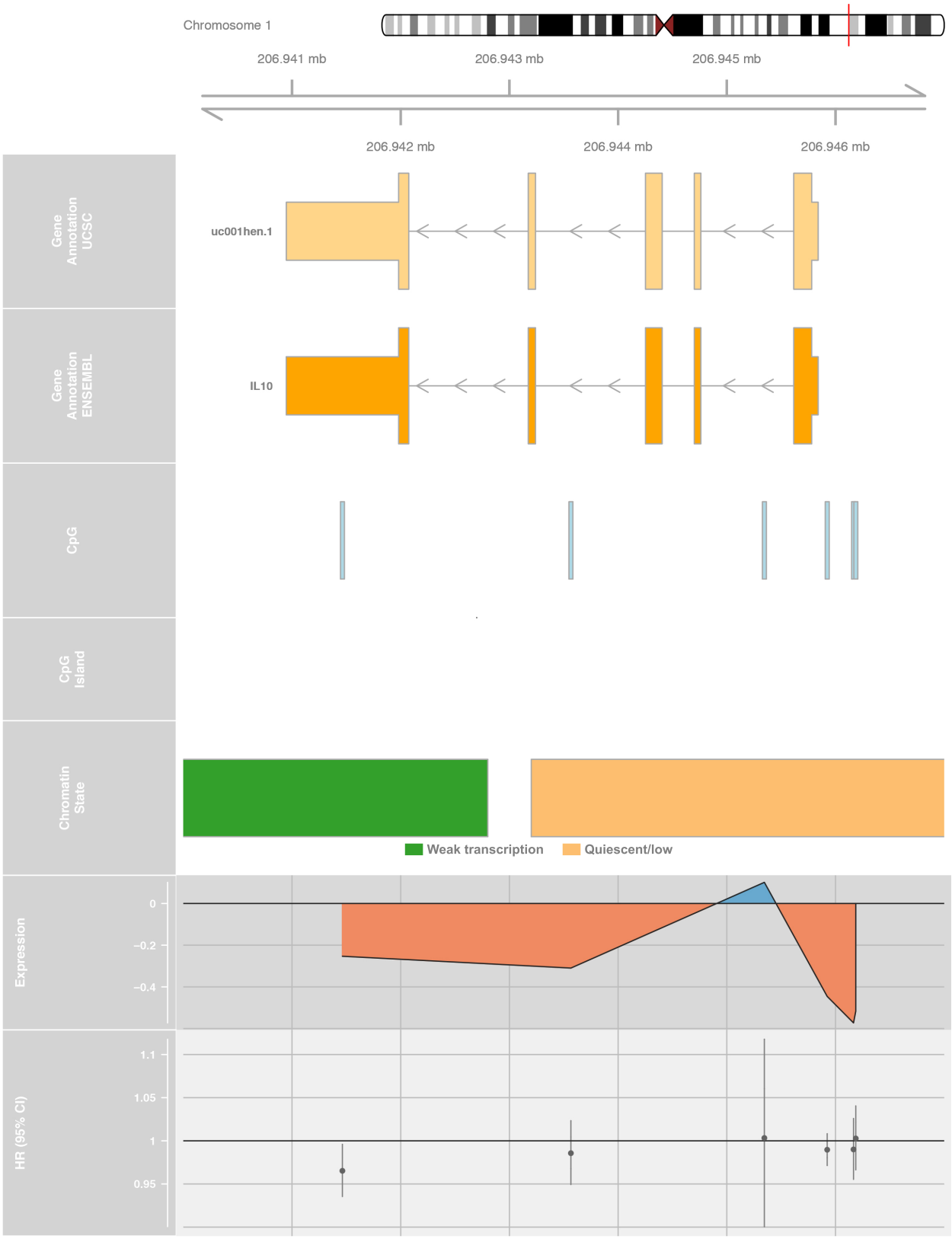


Figure 5-5. Genomic information, Spearman correlation coefficients for expression, and hazard ratios and 95% confidence intervals for CpG loci in *IL10*.



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