Genes, the environment, and depressive symptom scores in the Multi-Ethnic Study of Atherosclerosis

by

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DEDICATION

This dissertation is dedicated to my sense of humor. I hope you're still there when I finish this…

I would also like to dedicate this to the many people who have been incredibly influential in my life:

To my brothers – you have been such inspirations to me for so many different reasons – keep being who you are.

To my parents – I hit the jack pot. You two have been the most supportive, amazing, creative, neurotic, inspirational, intelligent, patient, caring, hilarious, open, incredible parents in existence. I know without a doubt I would not be where I am without you. Thank you.

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"As you navigate through the rest of your life, be open to collaboration. Other people and other people's ideas are often better than your own. Find a group of people who challenge and inspire you, spend a lot of time with them, and it will change your life."

-Amy Poehler

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LIST OF ABBREVIATIONS

ABBREVIATIONS

AA ..... African Americans
bp ..... Base Pair
CARe ..... Candidate-gene Association Resource
CA ..... Chinese Americans
CB ..... Chronic burden
CES-D ..... Center for Epidemiologic Studies –Depression
CEU ..... Population panel for Northern and Western European ancestry
CIDR ..... Center for Inherited Disease Research
Chr/CHR ..... Chromosome
dbGAP ..... Database of Genotypes and Phenotypes
dbSNP ..... Database of Single Nucleotide Polymorphisms
DSM-IV ..... The Diagnostic and Statistical Manual, version IV
E ..... Environment
EA ..... European Americans
G ..... Gene
GEE ..... Generalized estimating equation
GESAT ..... Gene x Environment Set association testing
GWA(S) ..... Genome-Wide Association (Study)
HA ..... Hispanic Americans
HRS ..... Health and Retirement Study
ICC ..... Intraclass Correlation
JPT+CHB ..... Combined population panel of Japanese and Han Chinese ancestry
kb ..... Kilo-base
LME ..... Linear mixed effects
Mb ..... Mega-base
MDD ..... Major Depressive Disorder
MESA ..... Multi-Ethnic Study of Atherosclerosis
NHLBI ..... National Heart, Lung, and Blood Institute
NIS ..... Neighborhood Index Score
PC ..... Principal Component
rGE ..... Gene-environment correlation
SEP ..... Socioeconomic Position
SHARe ..... SNP Health Association Resource
SKAT ..... Sequence Kernel Association Test

SLE ..... Stressful Life Events
SNP ..... Single Nucleotide Polymorphism
SS ..... Social Support
YRI..... Population panel for African ancestry (Yoruba in Ibadan, Nigeria)

ABSTRACT

Genetic factors, stressors operating over the life course, and various aspects of social context have been shown to play a role in the etiology of depressive symptoms. However, empirical studies that investigate environmental and genetic factors, as well as their interactions, remain rare. First, traditional genetic analysis methods were employed to investigate the genetic determinants of depressive symptoms at the single-nucleotide polymorphism (SNP) level, incorporating different approaches to analyzing longitudinal outcomes (baseline measure, measures averaged over exam visits, and a repeated measures); secondly, state-of-the-art genomic region-level analysis methods were utilized to identify genomic regions associated with depressive symptoms; and finally, genomic region by environment (G x E) analysis methods were used to examine of the extent to which individual- and neighborhood-level social exposures modify the genetic effects of depressive symptoms. All analyses were performed both within and across multiple ethnicities (African, European, Hispanic, and Chinese Americans). This work includes evidence that incorporating longitudinal measures (through the averaged or repeated measures approach) results in smaller p-values and an increase in the number of single-nucleotide polymorphisms (SNP) reaching genome-wide suggestive level, as well as both genomic-region determinants of depressive symptom scores and modification of those regions by social environments at both an individual- and neighborhood-level (chronic burden, social support, or neighborhood index score). This dissertation represents an

important contribution to life sciences in several ways: first, this is the first analysis that incorporates novel methods with depressive symptom outcomes; second, through the investigation of the association of genetic variants and depressive symptoms across multiple ethnicities; third, through a detailed comparison of how longitudinal data can be used to define a mental health phenotypes in the context of genetic studies; and finally through the use of both individual- and neighborhood-level interactions with genetic information at both an individual SNP level and a region level. Replicating these initial findings in other studies will help motivate efforts to reduce depressive symptom burden through modifiable environmental factors in individuals with certain genetic profiles.

CHAPTER I

I. Introduction

1.1 Introduction

Genetic factors, stressors operating over the life-course, and various aspects of social context (including neighborhood environments) have been proposed to play a role in the etiology of depression. However, empirical studies investigating the joint effects of these factors as well as their interactions remain rare. Using datasets collected by the Multi-Ethnic Study of Atherosclerosis (MESA), SNP Health Association Resource (SHARe) Project, and the associated MESA Neighborhood Ancillary Study, which includes extensive social and psychosocial assessments, I conducted a novel investigation of the extent to which individual- and neighborhood-level social exposures interact with genetic predispositions to affect levels of depressive symptoms in population-based samples.

1.1.1 Goal

My goal was first to investigate the association of single nucleotide polymorphisms (SNP) with depressive symptom scores in a longitudinal setting to elucidate genetic predictors of depression across four ethnicities (African American, European American, Chinese American, and Hispanic American); second to test whether regions around the most strongly associated SNPs (lowest p-values) from meta-analysis across ethnicity for averaged depressive symptom scores were associated with averaged

depressive symptom scores within and across ethnicity; and third to determine whether any observed genetic effects are modified by individual-level or neighborhood social environments in a large, population-based epidemiologic study with detailed measures of social contexts. A detailed analysis plan can be found in Figure 1. These analyses were undertaken with specific attention to population stratification and gene- and SNP-level inference within a repeated measures frame work. This dissertation has several major strengths, including the availability of detailed social environment measures available in MESA, which have been previously linked to depression in the same sample;[1] the use of novel methods to evaluate gene-level associations;[2, 3] and the use of a large population-based multiethnic sample with multiple waves of data, allowing for longitudinal analyses. This dissertation takes advantage of a unique dataset, and contributes to the intersection of the fields of genetic, social and psychiatric epidemiology.

1.2 Aims

Aim 1: Investigate genetic associations with depressive symptoms scores using genome wide association studies (GWAS) within and across four ethnicities contrasting different methods of incorporating repeated outcome measures. Compare these results to previously published GWAS on depressive symptoms.

Aim 2: To conduct meta-analysis across ethnicities to further define genomic regions and then evaluate these regions' association with depressive symptoms, within and across four ethnicities.

Aim 3: To investigate whether social context (chronic burden, social support, or neighborhood index score) modifies genetic risk of depressive symptom score for those genomic regions identified in Aim 2. If any significant associations are discovered, follow up regional analysis with individual SNP-level analysis for each SNP by environment interaction within the region.

1.3 Background and public health significance

1.3.1 Depression

Depression is one of the most common mental illnesses and is characterized by feelings of guilt, disturbed sleep or appetite, poor concentration, low energy, persistent sadness, and loss of interest among other symptoms.[4] Depression is expected to rank second among the leading contributors of disease burden by 2020, according to the World Health Organization; it is also expected to be the second largest cause of disability after heart disease by the same year.[5] It was estimated that the economic burden of depression was around $83 billion dollars per year as of 2000.[6] Depression – specifically, major depressive disorder (MDD) – is characterized by the presence of the majority of the above symptoms, for a duration of at least two weeks.[7] The prevalence of MDD in adults 18 and over in the United States is higher than any other DSM-IV mental disorder, with a lifetime prevalence of 16.6% and 12-month prevalence of around 6.7%.[8] Rates of subclinical depression vary based on sample definitions, with community sample rates between 8.4 and 9.9% and primary care patient rates of 5 to 16%.[9, 10] Subclinical depression has been associated with both clinical and functional

impairment and increased use of health services, as well as higher degrees of morbidity, poorer social functioning, and poorer quality of life than those without depression.[11-13]

Though MDD is characterized as a dichotomous trait, depression exists on a continuum with different levels of severity and duration. Binary phenotypes of depression are usually based on questionnaire data, such as the CES-D, or even a particular number of endorsed symptoms from the Diagnostic and Statistical Manual (DSM) for mental illness.[7] The CES-D consists of 20 items and includes items on depressed mood, feelings of worthlessness, hopelessness, loneliness, sleep disturbance, loss of appetite, concentration problems and psychomotor retardation (see Appendix 1 for full CES-D inventory). The scale is a sum of the 20 items (items rated from 0 to 3) and ranges from 0 to 60. Internal consistency was initially reported as 0.90 (Cronbach's alpha).[14]

MDD is a diagnostic convention applied to the very extreme end of the depressive continuum[15] and is characterized as a dichotomous measure, differentiating between cases and non-cases.[16-18] Often, a cutoff of 16 is used for the CES-D to distinguish individuals in the "normal" range from those considered to be "probable cases."[19] The CES-D has a sensitivity and specificity for MDD that has been reported anywhere in the range of 64%-90% and 70% - 93% respectively.[16, 20-22] The positive predictive value from the CES-D (the proportion of true cases among those exceeding the cutoff) for MDD is around 30%.[20, 21, 23] The binary nature of these measures, however, gives minimal information about those who do not present with MDD, who often do not report extreme events in the numbers that would lead to a diagnosis of MDD.[24]

While other instruments exist that could provide a more valid assessment of depression (e.g. Composite International Diagnostic Interview,[25] Beck Depression

Inventory[26]), these instruments often require a trained interviewer or clinician to administer. The CES-D can be self-administered and taken in a very short amount of time. Symptoms assessed on the CES-D are among those on which a diagnosis of clinical depression is based.[14]

Depressive symptoms, which are measured on a continuous scale, are thought to have similar patterns of risk factors to MDD, suggesting that MDD and depressive symptoms share a common origin.[27] Due to the hypothesized shared etiology between MDD and depressive symptoms, the paucity of literature on MDD risk factors and the relative lack of established risk factors for depressive symptoms, information from risk factors for MDD was used to inform investigation of depressive symptoms in MESA and HRS – noting that the outcome in this dissertation, CES-D score, represents depressive *symptoms*, not depression.

1.3.2 Depression and environmental exposures

1.3.2.1 Individual-level social exposures

Any environmental, social, or internal stimulus that requires an individual to change his or her usual behavior pattern can be referred to as a "stressor" or "stress."[28] Reactions from these perceived "stressors" can often lead to a state of physiological or emotional arousal that can be considered a "stress reaction." Over time, stressors can accumulate, resulting in the decreased ability of an individual to cope or readjust his or her behaviors. This can lead to depletion of physical or psychological resources, and thus an increase in the chance of psychological distress or even physical illness.[29-32]

Social stress theory often encompasses two broad basic principles: exposure to stressors and vulnerability to stress.[32, 33] Figure 2 presents a heuristic model of stressors included in this dissertation within the framework of existing social stress theory. Many studies have documented associations between social and psychosocial exposures and depression or depressive symptoms at an individual-level. Specific exposures that have been investigated include adult socioeconomic position (SEP), measured using education, income, and wealth (e.g. [34-38]); individual-level stressors, such as chronic burden (e.g. [29, 39, 40]); and social support (e.g. [41, 42]). I investigated chronic burden, which represents exposure to a set of potential stressors. To examine differential exposure to stressors, I included adult SEP measured by a summary index (including education, income, and wealth) in the region-level and interaction analyses. Personal and social resources, particularly social support, can reduce vulnerability to stress, and so social support is also included as a individual-level social environment as a measure of *susceptibility* to stress.

Socioeconomic predictors

Many leading causes of ill health in the United States and other countries are associated with SEP. Often, the least affluent suffer a disproportionate share of disease burden, including depression.[43] Major depressive disorder and greater depressive symptomatology have been found to be more prevalent at lower levels of SEP in several studies.[37, 38]

The Alameda County Study (ACS) has published several articles highlighting the relationship between SEP and disease, including depression.[44-46] The ACS is a community-based longitudinal study of psychological and social factors and their role in

health and well-being in approximately 7,000 adults from Alameda County, California.[47] Data from this study has demonstrated a graded relationship between SEP, measured by education or income, and prevalent and incident depression.

In the accompanying Figure 3, adapted from Everson, et al. 2002,[43] the prevalence of depression, defined as having five or more symptoms based on an 18-item self-report questionnaire, is almost half that in men and women with a high school degree or more (12%) compared to men and women who have not obtained a high school education (21%).[48] Better educated groups have lower prevalence of depression compared with those who have only nine to 11 years of education.

When income is the primary measure of SEP, the same pattern can be seen, with 11% of higher income respondents experiencing depressive symptoms compared with 19% of lower income respondents.[48] Evidence from earlier studies using the ACS data has also shown relationships between SEP and incident depression. Participants with less than nine years of education who were not depressed at the start of the study were nearly twice as likely to become depressed over the subsequent nine years of follow-up relative to those with a high school education or more (odds ratio (OR) = 1.86, 95%CI = 1.36-2.55).[49]

Many other studies have shown pronounced socioeconomic gradients in depression. For example, Figure 4 compares findings from four different studies highlighting the relationship between SEP and tertiles of depressive symptoms (adapted from Everson, et al 2002[43]). This figure shows the proportion of respondents within each education tertile (low, middle, or high, defined in each study by number of years of education) who reported a high number of depressive symptoms. These studies are

composed of exceedingly diverse participant bases and so the demonstrated gradients are particularly remarkable because they demonstrate consistency across the four unique study populations (Consumers Survey, Detroit Study, Alameda Study, and the Kuopio Study).[43] The Consumers' Survey included 1,423 randomly sampled men and women from the contiguous 48 states using random-digit-dialing.[50] This sample is weighted to be representative of the US population by age, race/ethnicity and gender. African Americans were oversampled in the Detroit study, which was conducted in 1995 and included 1,139 participants from a three-county region surrounding Detroit, Michigan.[51] The final sample included approximately equal numbers of Europeans and African Americans, and was collected to explore attitudes, psychosocial characteristics, behaviors, and health.

The ACS study was intended to represent the demographic makeup of Alameda County, California, in 1965, when the study commenced.[47] The study population includes 78.9% Europeans, 12.4% African Americans, and 3.9% Hispanics (with 4.8% categorized as "other" racial/ethnic groups or not identifying with a single racial/ethnic category) aged 17 – 94 years in 1965. The Kuopio Ischemic Heart Disease Risk Factor Study included 2,682 men between ages 42 and 60 when the study began (between 1984 and 1989) from the Kuopio region of eastern Finland. All participants in this study are European.[52]

Despite such a diverse range of study populations, the socioeconomic gradient persists. I incorporated the theory generated from this broad literature by using data from MESA to investigate individual SEP and its relation, and possible modification by

genes/gene-regions, to depressive symptoms using education, income, wealth, and childhood SEP measures.

Stressors

According to social stress theory, low SEP increases the risk of depression, in part, by increasing exposure to chronic and acute stressors. I focused on one measure of chronic stress: chronic burden. A majority of research supporting relationships between stress and depressive episodes has been based on episodic stressors, or discrete events having a beginning and an end, that have negative or undesirable content (e.g.[29, 53, 54]). On the other hand, chronic stress has been inconsistently defined and comparatively less studied.

Early studies investigating chronic stress and depression defined chronic stress as "ongoing difficulties lasting at least four weeks" and found that depressed subjects were more likely to have experienced either an ongoing difficulty or at least one severe life event prior to the onset of depression.[29, 40] These earlier studies often did not differentiate between the effects of chronic and acute stress.[55] Other research has reported that continuing adverse conditions, such as poverty, medical disabilities, and lasting marital discord are associated with risk of depression.[29, 56-58] Researchers have also found an association with depression when chronic stress is defined as the continued absence of social support (e.g.,[59]).

The development of a chronic stress profile covering domains such as intimate relationships, close friendships, family relations, finances, and the health of self and family members in the past six months found that chronic stress increased depression in adult patients[60] and in youth already at risk for depression.[61] There is some evidence

to suggest that chronic stress (defined as stress ongoing for more than 12 months) is a stronger predictor of depressive symptoms than acute stressors.[62] Chronic stress is an important area in need of further study, with implications in the association between individual stressors and depression. In this dissertation, I utilized an index of chronic burden as a proxy for individual-level social environment stressors.

Stress buffers

Low SEP may also increase the risk of depression by decreasing access to stress-buffering resources, such as self-esteem and social support.[32] Much research on social support and its association with depression was published in the 1970s and 1980s. Though the meaning, nature, and measurement of social support have been debated in the literature, there has been movement to identify several distinct types of components of support.[63] Some of these components include structural aspects of relationships (e.g., living alone), frequency of social contact, participation in social activities, and involvement in social networks. In a conceptual analysis, the four most frequently used defining attributes of social support included emotional, instrumental, informational, and appraisal. Social networks, social embeddedness, and social climate were all identified as antecedents of social support.[64, 65]

One of the most robust relationships between social support and depressive symptoms in late life has been found with perceived emotional support.[41] A community study in Hong Kong found that impaired social support, (including network size, network composition, social contact frequency, satisfaction with social support, and instrumental-emotional support) and depressive symptoms were associated in bivariate analysis.[42] Various aspects of social support have also been associated with depression

or depressive symptoms in longitudinal studies. In a community-based study of Australians, poor social support was predictive of the number of depressive symptoms at follow-up, three to six years after baseline measurements.[66] Additionally, insufficient social networks predicted the incidence of major depression in a sample of 875 non-depressed elderly people over a three-year follow-up.[67] Measured social support indices within MESA are used as a measure of vulnerability to stress in this dissertation. Both the chronic burden and social support constructs are consistent with aspects of proximal determinants of depression using the social stress model.[32]

1.3.2.2 Neighborhood-level social exposures

It has been postulated that neighborhood contextual characteristics may be related to mental health outcome over and above the effects of individual characteristics. A large number of studies have documented associations of various features of neighborhood environments with depression or depressive symptoms (reviewed in [68, 69]). The neighborhood constructs investigated have included general measures of neighborhood SEP, as well as more specific measures of chronic stressors (including violence, disorder, and aesthetic quality) and measures of neighborhood social cohesion/social support. Mair et al, 2008, evaluated 45 observational studies published between January 1990 and August 2007.[70] Studies included sample sizes ranging from 117 to 56,428, adult populations, children or teenagers, the elderly, mixed ethnic/racial groups, African-American-only, and Mexican-American-only studies. These studies also included metropolitan or urban areas, non-urban areas, different depression measures, different study designs, and differing definitions of neighborhood. Review of these studies showed an overwhelming majority (37 of the 45 studies) found support of an association between

neighborhood characteristics and depression or depressive symptoms after controlling for various individual-level characteristics. Common covariates in these studies included age, race/ethnicity, gender, marital status, education, and income.[1, 70] A more recent study using the MESA data, not included in the review by Mair et al, 2008, found that key features of the environment including lower levels of social cohesion were significantly associated with depressive symptoms.[1, 68] Here, I describe the evidence for including neighborhood predictors in the context of depression and depressive symptoms in the context of social stress theory.

Potential neighborhood stressors may include residential stability, deprivation, violence, disorder, and aesthetic quality. Depressive symptoms were found to be associated with residential stability (β (SE) = 0.72 (0.27)) after controlling for individual-level characteristics in 3,442 elderly individuals living in urban areas of the U.S.A. where census tracts are defined as the neighborhood.[71] Depressive symptoms were also associated with neighborhood-level measures of deprivation (OR for highest vs. lowest fifth 2.4 (1.28 to 4.48)), lack of social support (OR = 2.51 (1.75 to 3.61)) and self-reported stress (OR = 10.42 (6.29 to 17.28)) in a model adjusting for all these characteristics plus social capital, receiving means-tested benefits, and having three or more kids 5 years of age or younger. This study was conducted in 2005, and investigated 846 mothers of young children living in deprived areas of Nottingham in the U.K., using British enumeration districts.[72] A study assessing neighborhood problems (too much traffic, excessive noise, trash and litter, smells, smoke) and using participant-defined neighborhoods reported that subjects in the top quartile of neighborhood problems were more likely to have depressive symptoms than the bottom quartile, after adjustment (OR

= 4.8 (2.4 to 9.5)), in adults living with asthma in northern California.[73] Another study found that being environmentally dissatisfied and living in neighborhoods with transportation problems were associated with increased levels of depressive symptoms. This study sampled 725 adults aged 55 and older from four metropolitan counties in Alabama and used the census tract as the definition of neighborhood.[74]

Though several studies found associations between neighborhood stressors and depression, some did not. Using census tract-defined neighborhoods, investigators conducting a study of 2,109 non-institutionalized people ages 65 and older in New Haven, Connecticut, found no association between racial/ethnic heterogeneity, residential stability, service density (services promoting social engagement, providing care, and undesirable amenities), and depressive symptoms.[75] Another study included in the review found no evidence for an association between neighborhood measures and depressive symptoms. This study involved 2,998 adults ages 65 and older in North Carolina and investigated racial/ethnic heterogeneity, residential stability, and neighborhood age structure. None of the factors was significantly associated with depressive symptoms conditional on census tract random effects, either before or after adjustment for individual characteristics.[76]

Certain aspects of neighborhoods can be thought of as buffering the effects of exposures on depression, such as social cohesion. An association between neighborhood cohesiveness and higher levels of depressive symptoms in adolescent and early adult females was reported. In the same study, neighborhood cohesiveness for adolescent males was noted. This study included 372 youth from the first and second waves of the Philadelphia family management study used a participant-defined neighborhood.[77] A

strong, prospective association between negative perceived neighborhood characteristics and subsequent depressive symptoms, after adjusting for baseline depression ($\beta = 0.28$, $p < 0.01$), was found in a sample of residents of high-drug-use areas in Baltimore, Maryland, using participant defined neighborhoods.[78, 79]

Another study found that having limited social supports within a neighborhood was associated with increased levels of depressive symptoms. This study sampled 725 adults aged 55 and older from four metropolitan counties in Alabama and used the census tract as the definition of neighborhood.[74] In another study, adolescents' perception of ambient hazards (β (SE) 0.022 (0.008)) and negative social cohesion (β (SE) -0.122 (0.032)) were both associated with depression symptoms in 877 adolescents from Los Angeles County using clustered census tracts as the neighborhood.[80] Simons et al, 2002, reported an association between community ethnic identification ($\beta = -0.392$, p-value = 0.04) and prevalence of discrimination ($\beta = 0.313$, p-value 0.04) with depressive symptoms, after controlling for individual- and community-level characteristics in a sample of 876 African-American children ages 10 to 12 in Georgia and Iowa.[81]

Incident depression/depressive symptoms, which can provide much stronger evidence of a causal effect of neighborhood on depression, has been assessed in several studies (reviewed in [70]). These studies defined incident depression as all subjects who did not have depression or presented with symptoms less than a certain cutoff at baseline, but who had depression or were above the cut-off at follow-up. Incident depression was associated with socioeconomic status[82], living in an impoverished area[83], and neighborhood disadvantage[84] in samples of New York residents, Alameda and Oakland County California residents, and African American women, respectively.

Though many studies found associations between social support and social cohesion, some studies failed to replicate these findings. Simons et al, 2002, found no association between community cohesion and depressive symptoms after controlling for individual- and community-level characteristics in a sample of 876 African-American children ages 10 to 12 in Georgia and Iowa. Neighborhoods were defined as "community groups," which were made up of census block group areas from cluster analysis.[81] Despite this finding of no association, much of the evidence in the literature supports some association between neighborhood social cohesion and depression.

Using the literature and available measures of neighborhood characteristics, I have included neighborhood safety, aesthetic quality and social cohesion as neighborhood dimensions included in the neighborhood index score. These measures stem were created with validated scales from an ancillary study of non-MESA participants residing in the same neighborhood as members of the MESA cohort. In using these measures and by aggregating responses across several respondents, I have reduced variability from individual subjectivity and measurement error and also circumvented same-source bias.[85] More details are presented in the individual sections.

1.3.3 Genetic mechanisms of depression

Genetic effects

The heritability (proportion of variation attributable to genetic factors) of depression as assessed by twin studies is estimated at around 30 to 50%.[86] Despite evidence for heritability of depression, the identification of vulnerability genes has not been as successful as it has been for many other complex disorders, such as obesity. Due to its complex nature, a number of genes are likely to be involved in the pathophysiology

of depression. Many genetic factors have been investigated, including candidate genes and SNPs, but little to none has been replicated to any acceptable level.

Interaction effects

Several different theories have been invoked in the analysis of mental health genes and the environment, including (but not limited to) gene-environment correlation (rGE)[87], Diathesis-Stress model (i.e. vulnerability genes)[88], and Differential Susceptibility (i.e. plasticity genes)[89, 90]. rGEs are different from G x E interactions in that rGE explains why individuals with certain genetic predispositions to exhibit sensation-seeking behaviors affiliate with individuals who are more likely demonstrate the same behaviors[91], while G x E would explain why individuals with a certain environmental exposure would lead to a specific phenotype only in individuals with a particular genotype.

Gene-environment correlation is broken down into three categories: passive gene-environment correlation (referring to the association between the genotype a person inherits from their parents and the environment in which that person is raised), evocative gene-environment correlation (also known as "reactive", referring to the association of an individual's genetically predisposed behaviors and other people's reactions to those behaviors), and active gene-environment correlation (also known as "selective", referring to the relationship between an individual's genetically influenced behaviors and the environmental exposures that an individual selects)[87, 92].

The prevailing framework has been that of the Diathesis-Stress model.[88] This view encompasses the idea that some individuals, due to a vulnerability – perhaps a

behavioral/temperamental or genetic – are disproportionately affected negatively by a particular environmental stressor leading to the manifestation of a psychopathological condition, such as depression.[93] Previously depression literature consistent with this theory has included environments of child maltreatment, negative life events and even parental discipline (e.g. [94-96]) and their interaction with genetic profiles.

Differential Susceptibility is a more recent framework of G x E interactions focusing on the idea that not only are some individuals more susceptible to negative environmental exposures, but those very same individuals may respond more positively to environmental support and enrichment, included the absence of a negative environment. Much literature to date has not explicitly investigated genes and environments in the context of this model – mostly by focusing only on negative environments and failing to measure the positive (excepting the absence of adversity) and investigating only a small range of psychological and behavioral outcomes, again focusing on negative psychopathologies.[93] However, there is a paucity of literature that supports this hypothesis, even if not explicit in the conclusions of the authors (e.g. .*MAOA*—Physical abuse—Mental health problems,[97] *5-HTTLPR*—stressful life events –depression symptoms[98]). Belsky and Pluess (2009)[93] outline several evidentiary criteria for determining differential susceptibility; (1) applying a conventional statistical criteria for evaluating an interaction, where interactions where regression lines do not cross are excluded, (2) evaluating the association between the environment and outcome (if they are related, then Diathesis Stress models are suggested), and (3) demonstrating differential susceptibility when there is a crossover interaction and the slope for the

susceptible subgroup is both significantly different than zero and significantly steeper that the non- (or less) susceptible subgroup.

These models are not necessarily mutually exclusive and evidence of one or more may be present in a study.[99] These different models may suggest that there is a profound influence of genes and the environment on epidemiologic and genetic parameters, including increased susceptibility to negative environments for certain genotypes. This can enhance our understanding of the pathways of risk leading to the occurrence of depressive symptoms in the general population. In the context of public health and in particular for depressive illness, risk-prevention efforts have tended to focus on behavior modification. Recognizing that risk for depression by be driven by genetic factors and modified by environments presents a complex paradigm for designing and testing intervention strategies for the future.

This dissertation exploits these theories by including environments that assess not only the negative end of an environmental spectrum, but also the positive end – and not only in terms of the absence of adversity.

1.3.3.1 Individual-level social and genetic predictors of depression

In recent years, there has been an upsurge of research literature showing the etiology of different types of psychopathology to be linked to both genetic and environmental factors working together in complex ways.[100-102] Though researchers have long known that both genetic and environmental risk factors independently contribute to the development of psychopathology, only recently has attention been focused on exploring how genes and environmental factors work in concert.[103, 104] Figure 5, adapted from Caspi and Moffitt 2006,[105] shows several approaches to

psychiatric genetics research (a) by showing a direct linear relationship between a gene and a disorder, (b) by showing the relationship between genes and disorders through an endophenotype, which is a heritable neurophysiological, biochemical, endocrinological, neuroanatomical, or neruopsycholocical antecedent of a disorder,[106] and (c) finally by showing a gene-environment interaction approach. True genetic and environmental effects can be obfuscated when gene-environment interactions are ignored, which can lead to false negative results and can be an explanation for inconsistent findings in the literature.[107]

G x E in psychiatric genetics have been reported for disorders such as attention deficit/hyperactivity disorder (ADHS), schizophrenia, substance use disorders, and depression (reviewed in [108]). Individual-level environmental outcomes include stressful life events, childhood maltreatment, institutional deprivation, stress, acute injury.[108] The most prominent and widely cited example of an individual-level G x E interaction in depression is that of Caspi et al, 2003.[95] This study used stressful life events and genetic variation in the promoter region of the serotonin transporter gene (*5-HTTLPR*) as the environment and genetic factors, respectively. Findings implicated a polymorphism (short allele) in the promoter region of the serotonin transporter gene, which predicted depression in interaction with major stressors (stressful life events (SLE)). This was the first study to identify a specific genetic locus ass ociated with depressive reactions to stressful life events.[95, 104]

After the initial findings of Caspi et al, 2003[95] a number of studies were undertaken to attempt to replicate the findings. Two meta-analyses of the *5-HTTLPR* x environment interactions with stress were conducted in 2007 and 2009 [109, 110]

concluding that there was no evidence of overall interaction between this variant and environments studied. However, these meta-analyses were limited to a selection of the literature due to strategical decisions about inclusion/exclusion criteria and methodological constraints. Inconsistencies in the findings from individual studies could be due to several reasons such as different definitions of outcomes, different study designs (longitudinal, cross-sectional , case-control, etc.), and varied exposure measurements. A more recent meta-analysis has included more current research (including studies investigated in the two previous reviews) and found strong evidence that the *5-HTTLPR* locus moderates the relationship between stress and depression (meta-analysis $p = 0.00002$).[111] This meta-analysis also stratified results by two integral sources of variation in individual studies: stress assessment methods (questionnaires, objective, and interviews) and stressor types (childhood maltreatment, stressful life events, and specific medical conditions). This meta-analysis concluded that studies with very defined stressors (childhood maltreatment and medical conditions) were more likely to find significant gene by environment interaction effects compared to those studies with more generally defined stressors – particularly those using interview or objective assessment measures.[108] Several other genetic regions (*COMT*, *TPH*, and *5-HTR2a*) have be identified through biological pathways (such as the hypothalamic-pituitary adrenal axis) as being potential regions of interest in the development of depression, though currently polymorphisms in these regions have failed to show interactions with SLEs.[112] Only one GWAS analysis of MDD has found a genome-wide significant hit [113], whereas a mega-analysis of all GWAS studies of MDD [114] has not found a single genome-wide significant hit, despite the fact that sample sizes were large enough

to expect several genome-wide hits if MDD behaved similar to diabetes or schizophrenia[115]. Hence, depression risk may need a different approach. This dissertation builds on previous literature by using questionnaires to evaluate interactions between well-defined individual-level stressors (chronic burden and social support) and genetic regions with depressive symptoms.

1.3.3.2 Neighborhood-level social and genetic predictors of depression

Most G x E studies have focused on individual traits or characteristics as the "E," or environmental, factor in the interaction. Very few studies have investigated neighborhood-level, or distal environments (particularly measures of social environment) when it comes to G x E studies. Despite the lack of literature on G x "*neighborhood E"* studies, many health-related outcomes have been associated with neighborhood-level environments. Existing literature remains limited in the range of contextual factors that have been considered in G x *"neighborhood E"* studies of depression.

Of note are differences between the use of objective and subjective measures of neighborhood-level social environments. A recent meta-analysis compares studies with objective versus subjective measures of the environment and concludes there is a systematic relationship between method of environmental assessment and the results of G x E studies with the length variant of the serotonin transporter.[109] This meta-analysis further states that all studies involving objective measures to assess stress replicated the G x E interactions either fully or in part, whereas non-replications relied on self-report measures.[109]

G x "*neighborhood-E"* interaction studies are exceedingly rare in depression, and non-existent in depressive symptoms. Interactions between the *5-HTTLPR* polymorphism

and social environment were detected in adolescent boys based on their residence in public versus privately owned house, with no significant interaction findings in adolescent females, despite other variables (e.g., traumatic conflicts in the family) showing significant G x E interactions exclusively in females.[116] Uddin et al, 2010, noted that the *5-HTTLPR* "sl" genotype conferred protection against depressive symptoms in adolescent females, independent of county-level social context (measured by county-level proportion of households receiving public assistance), though in adolescent males, the same genotype only conferred protection against depressive symptoms within the context of county-level deprivation.[117]

There is a large gap in the G x E literature in which studies of interactions between whole genes or gene regions (not individual loci, as have been investigated previously) and individual- or neighborhood-level social environment could greatly advance the field. MESA provides us the opportunity to use objective measures of neighborhood social environment to obtain a more robust measure of environment. In addition, new statistical methods allow us to investigate whole gene/gene-region interactions in the context of G x E interactions, both at the individual- and neighborhood-levels.[2, 3]

Figure 1 Analysis plan for dissertation

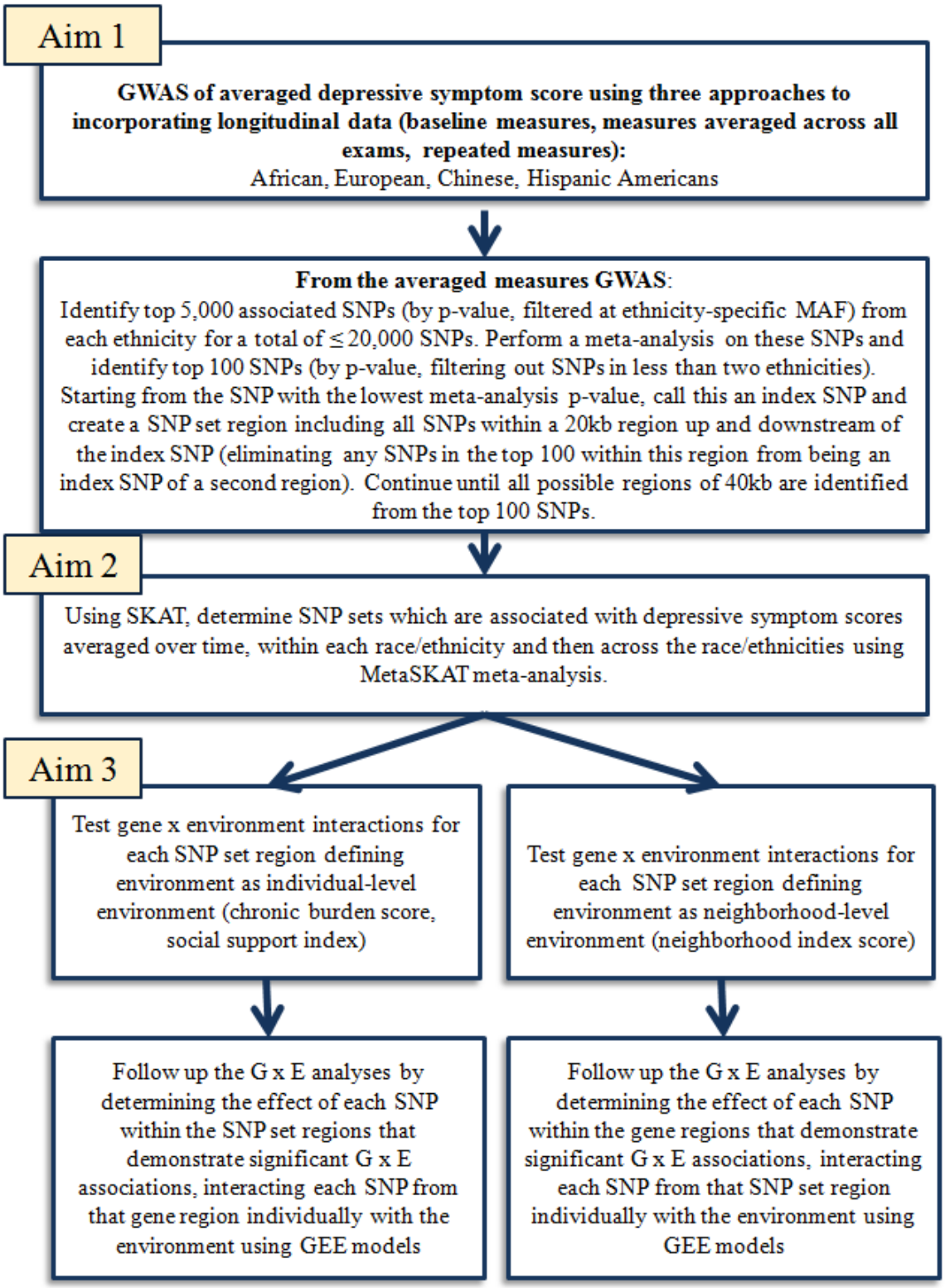


Figure 2 Heuristic model of social stress theory

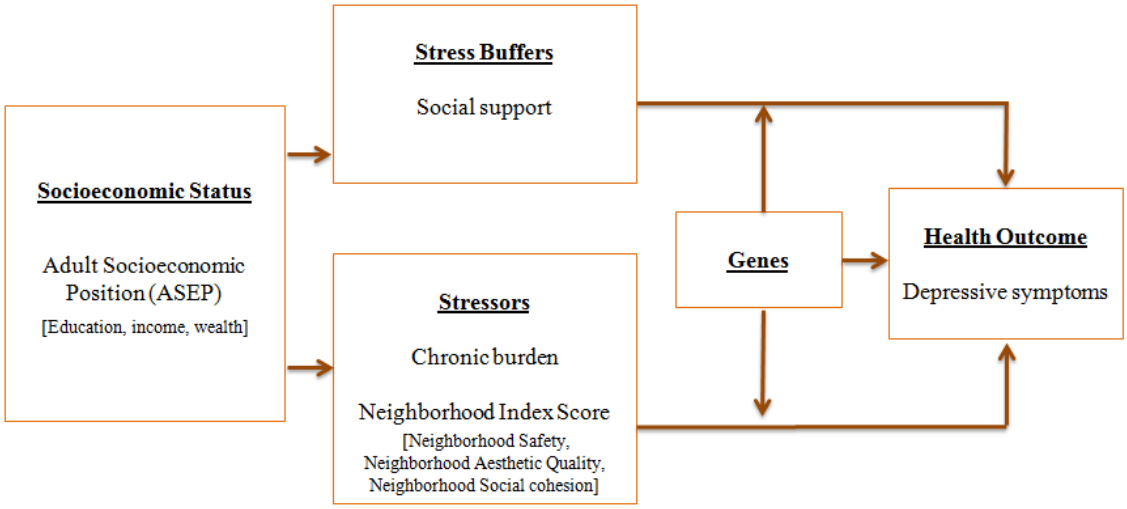


Figure 3 Prevalence of depression by levels of education and income: Alameda County Study, 1965 adapted from Everson, et al 2002.[3]

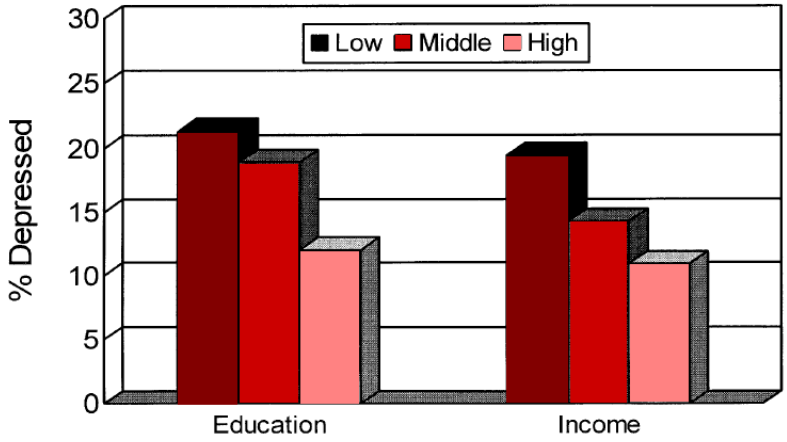
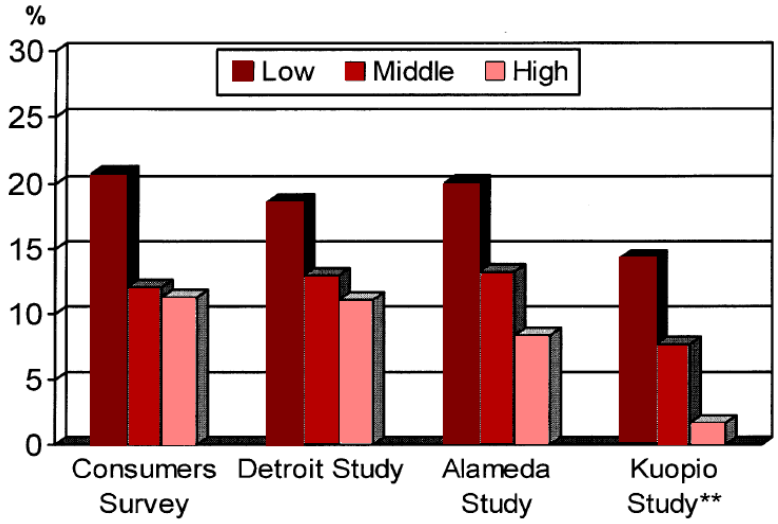
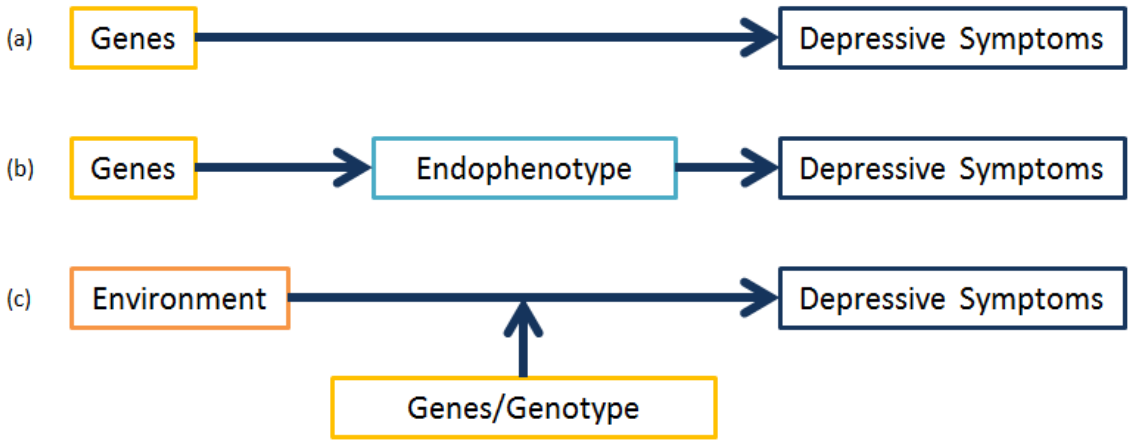


Figure 4 Prevalence of depressive symptoms by education in four epidemiological studies. Education categories defined within each study



Data from the Kuopio Study represent hopelessness rather than overall depressive symptoms. Adapted from Everson, et al 2002.[3]

Figure 5 Approaches to psychiatric genetics research.



a | The gene-to-disorder approach assumes direct linear relations between genes and disorder. b| The endophenotype approach replaces the disorder outcomes with intermediate phenotypes. c | The gene–environment interaction approach assumes that genes moderate the effect of environmental pathogens on disorder.[105]

CHAPTER II

II. Study population and descriptive statistics

2.1 The Multi-Ethnic Study of Atherosclerosis

The Multi-Ethnic Study of Atherosclerosis [118] is a longitudinal study supported by the National Heart, Lung, and Blood Institute (NHLBI) designed to identify risk factors for subclinical atherosclerosis. The MESA cohort was recruited in 2000-2002 from six Field Centers: Baltimore MD (Johns Hopkins University Field Center); Chicago, IL (Northwestern Field Center); Forsyth County, NC (Bowman Gray Field Center); Los Angeles, CA (UCLA Field Center); New York, NY (Columbia Field Center); and St. Paul, MN (University of Minnesota Field Center). Participants were 45-84 years of age and free of clinical cardiovascular disease at baseline. At each site a probability sample of approximately 1100 participants was selected at each site to represent specific populations. At baseline the MESA cohort included 6814 men and women, with 38.5% non-Hispanic whites, 27.8% non-Hispanic African-Americans, 22% Hispanics, and 11.8% Chinese. Participants attended a baseline examination (2000-2002) and three additional follow-up examinations approximately 18-24 months apart. At each clinic visit, participants completed a series of demographic, personal history, medical history, access to care, behavioral, and psychosocial questionnaires in English, Spanish, or Chinese. These visits included three measured of depressive symptoms using the Center for Epidemiologic Studies Depression (CES-D) scale. Overall, cohort follow-up has been excellent. The sample at each exam and the response rates (of participants alive)

were: exam 1 (*n*=6,814), exam 2 (*n*=6,239, 92%), exam 3 (*n*=5,946, 89%), exam 4 (*n*=5,704, 87%).

Since MESA's primary hypotheses relate to determinants of subclinical cardiovascular disease, certain exclusion criteria were applied (Table 1). Institutional Review Board approval was obtained at each of the six MESA centers where participants were seen for clinical exams.

2.1.1 Depressive symptoms

MESA uses an instrument widely accepted for assessment of depressive symptoms in the general population, the CES-D scale.[14] The CES-D is advantageous in such a large sample due to the quickness with which it can be completed (5-10 minutes) and that it has been translated and validated into several languages. Depressive symptoms were measured in MESA participants at baseline and at two follow-up visits using the 20-Item CES-D Scale.[14] Higher CES-D scores represent more/more severe depressive symptoms. The first follow-up visit including an assessment of the CES-D (exam 3) was 3-4 years after baseline, and the second 4-5 years after baseline, at exam 4.

2.1.2 Anti-depressant adjustment

Rather than removing individuals taking anti-depressant medication and losing valuable genetic information, CES-D scores were adjusted for treatment effect using a similar algorithm used for adjustment of blood pressure for persons taking anti-hypertensive medications.[119] Since response to anti-depressant medication is highly variable and information on compliance to medication is not always available in population-based studies, there were two assumptions made when adjusting CES-D

scores. First, CES-D scores of anti-depressant users are right-censored. That is, the CES-D score while *on* anti-depressant medication is lower than the score while not taking anti-depressant medication. Second, participants with low depressive symptoms scores respond less to anti-depressant medication than persons with high depressive symptom scores, on average.

The algorithm for adjustment of anti-depressant use was run separately for multiple factors: gender, race, and exam period, on a total of 6,438 individuals. The nonparametric imputation algorithm replaces the CES-D score of a person using anti-depressants with the mean depressive symptom score for all persons taking anti-depressants with the same or higher depressive symptom score. This method has recently been used in a large depressive symptom GWAS consortium.[120] Anti-depressant use was defined at each exam by self-reported monoamine oxidase inhibitor (i.e. isocarboxazid, phenylzine, tranylcypromine), tricyclic anti-depressant (i.e. amitriptyline, doxepin, nortriptyline) and/or non-tricyclic anti-depressant (i.e. citalopram, escitalopram, fluoxetine, sertraline) use coded as yes/no. Descriptive statistics on depressive symptom scores and anti-depressant use for each ethnicity for participants not on anti-depressant and also for those on anti-depressant medication both before imputation and after imputation are available in Table 2. Since those who had missing information on anti-depressant use were not significantly different on exam-specific mean CES-D scores than those who did not take anti-depressants (exam 1: p-value = 0.5955, exam 3: p-value = 0.1476, exam 4: p-value = 0.1103), individuals with missing information on anti-depressants were classified as "0 – not taking anti-depressants" for imputation purposes. This allowed the increase of sample sizes for each exam and thus an increase in statistical

power. Missing information on anti-depressant use was observed on two participants from exam 1, 91 participants from exam 3, and 167 participants from exam 4. The distribution (histogram and box-plots) of CES-D for each exam, for those missing anti-depressant use compared to those with no anti-depressant is shown in Figure 6-Figure 9. The distribution of CES-D in this sample is skewed right, with the majority of values being less than 10. The distributions of CES-D scores are broken down by race and phenotype (baseline, averaged, repeat measure) as well as by log-transformation status. The (CES-D scores + 1) were log transformed to improve consistency with linear regression assumptions after anti-depressant imputation for use in MESA.

2.1.3 Genetic data

All genotype collection and laboratory analyses were done by MESA. MESA provides genotypes to the SNP Health Association Resource (SHARe) Project. DNA isolated from whole blood or packed cells are frozen at -70^{o}C. The DNA extraction and purification method uses sodium dodecylsulfate cell lysis followed by a salt precipitation method for protein removal using commercial Puregene® reagents (formerly Gentra Systems, Inc., Minneapolis, MN 55447; currently Qiagen Instrument Service, Germantown, MD 20874). DNA is quantitated using the NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Quantitation by Picogreen analysis (Molecular Probes, Eugene, OR) is also available. A mean yield of 200 µg or 40 µg DNA/mL packed cell is obtained, and DNA is of high quality (mean purity A260/280=1.77) and high molecular weight as determined by gel electrophoresis.

Approximately one million SNPs were typed using the Affymetrix Genome-Wide Human SNP Array 6.0. For quality control, SNPs were filtered for SNP level call rate

<95% and individual level call rate <95%, and monomorphic SNPs were removed. The IMPUTE 2.1.0 program was used in conjunction with HapMap Phase I and II CEU+YRI+CHB+JPT as the reference panel (release #22 - NCBI Build 36 (dbSNP b126)) for African American, Chinese and Hispanic participants, and HapMap Phase I and II - CEU as the reference panel (release #24 - NCBI Build 36 (dbSNP b126)) for Europeans to increase the number of available SNPs to approximately 2.5 million markers per each ethnicity. Genotyping was completed by Affymetrix in September 2009 and released on dbGaP in February 2009.

To account for population substructure, ethnic-specific principal components were taken from the genome-wide data. Principal components (PC) were computed through the MESA SHARe project by the Wake Forest analysis team. The PCs were computed on 8,227 individuals who self-reported ethnicity: 2,590 African Americans, 2,174 Hispanics Americans, 2,686 European Americans, and 777 Chinese Americans. PCs were computed separately in each self-reported ethnic group. The Wake Forest analysis team excluded the 23,428 SNPs that were already flagged in the data that was downloaded from dbGaP. They also removed 6,849 SNPs in genomic regions that have been shown to harbor long range linkage disequilibrium (LD), as these regions have been shown to influence the choice of PCs. Each ethnicity was adjusted for population substructure through the top four ethnic-specific PCs as proposed previously in MESA and elsewhere.[121, 122]

For the baseline and averaged phenotype genetic information, genotype probabilities were converted to dosages within the PLINK platform using command-line options to specify the probabilities.[123, 124] For the generalized-estimating equation

repeated measures analyses, probabilities were converted to dosages using R software.[125] Dosages were used for the GWAS analyses in Aim 1. Since the PLINK package models the minor allele (which is not always the coded allele), and the GEE models the coded allele, the sign of the beta estimates for the results from the GEE models have been reversed if required (+ to –, or – to +) to allow for consistency across models.

2.1.3 Environmental data

2.1.3.1 Individual-level social environment

Two dimensions of individual-level social environment, chronic burden (CB) and social support (SS), were investigated in this dissertation. The CB scale was available at exam one and exam three.[118, 126] The summed "yes" responses for the CB scale were calculated for the following questions: 'have you experienced ongoing health problems (self) greater than six months'; 'has someone close to you experienced ongoing health problems greater than six months'; 'have you experienced ongoing job difficulties greater than six months'; 'have you experienced ongoing financial strain for greater than six months'; and 'have you experienced ongoing relationship problems greater than six months'. If an individual's response to any of the five aspects of the individual exam's CB was missing, the exam-specific CB score was set to missing.

To capture the longitudinal aspect of CB, the scores from exams one and three were averaged for each individual. If an individual was missing either CB value, then the averaged CB was calculated from the existing measure. To increase the sample size, the average CB score was set to missing only if the measure was not calculated on both exams. The distribution of averaged CB for the overall sample and for each race

specifically (African, European, Chinese, and Hispanic Americans) is shown in Table 3(a). For analyses, CB was mean centered to aid in interpretability. Higher values of CB score indicate a higher chronic burden (i.e. *more* burdens). The distribution of chronic burden, averaged over exams one and three is shown in Figure 10.

The emotional social support scale in MESA is composed of six questions on a five-point Likert scale (1 = "none of the time" to 5 = "all of the time"), and measured at exam one and exam three.[118, 127] The questions measured SS by asking whether 'someone available to listen to you'; 'someone available to give you advice'; 'someone available to show you love and affection'; 'someone available to help with daily chores'; 'someone available to provide emotional support'; and 'sufficient contact with someone you can confide in'. If any of the component items were missing, then the score was set to missing for that exam.

To capture the longitudinal aspect of social support, the SS scores from exams one and exam three were averaged together within an individual. If a participant was missing either exam-specific SS measure, then the averaged SS was calculated from the existing measure. Only if both SS measures were missing was the averaged value set to missing. The distribution of averaged SS for the overall sample and for each race specifically (African-, European-, Chinese-, and Hispanic-Americans) is shown in in Table 3(b). For analysis, SS was mean centered to aid in interpretation. Higher scores indicate more social support. The distribution of chronic burden, averaged over exams one and three is shown in Figure 11.

For the overall sample, the correlation between CB and SS is significantly different than zero ($r = -0.27$, $p < 0.0001$). Race-specific correlations are also significant

and range from -0.26 and -0.29 (European: -0.29, $p < 0.0001$; African: -0.27, $p < 0.0001$; Chinese: -0.29, $p<0.0001$; Hispanic: -0.26, $p<0.0001$). Though they are correlated, they represent two different dimensions of individual-level social environment and are analyzed in separate models.

Preliminary race-specific linear models for the association between depressive symptoms and social environment, both unadjusted and adjusted (adjusting for age, sex, adult socioeconomic position and individual-level social environment) are displayed in Table 4 and Table 5. The CB and SS scores were included in separate models. Averaged chronic burden score was significantly associated with averaged depressive symptom score in each ethnic group in both the unadjusted and adjusted models (p-value < 0.0001 in all models; Table 4). Averaged social support was significantly associated with averaged depressive symptom score in each ethnic group in both the unadjusted and adjusted models (p-value < 0.0001 in all models; Table 5). These models support the need to investigate these factors in interaction with genes.

2.1.3.2 Neighborhood-level social environment

This dissertation uses three dimensions of neighborhood social environment: aesthetic quality (AQ), safety (SF), and social cohesion (SC) measured with a 1-mile radius as the definition of neighborhood. The 1-mile neighborhood radius around individual respondents was selected after examining Pearson's correlations, linear models and clustered mixed models for the 1-mile and conditional empirical Bayes (CEB) neighborhood estimates for each MESA exam with CES-D measures (one, three, and four), and averaged across all three of the exams for the separate neighborhood characteristics as well as for an index score created by combining the three

characteristics. Evidence of significant, strong correlation between the CEB and 1-mile measures and significant association between depressive symptoms and each measure were used to determine if 1-mile measures would be appropriate for analysis. Since the correlation between the CEB and 1-mile measures was strong (>0.70) the 1-mile measures were used for analyses. The 1-mile neighborhood buffer is also consistent with the neighborhood definition of 1-mile from the Health and Retirement Study (which is being used as a replication sample in future analyses), which aids in interpretation and comparability across the two studies.

The longitudinal scales for these measurements were created using the MESA Neighborhood Survey embedded within the MESA survey conducted at exam one, the MESA Neighborhood Activities Survey (MESAN) conducted at exams two and three, and MESA exam five (MESA5). Each MESA participant self-reported information about their neighborhood for these scales. Additionally, scales utilized information from a random sample of people in the 1-mile radius where MESA participants were living using the Community Survey (CS). The community surveys were conducted between January 2004 and August 2004 for the Baltimore, New York, and Los Angeles sites (CS1), between August 2006 and February 2008 for the New York and Los Angeles sites (CS2), and August 2011 and May 2012 for all six of the MESA study sites (CS3). See Table 6 for a timeline of surveys.

These combined scales were created for the 1-mile neighbors using only questions that were common between MESA, MESA5, CS1, CS2, and CS3. The respondent's own answer was not included in the crude means estimates. The neighborhood measures created from these combined surveys link to different MESA exams through similar

collection periods and allow for time-varying neighborhood information. The scales created from MESAN+CS1 are linked to exams one and three while the neighborhood scales created from MESA5+CS3 and are linked to exam four.

Neighborhood measures were linked with individual MESA participants through address data. The address data is compiled for each month starting in 2000 and ending in January 2012 which coincides with the time the MESA study exams 1 – 5 were collected. Only MESA participants who agreed to participate in the neighborhood study are included in these analyses. Addresses were geocoded using a 5-foot offset from major roadways either using a batch process sending addresses directly to TeleAtlas to geocode (addresses added in 2007) or using EZ-Locate software at the University of Michigan (addresses added in 2010 or later). After geocoding, the neighborhood level data was linked to the addresses within a 1-mile buffer by matching each participant of the survey within 1 mile based on the latitude/longitude of the address.

We have used measures pooling all MESA and CS data into crude means. Per MESA recommendation, when choosing which scales to include, I performed sensitivity analysis excluding neighborhoods where scales were based on less than five neighbors. The neighborhood scales and all preliminary analyses were calculated using SAS 9.2. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.[128]

In creating the neighborhood scales, “Don’t Know” or “Refused” values were set to missing for each of the original variables in each of the surveys. Several questions

were reverse coded so that questions reflected better social outcomes with increasing scores. The original coding and which questions were reverse-coded is shown in Table 7.

The correlations between the averaged neighborhood measures and the neighborhood index score (NIS) are shown in Table 8. All correlations were significantly greater than zero, with p-values <0.0001. The smallest correlation between the NIS and an averaged neighborhood dimension was with the SC score ($r = 0.67$).

The model information from adjusted neighborhood dimension mixed models predicting depressive symptom score are presented in Table 9. Using the neighborhood summary score allows us to obtain a more reliable estimate, combining all the neighborhood dimensions into one value. Additionally, we are avoiding the issue of colinearity in our final models.

The distributions of each component score (AQ, SF, SC), averaged over exams one, three, and four is shown, paneled by race, in Figure 13-Figure 15 along with the combined neighborhood index score in Figure 16. The index score was created by averaging the 1-mile means for the three variables across the three exams by neighborhood dimension and then averaging the three averages. If any one of the nine variables (AQ exam one, three, and four; SF exam one, three, and four; or SC exam one, three, and four) was missing then the index score is set to missing. The index score was then mean-centered by the overall mean to aid interpretability. Higher index scores indicate "more positive" overall neighborhood environments, such as a high degree of SF, good AQ, and/or good SC. The index scores range from 2.34 to 4.58 (mean centered (mc): -1.30 to 0.95), with a mean of 3.64 (mc: 0) and a standard deviation of 0.33). The

analysis sample includes 5,023 total individuals (2,047 European Americans, 559 Chinese Americans, 1,391 African Americans, and 1,026 Hispanic Americans) and is presented in Table 10.

2.1.4 Covariates

Information on age (in years), gender (male/female) and ethnic group was obtained from all MESA participants at baseline. Ethnic group was characterized using participants' responses to questions modeled on the Year 2000 Census. Participants were classified as Hispanic, non-Hispanic white, Chinese and non-Hispanic black. These ethnicities will be referred to as Hispanic American (HA), European American (EA), Chinese American (CA), and African American (AA). Study sites included Baltimore MD (Johns Hopkins University Field Center); Chicago, IL (Northwestern Field Center); Forsyth County, NC (Bowman Gray Field Center); Los Angeles, CA (UCLA Field Center); New York, NY (Columbia Field Center); and St. Paul, MN (University of Minnesota Field Center). For the baseline analyses and the averaged analysis, baseline age, sex, ethnic group and study site were used. For the repeat measures analyses, age and study site were treated as time-varying, while sex and ethnic group were obtained from baseline.

Adult socioeconomic position (ASEP) was used in aims two and three. Since several measures of ASEP were available (measuring different dimension of socioeconomic position), we summarized indicators into an ASEP score. The methods are based on previous work and combine information on income, education, and wealth (ownership of a home, car, land/property or investments).[129, 130] Income was defined in four categories (<$25,000, $25,000–39,999, $40,000–74,999, or +$75,000) and

collapsed from the original 13 categories in MESA. At the baseline examination, highest level of education completed was reported and for these analyses operationalized into four categories (completed high school or less, some college but no degree/technical school certificate, associate or bachelor's degree, or graduate/professional degree). The four wealth indexes included: (1) whether the participant, or their family, had investments such as stocks, bonds, mutual funds, retirement investments, or other investments (yes/no), (2) whether the participant owned their home (yes/no), (3) whether the participant owned a car (yes/no), (4) whether the participant owned land or another property that was not their primary residence (yes/no). To create the summary score for ASEP, the individual measures for income, education and wealth were summed (income variable (0 – 3, low to high), education (0 – 3, low to high), and for each wealth indicator, a single point was added). The ASEP score ranged from 0 – 10, with higher scores indicating greater ASEP (Figure 17).

2.1.5 Analysis data set and descriptive statistics

There are 8,227 individuals in the MESA genotype database. After removing participants with missing genetic data, depressive symptom score, or covariates used for analysis (Aim 1: age, sex, site, top four ethnicity-specific principal components), we had an analysis sample size of 6,335 (EA: 2,514; AA: 1,603; CA: 775; HA: 1,443) individuals. For aims two and three, the sample size was further reduced removing individuals with missing ASEP and CB, SS, or NIS.

2.2 Health and Retirement Study

HRS is a dynamic cohort, national panel survey and includes measures collected every two years on more than 22,000 Americans over the age of 50 in multiple race/ethnic groups. It is the largest, most representative longitudinal study of Americans over age 50. HRS began collecting data in 1992 using a probability sample with oversamples of minorities. HRS is supported by the National Institute on Aging (NIA U01AG009740) and the Social Security Administration. The baseline HRS cohort consists of people who were born in 1931 through 1941 and were household residents of the contiguous United States in the spring of 1992, and their spouses or partners at the time of the initial interview in 1992 or at the time of any subsequent interview. The HRS is an ideal sample for joint analysis because HRS participants are roughly the same age as MESA participants, both samples are multi-ethnic, and both studies have similar measures of the outcomes and environments of interest. The HRS study contains several different facets including a core survey,[131] a psychosocial leave-behind participant questionnaire (LBQ),[132] and genetic data.[133]

2.2.1 Depressive symptoms

Depressive symptoms were measured in HRS participants at multiple follow-up visits using the 8-Item CES-D Scale. Each self-respondent was asked the following questions with ‘yes’ or ‘no’ response options: 1) Much of the time during the past week, I felt depressed; 2) I felt everything I did was an effort; 3) My sleep was restless; 4) I was happy; 5) I felt lonely; 6) I enjoyed life; 7) I felt sad; 8) I could not “get going”. The total number of “yes” responses to questions 1, 2, 3, 5, 7, and 8, and the “no” responses to questions 4 and 6 were summed to be the total depressive symptom score ranging from 0

to 8 (Figure 18). Since there were no assessments of anti-depressant medication in HRS, the scores were not adjusted for medication use.

2.2.2 Genetic data

Over 16,000 HRS respondents were genotyped in 2006 or 2008 using the Illumina Human Omni-2.5 Quad beadchip methodology to collect information on ~2.5 million SNPs .[133] Samples originated from either buccal swabs (collected in 2006) in phase I or from saliva samples (collected in 2008) in phase II. Though these phases were genotyped separately, the data was clustered and called together. Genotyping was conducted by the Center for Inherited Disease Research (CIDR) at Johns Hopkins University. Individuals with missing call rates >2%, SNP with missing call rates <85%, chromosomal anomalies, and first degree relatives in the HRS were removed from the database before posting to dbGaP. The genotyping data is consistent with build 37/hg19.[134] Respondents who consented to provide DNA samples and answered at least one of the CES-D8 assessments were used in the analysis.

All HRS respondents who provided DNA samples and completed at least one CES-D8 depressive symptom assessment (N = 10,163) were used in the analyses. Nearly 10,000 members of this sample responded to the CES-D8 items on five or more interview occasions between 1993 and 2010.

2.2.3 Covariates

Age (in years) and gender (male/female) were assessed for all HRS participants at the first exam for which they had a valid measure of CES-D8. The first exam for which a participant had a valid measure of CES-D was characterized as the “baseline” measure.

Genetic ancestry in HRS was identified through principal component analysis on genome-wide SNPs calculated across all participants. The final European American sample included all self-reported non-Hispanic whites that had PC loadings within ±one standard deviations for eigenvectors 1 and 2 in the PCA of all unrelated study subjects. The final African American sample included all self-reported African Americans within two standard deviations of all self-identified African Americans for eigenvector 1 and ± one standard deviation for eigenvector 2 in the PCA of all unrelated study subjects.

2.2.4 Analysis data set and descriptive statistics

There are 12,507 individuals with phenotype information in dbGaP. After removing individuals with missing information on phenotype, genotype, or the covariates of interest, the final analysis subset for HRS consisted of 10,163 individuals. Of those 10,163 individuals, 85.1% were European American and 14.9% were African American.

Table 1 Exclusion criteria for the Multi-Ethnic Study of Atherosclerosis[118]

Age younger than 45 or older than 84 years
Physician-diagnosed heart attack
Physician-diagnosed angina or taking nitroglycerin
Physician-diagnosed stroke or TIA
Physician-diagnosed heart failure
Current atrial fibrillation
Having undergone procedures related to cardiovascular disease (CABG, angioplasty, valve replacement, pacemaker or defibrillator implantation, any surgery on the heart or arteries)
Active treatment for cancer
Pregnancy
Any serious medical condition which would prevent long-term participation
Weight >300 pounds
Cognitive inability as judged by the interviewer
Living in a nursing home or on the waiting list for a nursing home
Plans to leave the community within five years
Language barrier (speaks other than English, Spanish, Cantonese or Mandarin)
Chest CT scan in the past year

Table 2 Depressive symptoms by ethnic group, imputed and non-imputed for each exam, MESA

	EA	CA	AA	HA	Total
	n (%)	n (%)	n (%)	n (%)	n
Exam 1					
No anti-depressant use	2214 (87.58)	756 (97.55)	1613 (96.07)	1363 (94)	5,946
CES-D [mean (sd)]	6.48 (6.45)	6.1 (6.42)	7.16 (7.15)	9.17 (8.65)	
Anti-depressant use	314 (12.42)	19 (2.45)	66 (3.93)	87 (6)	486
CES-D before imputation [mean (sd)]	10.36 (8.81)	8.05 (10.67)	11.95 (10.59)	13.7 (11.3)	
CES-D after imputation [mean (sd)]	18.56 (7.46)	16.05 (10.31)	20.92 (8.75)	23.76 (8.23)	
Exam 3					
No anti-depressant use	2185 (86.43)	761 (98.19)	1616 (96.25)	1365 (94.14)	5,927
CES-D [mean (sd)]	6.38 (6.58)	6.00 (7.01)	6.81 (7.05)	9.27 (9.15)	
Anti-depressant use	343 (13.57)	14 (1.81)	63 (3.75)	85 (5.86)	505
CES-D before imputation [mean (sd)]	10.84 (9.19)	8.36 (8.85)	13.1 (11.34)	13.87 (10.68)	
CES-D after imputation [mean (sd)]	19.35 (7.58)	14.57 (7.97)	22.99 (8.36)	22.95 (7.71)	
Exam 4					
No anti-depressant use	2184 (86.39)	764 (98.58)	1608 (95.77)	1356 (93.52)	5,912
CES-D [mean (sd)]	6.91 (6.8)	5.98 (6.67)	6.94 (6.49)	9.19 (8.93)	
Anti-depressant use	344 (13.61)	11 (1.42)	71 (4.23)	94 (6.48)	520
CES-D before imputation [mean (sd)]	11.34 (9.29)	11.55 (11.29)	13.51 (11.76)	16.56 (12.49)	
CES-D after imputation [mean (sd)]	19.91 (7.55)	19.92 (7.66)	23.88 (8.58)	27.03 (10.21)	

* CES-D scores were imputed within each race and gender separately, within each exam separately. EA: European American, CA: Chinese American, AA: African American, HA: Hispanic American

Table 3 Descriptive statistics of averaged chronic burden and averaged social support by race and for the overall MESA sample

	N	Mean	Standard Deviation	Minimum	Maximum
(a) Averaged Chronic Burden					
Overall	5967	1.06	1.02	0	10
European Americans	2520	1.10	1.02	0	5
Chinese Americans	774	0.65	0.82	0	4.5
African Americans	1664	1.17	1.09	0	5
Hispanic Americans	1445	1.06	1.01	0	5
(b) Averaged Social Support					
Overall	6421	24.15	4.86	6	30
European Americans	2524	24.06	4.94	6	30
Chinese Americans	775	23.86	4.31	6	30
African Americans	1672	24.27	4.73	6	30
Hispanic Americans	1450	24.34	5.13	6	30

Table 4 Race-specific linear models predicting averaged depressive symptom score for unadjusted and adjusted linear models

	African American			European American			Chinese American			Hispanic American		
	Beta	**Std Err**	**p value**	**Beta**	**Std Err**	**p value**	**Beta**	**Std Err**	**p value**	**Beta**	**Std Err**	**p value**
Intercept	1.54	0.03	<.0001	1.60	0.02	<.0001	1.41	0.04	<.0001	1.79	0.03	<.0001
Averaged Chronic Burden	0.31	0.02	<.0001	0.33	0.02	<.0001	0.40	0.03	<.0001	0.32	0.02	<.0001
Intercept	1.95	0.14	<.0001	2.11	0.13	<.0001	1.05	0.23	<.0001	1.87	0.14	<.0001
Age	0.00	0.00	0.41	0.00	0.00	0.15	0.00	0.00	0.62	0.00	0.00	0.96
Female	0.08	0.04	0.03	0.16	0.03	<.0001	0.23	0.06	<.0001	0.22	0.04	<.0001
Male	(ref)			(ref)			(ref)			(ref)		
ASEP	-0.06	0.01	<.0001	-0.07	0.01	<.0001	0.04	0.01	0.00	-0.06	0.01	<.0001
Averaged Chronic Burden	0.27	0.02	<.0001	0.30	0.02	<.0001	0.38	0.03	<.0001	0.31	0.02	<.0001

ASEP: Adult Socioeconomic Position

Table 5 Race-specific linear models predicting averaged depressive symptom score for unadjusted and adjusted linear models

	African American			European American			Chinese American			Hispanic American		
	Beta	**Std Err**	**p value**	**Beta**	**Std Err**	**p value**	**Beta**	**Std Err**	**p value**	**Beta**	**Std Err**	**p value**
Intercept	3.45	0.09	<.0001	3.60	0.08	<.0001	3.83	0.15	<.0001	3.78	0.10	<.0001
Averaged Social Support	-0.06	0.00	<.0001	-0.07	0.00	<.0001	-0.09	0.01	<.0001	-0.07	0.00	<.0001
Intercept	4.05	0.16	<.0001	4.08	0.13	<.0001	3.23	0.24	<.0001	3.86	0.16	<.0001
Age	-0.01	0.00	<.0001	-0.01	0.00	<.0001	0.00	0.00	0.14	0.00	0.00	0.08
Female	0.13	0.04	0.00	0.21	0.03	<.0001	0.25	0.05	<.0001	0.24	0.04	<.0001
Male	(ref)			(ref)			(ref)			(ref)		
ASEP	-0.06	0.01	<.0001	-0.06	0.01	<.0001	0.04	0.01	<.0001	-0.04	0.01	<.0001
Averaged Social Support	-0.06	0.00	<.0001	-0.06	0.00	<.0001	-0.09	0.01	<.0001	-0.06	0.00	<.0001

ASEP: Adult Socioeconomic Position

Table 6 Timeline of MESA surveys

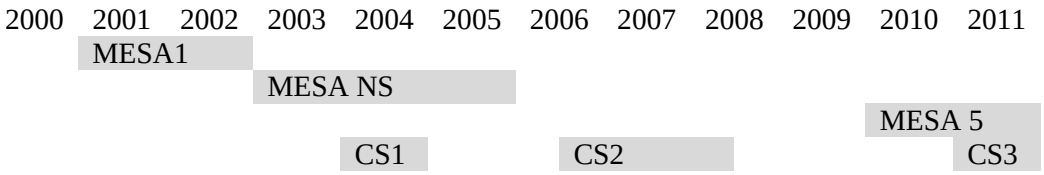


Table 7 Neighborhood scales common questions for Social Cohesion, Aesthetic Quality and Neighborhood Safety, MESA

Scale	Component	Question
Social Cohesion (higher score is better cohesion)	SocCo1	People around here are willing to help their neighbors – Reverse coded
	SocCo2	People in my neighborhood generally get along with each other – Reverse coded
	SocCo3	People in my neighborhood can be trusted – Reverse coded
	SocCo4	People in my neighborhood share the same values – Reverse coded
Aesthetic Quality (higher score is better Aesthetic Quality)	AeQual1	There is a lot of trash and litter on the street in my neighborhood.
	AeQual2	There is a lot of noise in my neighborhood.
	AeQual3	My neighborhood is attractive – Reverse coded
Safety (higher score is more safety)	Safe1	I feel safe walking in my neighborhood day or night – Reverse coded
	Safe2	Violence is a problem in my neighborhood.

Original coding for all components is 1=Strongly Agree, 2=Agree, 3=Neither Agree Nor Disagree, 4=Disagree, and 5=Strongly Disagree.

Table 8 Pearson's correlations between averaged neighborhood measure and neighborhood index score

Simple Statistics

Variable	N	Mean	Std Dev	Minimum	Maximum
NIS	4754	0.00	0.33	-1.30	0.95
Averaged aesthetic quality	4846	3.70	0.42	2.17	5.00
Averaged safety	4848	3.67	0.42	2.00	5.00
Averaged social cohesion	4761	3.56	0.25	2.42	5.00

	NIS	Averaged aesthetic quality	Averaged safety	Averaged social cohesion
NIS	1	**0.95**	**0.91**	**0.88**
Averaged aesthetic quality		1	**0.77**	**0.84**
Averaged safety			1	**0.67**
Averaged social cohesion				1

NIS: Neighborhood Index Score,

Bolded values are significant at $p<0.001$ for the test Prob > |r| under H_0: Rho=0

Table 9 Parameter estimates for log transformed (CES-D score + 1), standard errors, and p-values for adjusted mixed-models, for each separate neighborhood dimension and for neighborhood index score

	Aesthetic Quality			Safety			Social Cohesion			Neighborhood Index Score		
Effect	**Beta**	**SE**	**P**	**Beta**	**SE**	**P**	**Beta**	**SE**	**P**	**Beta**	**SE**	**P**
Intercept	2.89	0.13	<.0001	2.88	0.14	<.0001	3.04	0.19	<.0001	2.54	0.08	<.0001
Age	-0.01	0.00	<.0001	-0.01	0.00	<.0001	-0.01	0.00	<.0001	-0.01	0.00	<.0001
Female	0.16	0.02	<.0001	0.16	0.02	<.0001	0.17	0.02	<.0001	0.17	0.02	<.0001
Male	(ref)			(ref)			(ref)			(ref)		
EA	-0.01	0.03	0.8731	-0.01	0.03	0.7111	-0.01	0.03	0.8323	0.00	0.03	0.9263
CA	-0.31	0.04	<.0001	-0.32	0.04	<.0001	-0.34	0.04	<.0001	-0.32	0.04	<.0001
AA	-0.09	0.03	0.0084	-0.11	0.04	0.0017	-0.10	0.04	0.0062	-0.10	0.04	0.0042
HA	(ref)			(ref)			(ref)			(ref)		
ASEP	-0.05	0.00	<.0001	-0.05	0.00	<.0001	-0.05	0.00	<.0001	-0.05	0.00	<.0001
Neighborhood Value												
AQ	-0.09	0.03	0.0038									
SF				-0.09	0.03	0.008						
SC							-0.14	0.05	0.0091			
NIS										-0.13	0.04	0.0019

ASEP: Adult socioeconomic position, AQ: Aesthetic Quality, SF: Safety, SC: Social Cohesion, NIS: Neighborhood Index Score, EA: European, CA: Chinese, AA: African American, HA: Hispanic, Adjusted models are adjusted for age, sex, race, adult socioeconomic position, and neighborhood value

Table 10 Mean centered summary neighborhood index score, combined sample and race-specific

	N	Mean	Std Dev	Minimum	Maximum
Overall	5023	3.63	0.34	2.34	4.58
Overall (MC)	5023	0.00	0.34	-1.29	0.95
European (MC)	1979	0.13	0.29	-1.19	0.95
Chinese (MC)	543	0.06	0.24	-0.87	0.82
African American (MC)	1239	-0.09	0.34	-0.77	0.83
Hispanic (MC)	993	-0.18	0.33	-1.30	0.92

MC: mean - centered using the overall mean

Figure 6 Distribution of CES-D score by exam for those with missing anti-depressant use (missing ad) compared to those with no anti-depressant use (no ad use), MESA

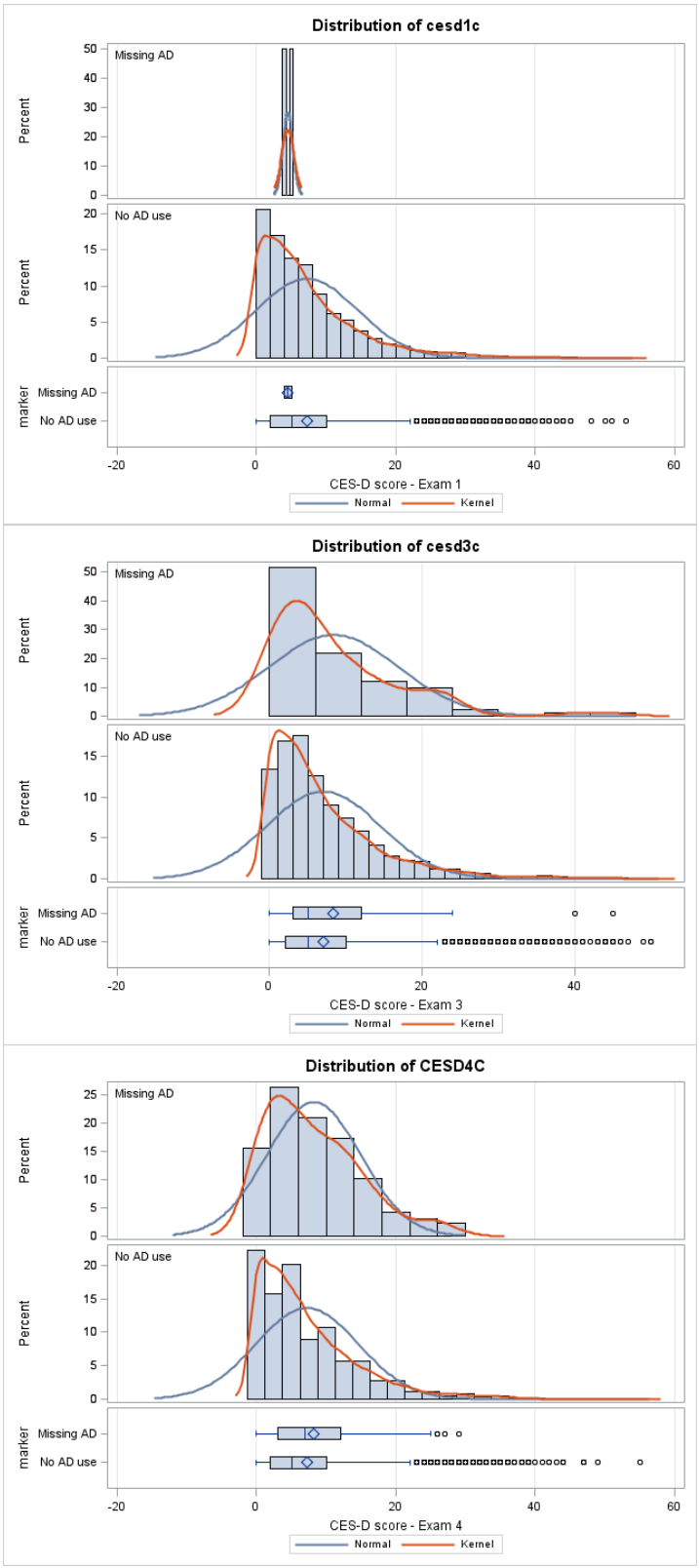
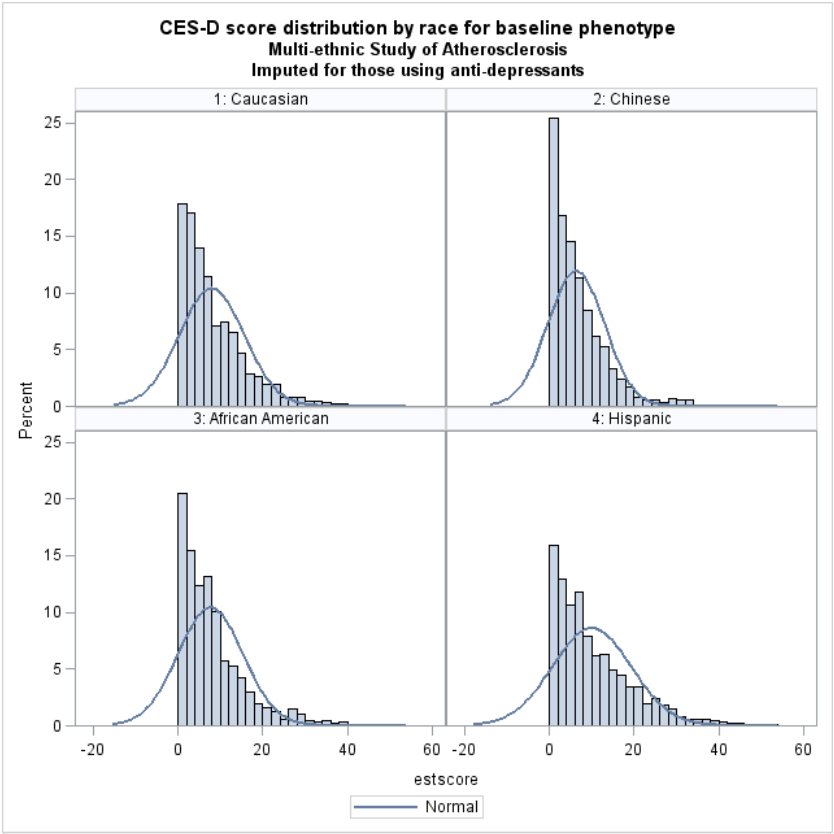


Figure 7 Distribution of CES-D score, raw and log-transformed, by ethnicity for Exam 1, MESA



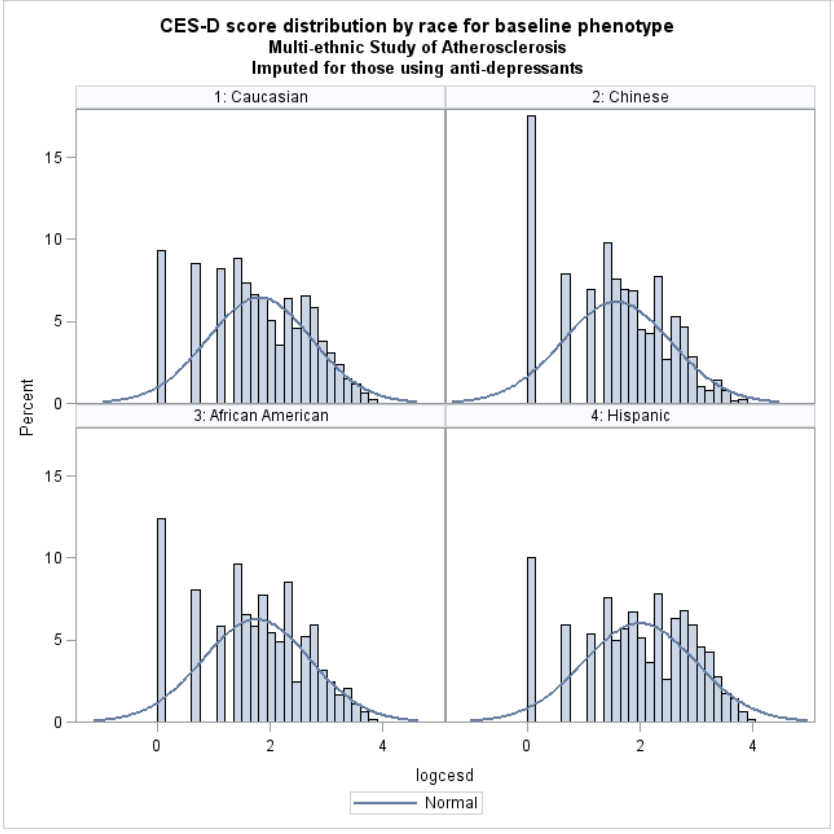


Figure 8 Distribution of CES-D score, raw and log-transformed, by ethnicity averaged across exams 1, 3, 4, MESA

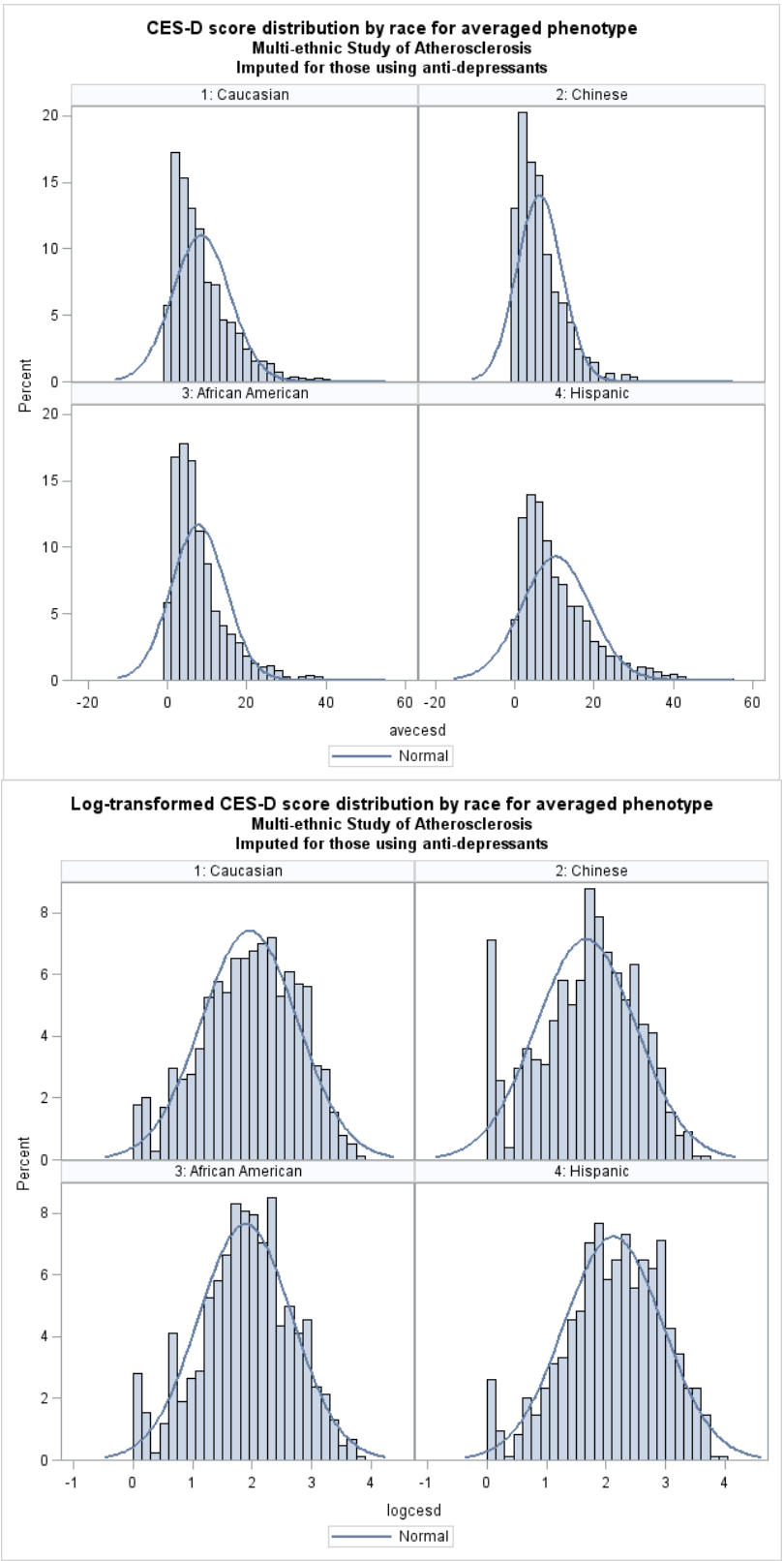
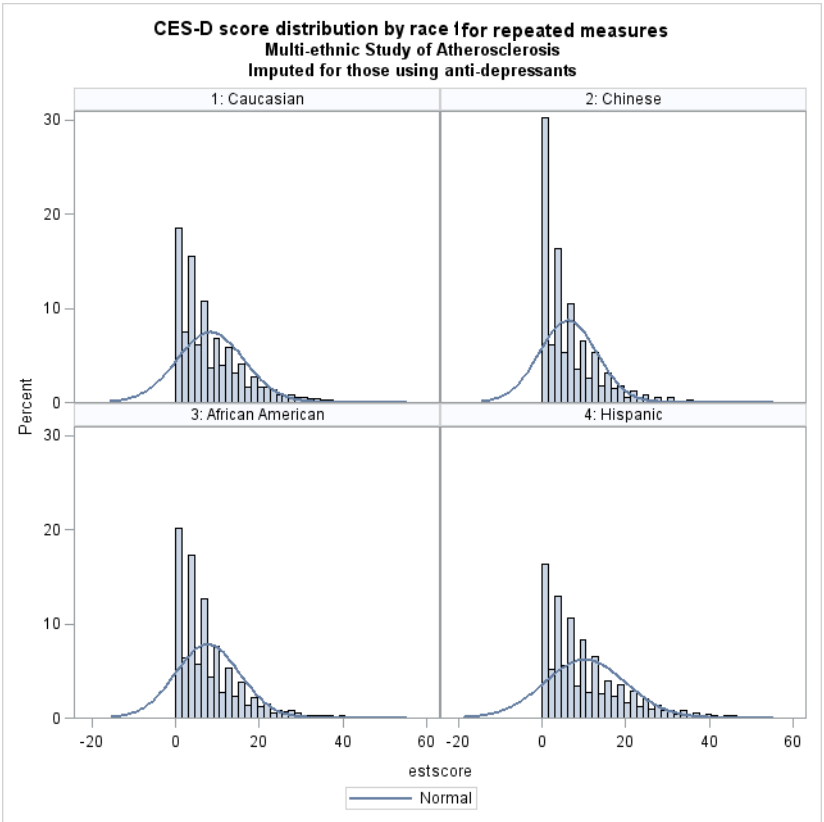


Figure 9 Distribution of CES-D score, raw and log-transformed, by ethnicity for repeated measures from exams 1, 3, 4, MESA



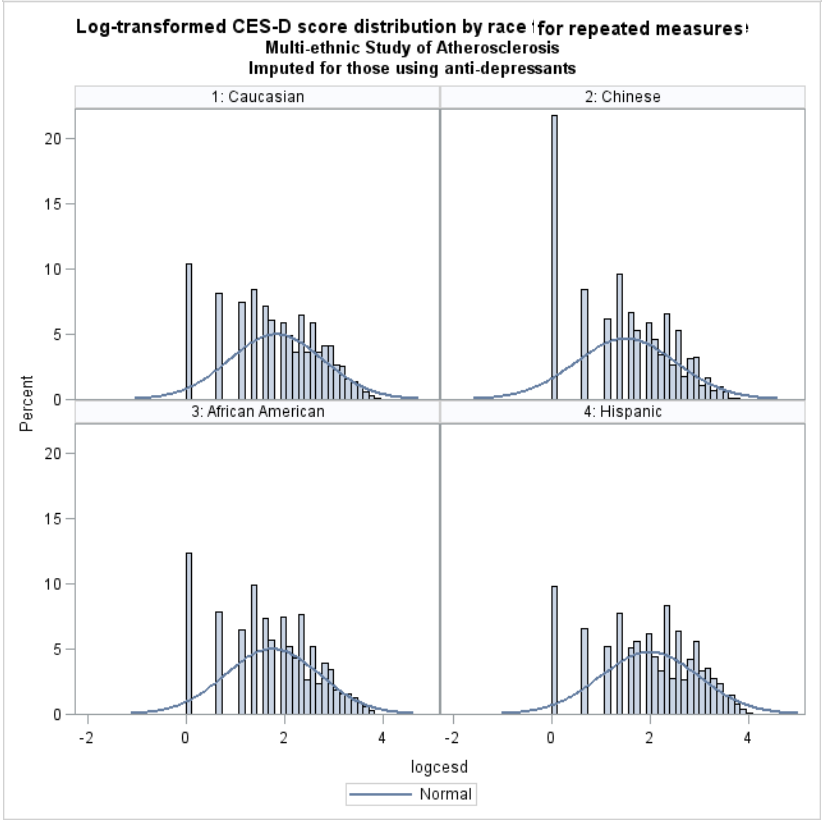


Figure 10 Distribution of chronic burden score, averaged over exams one and three by ethnicity

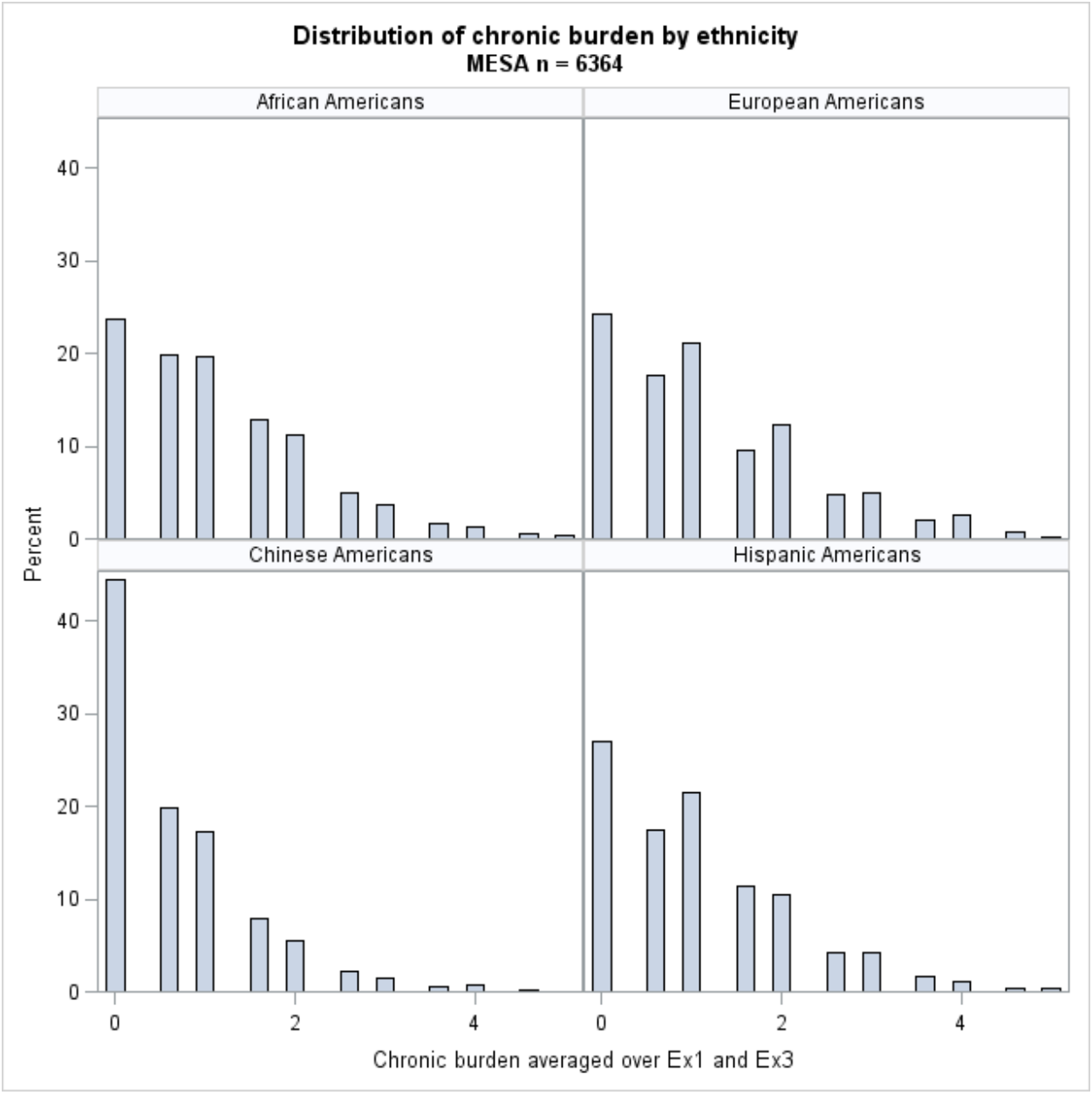


Figure 11 Distribution of social support score, averaged over exams one and three by ethnicity

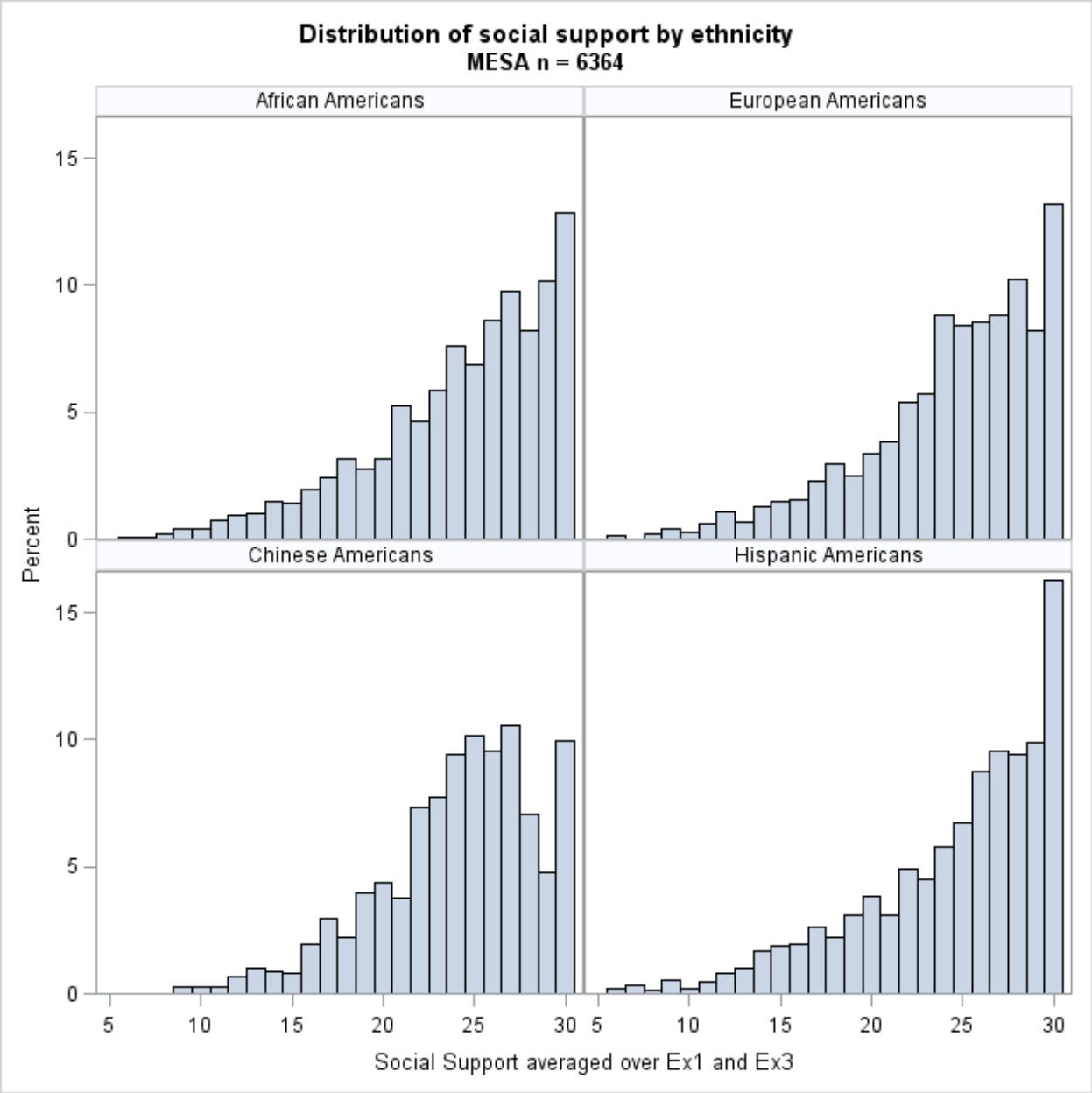


Figure 12 Distribution of age in 2000 for MESA and HRS participants

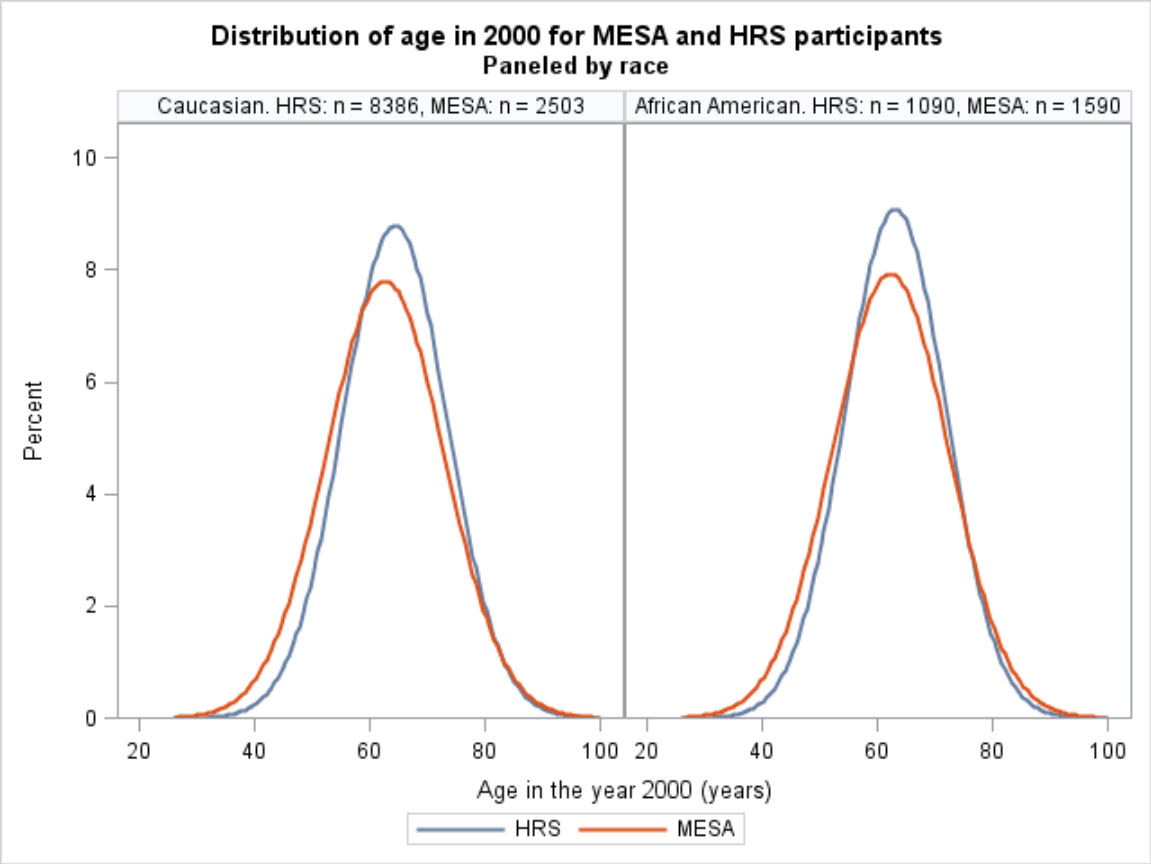


Figure 13 Distributions of aesthetic quality, averaged over Exams one, three, and four, paneled by race

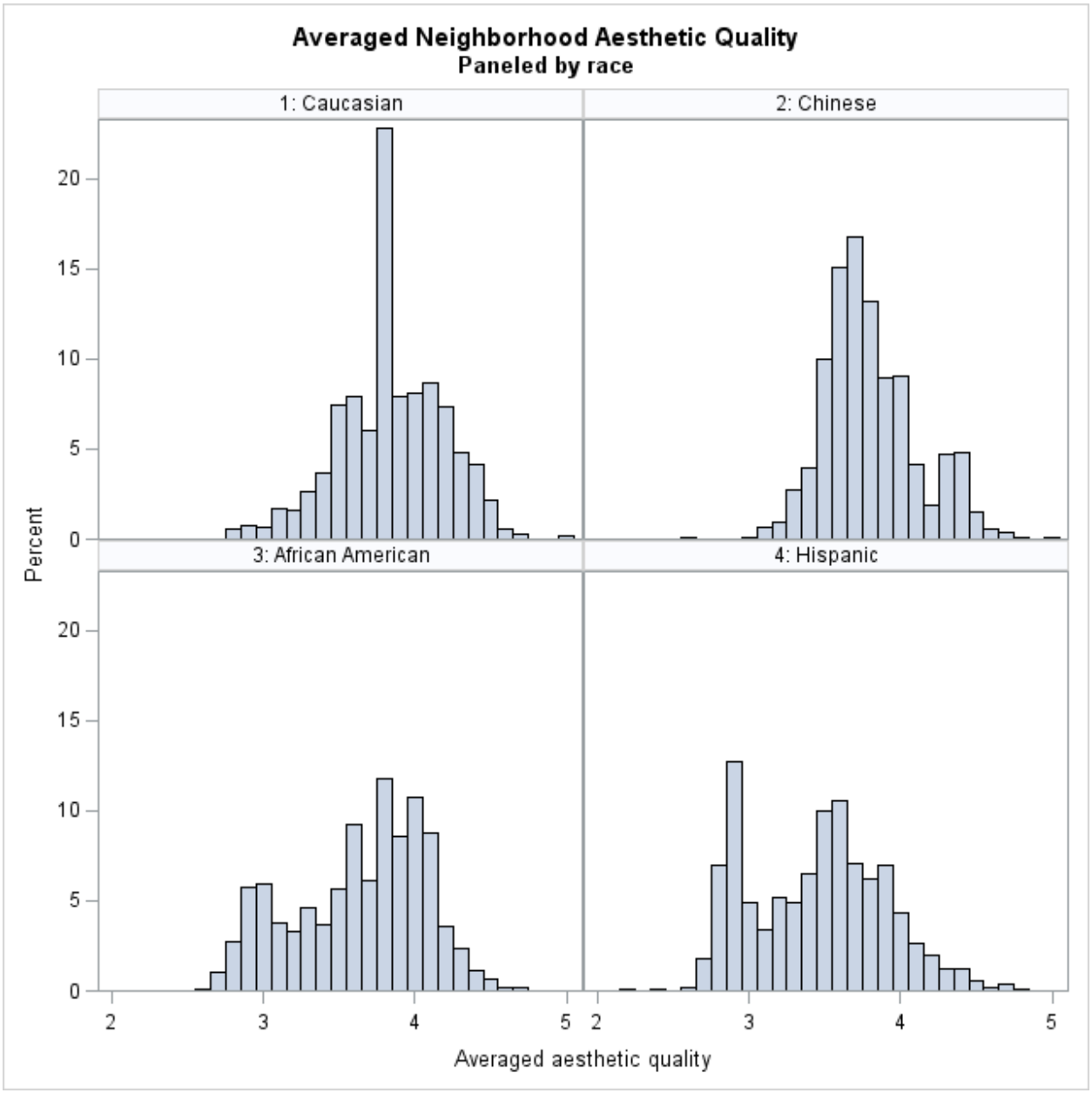


Figure 14 Distributions of neighborhood safety, averaged over Exams one, three, and four, paneled by race

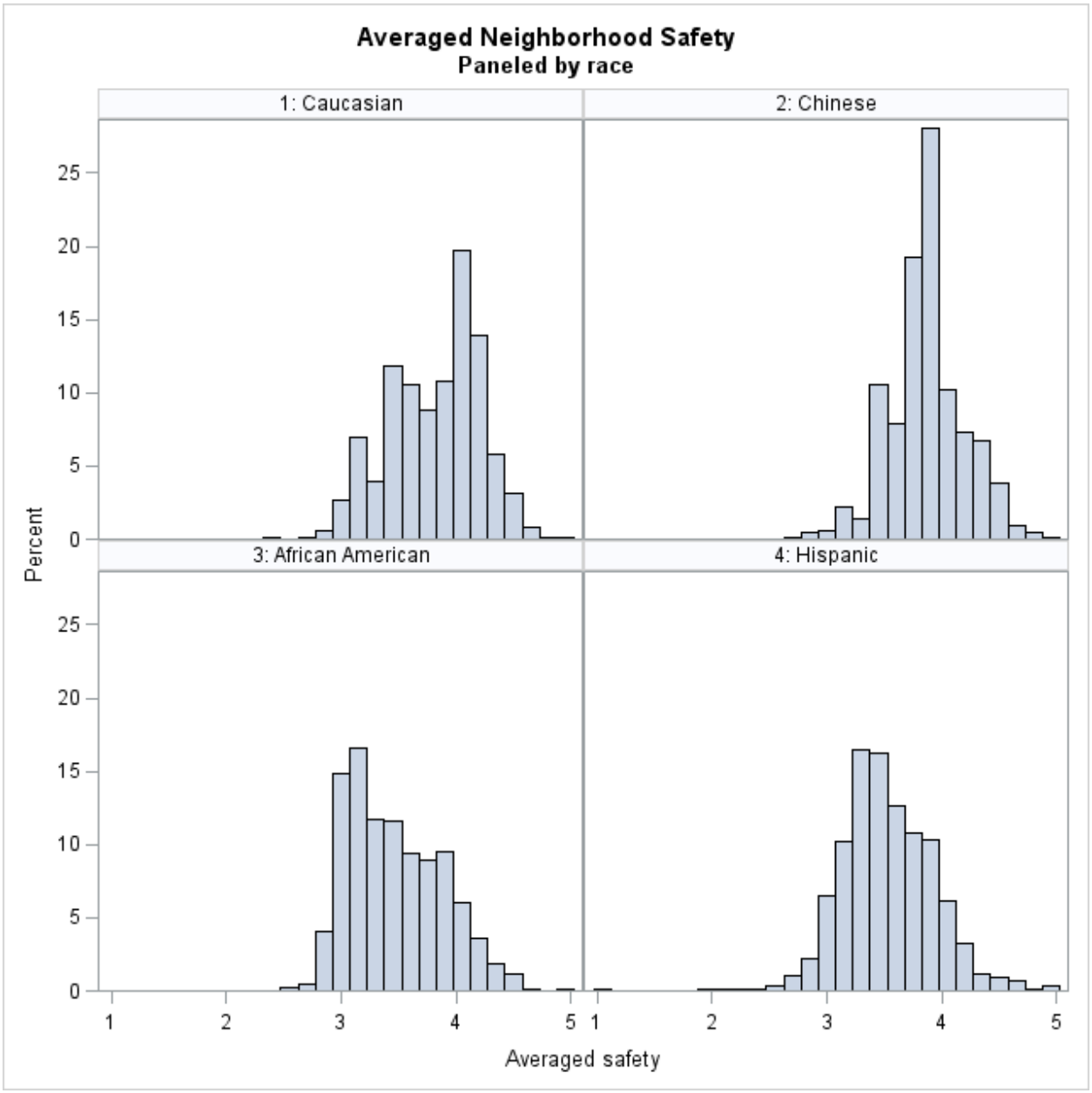


Figure 15 Distributions of social cohesion, averaged over Exams one, three, and four, paneled by race

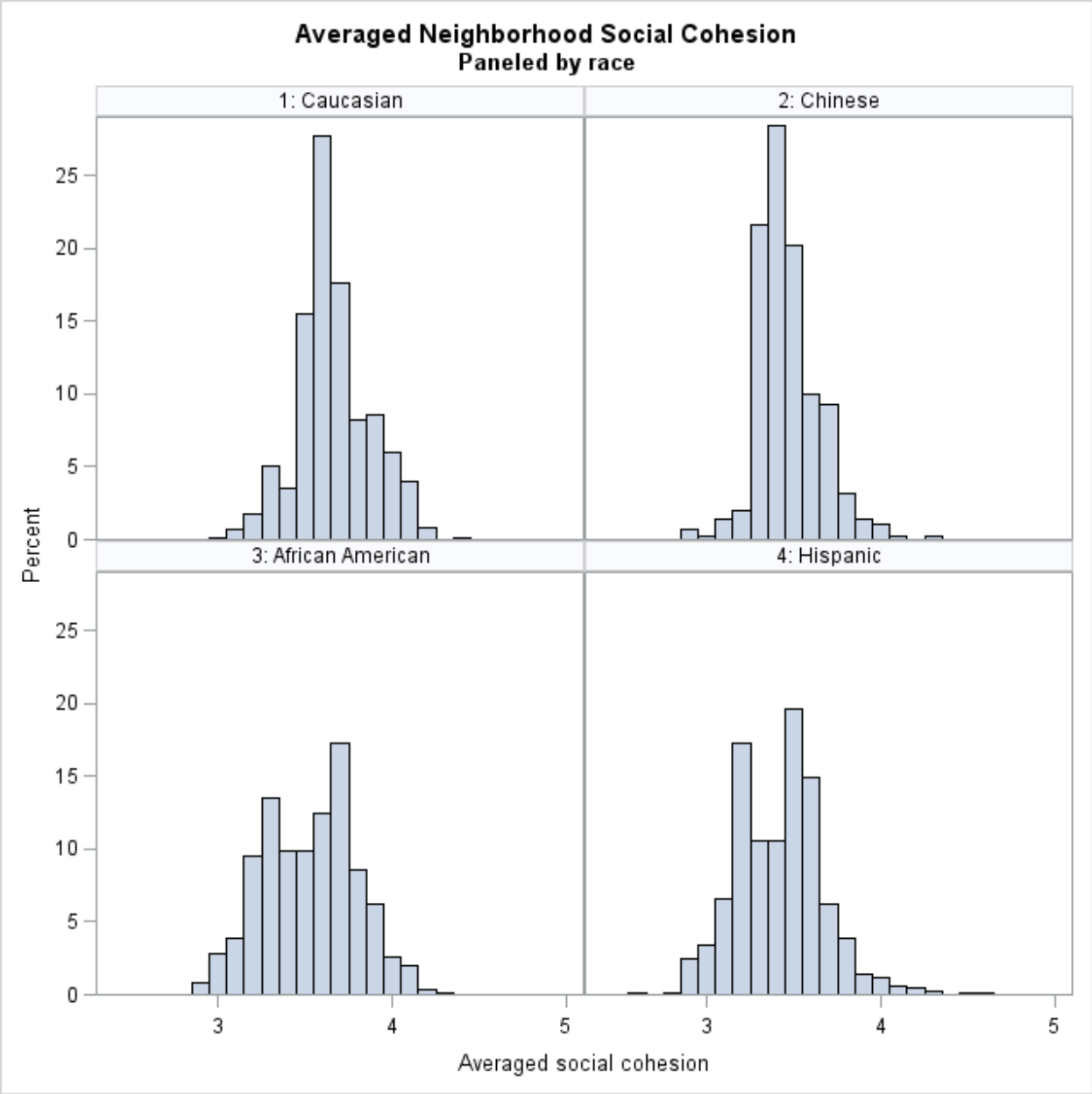


Figure 16 Distributions of neighborhood index score, averaged over Exams one, three, and four, paneled by race

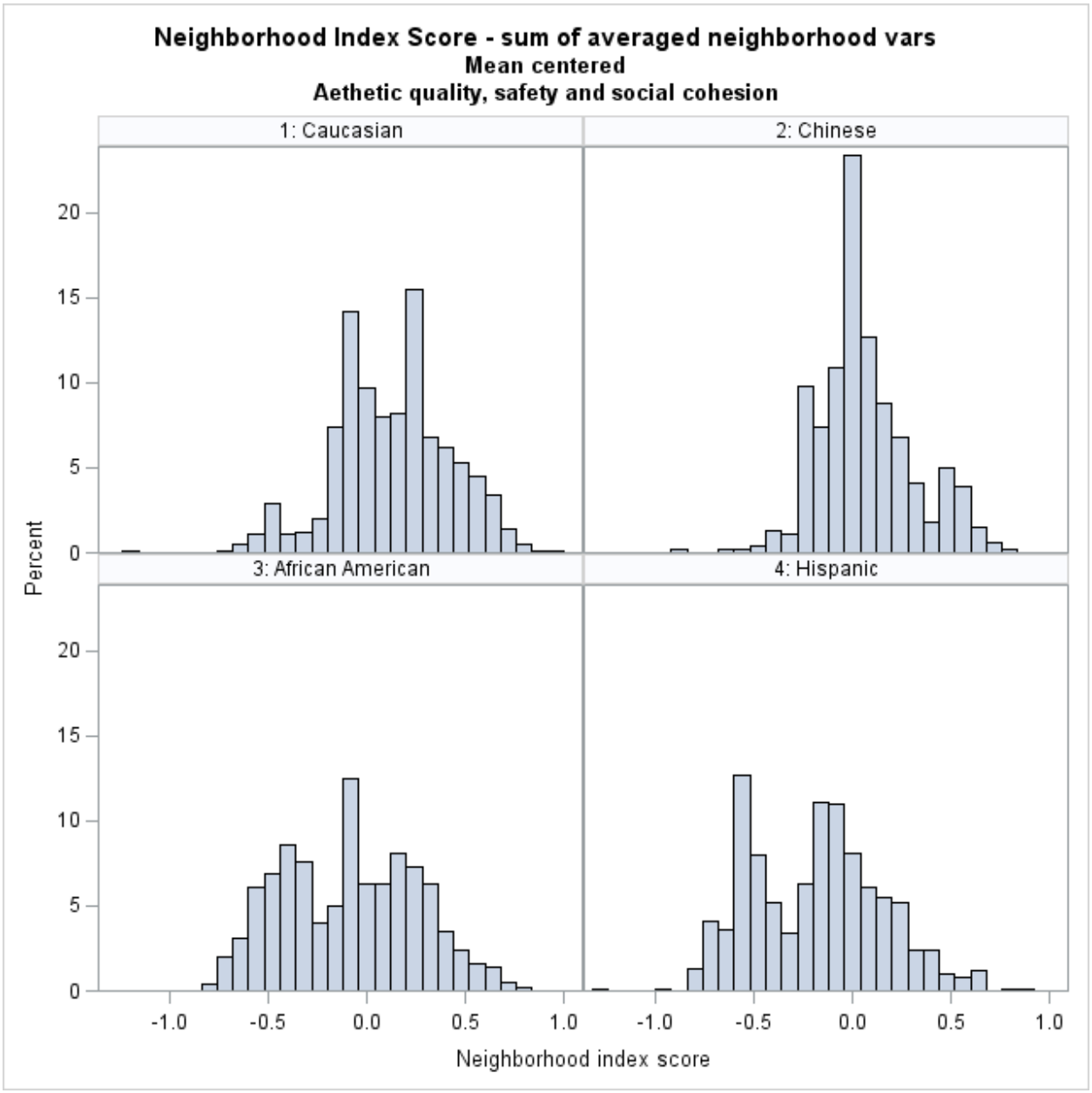


Figure 17 Distribution of adult socioeconomic position by ethnicity

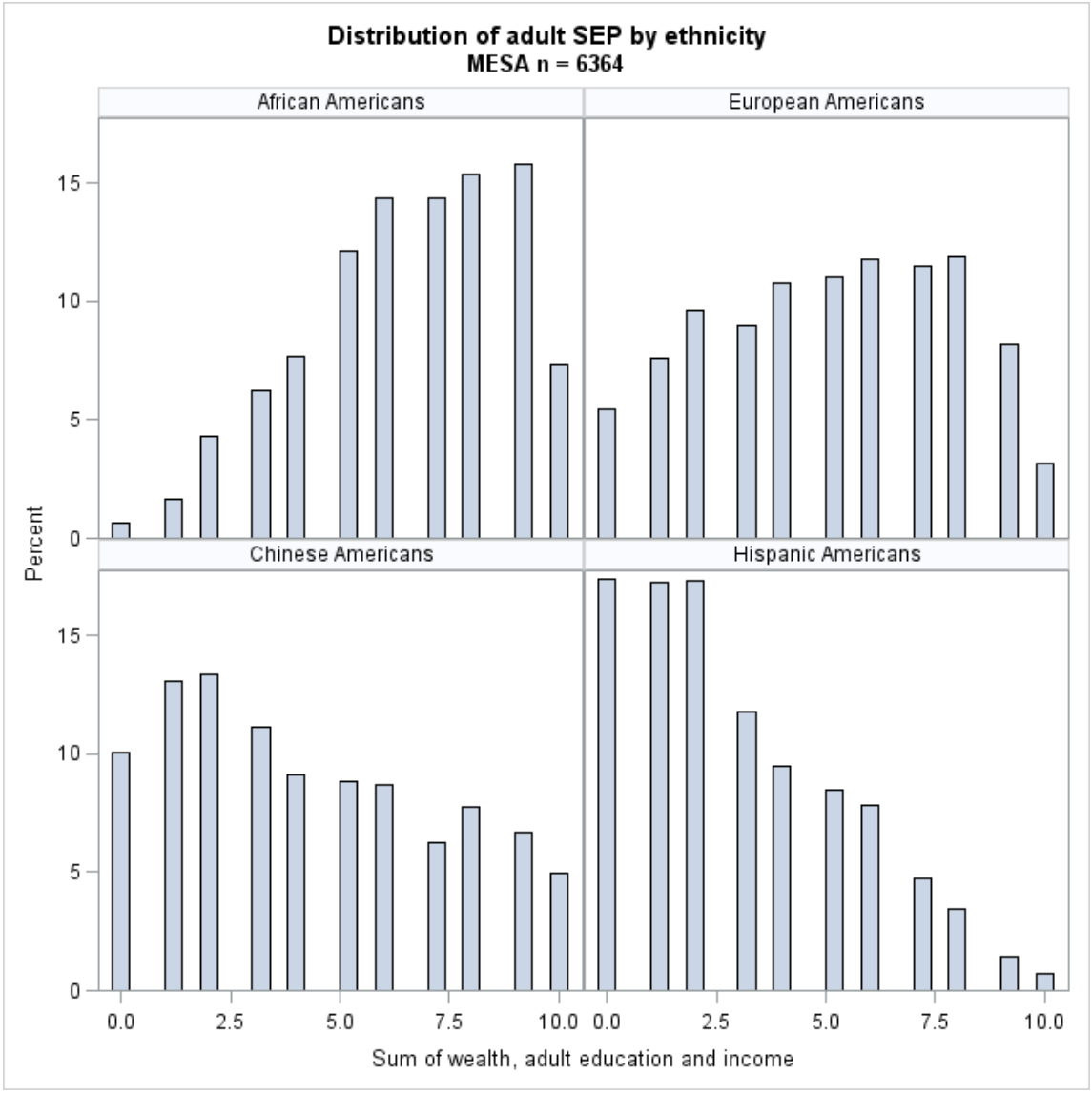
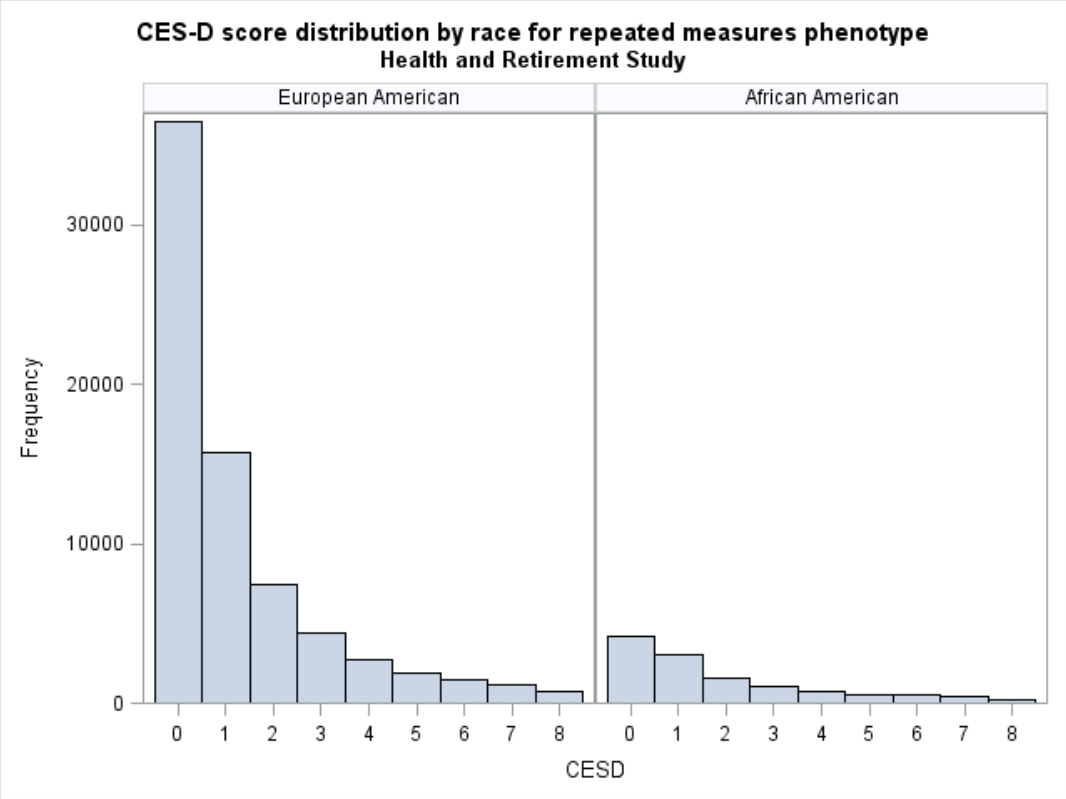
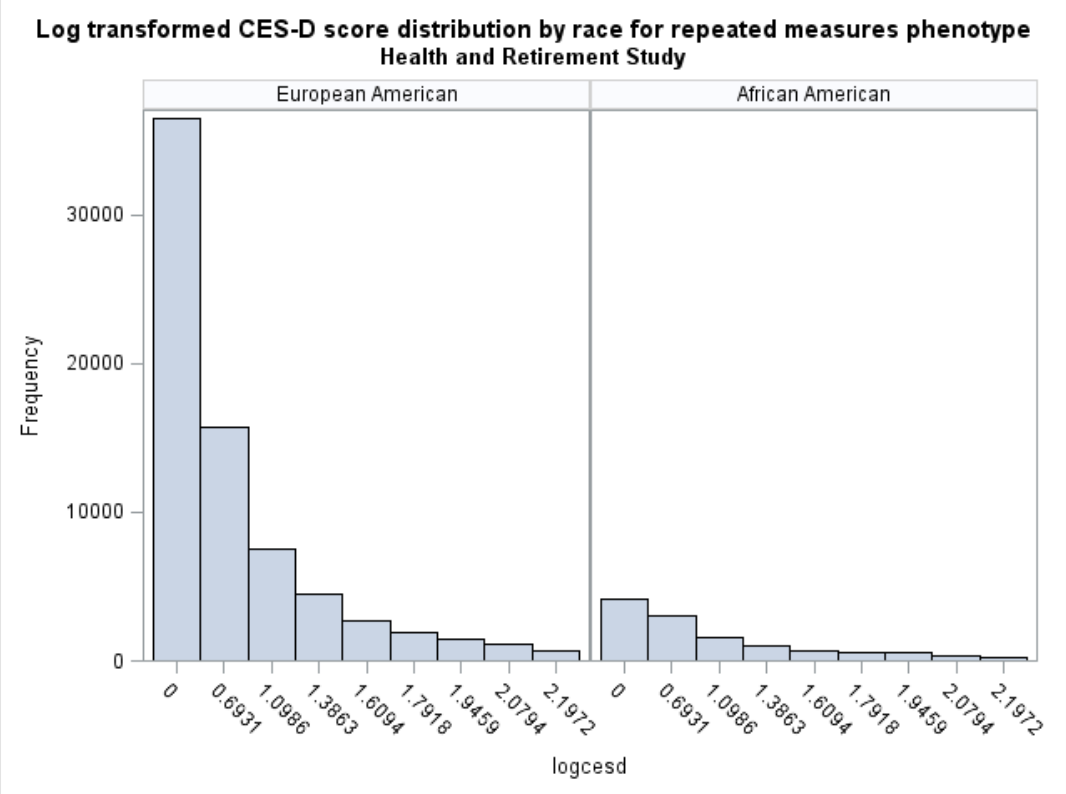


Figure 18 Distribution of CES-D score, raw and log-transformed, by ethnicity, averaged across exams 2 – 10, Health and Retirement Study





CHAPTER III

III. Comparative genome-wide association studies of depressive symptom phenotype in a repeat measures setting by ethnic group in the Multi-Ethnic Study of Atherosclerosis

3.1 Introduction

Major Depressive Disorder (MDD) is the most prevalent mental disorder in the United States [7]. The lifetime prevalence of MDD is approximately 16.6% with a 12-month prevalence of approximately 6.7% in US adults aged 18 and over [8, 135]. MDD is associated with high morbidity [136-138]. It is estimated that the heritability of MDD is in the range of 31% to 42%, indicating a strong genetic contribution to disease etiology.[139, 140]

Though several linkage studies of MDD have been performed, only a single locus has been identified in the 17q-31 chromosomal region [141, 142]. Genome wide association studies (GWAS) have also had limited success in identifying new associated loci. Nine GWAS on MDD have been published to date [9-13, 136, 139, 143, 144] as well as a meta-analysis of the nine GWAS that included almost 19,000 European unrelated individuals [114]. Only one loci reached genome wide significance in individual studies[113], but this loci was not significantly associated with MDD in the meta-analysis [114]. Meta-analyses of genetic predictors of MDD are currently consistent with chance findings and hypothesized candidate genes identified from physiological

pathways (such as *TPH2*, *HTR2A*, *MAOA*, *COMT*) have rarely been identified/replicated as predictors of MDD in GWAS [136, 145-147].

It is possible that measurement error in the assessment of MDD could be contributing to the largely null findings. Depressive symptoms exist on a spectrum, varying in both severity and duration. The ability to detect genetic predictors of depression may be enhanced by analyzing depressive symptoms quantitatively [148], rather than applying cutoffs or defining disorders such as MDD at the extreme of the continuum [15]. Depressive symptoms are often measured using the 20-item Center for Epidemiologic Studies Depression scale (CES-D). Because depressive symptoms may vary over time in relation to a variety of circumstantial factors, repeated measures of depressive symptoms may provide a better characterization of an individual's phenotype than a single measure, thus increasing power to detect underlying genetic predictors.

The Multi-Ethnic Study of Atherosclerosis (MESA) was recently part of a discovery sample for a cross-sectional GWA study of depressive symptoms conducted by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium [120]. This GWAS focused on a single measure of depressive symptoms (as assessed by CES-D) in individuals of European descent. Though no loci reached genome-wide significance in the discovery sample (composed of 34,549 individuals), one of the seven most significant SNPs had a suggestive association in the replication sample (rs161645, 5q21, $p = 9.19 \times 10^{-3}$). This SNP reached genome-wide significance ($p = 4.78 \times 10^{-8}$) in overall meta-analysis of the combined discovery and replication samples (n = 51,258) [120]. Important limitations of this GWAS include the reliance on a single measure of depressive symptoms and the focus on a single race/ethnic group. The present

study improves the characterization of the phenotype through the incorporation of repeated measures of depressive symptoms over time and extends the GWAS to multiple ethnicities.

We use longitudinal data on depressive symptoms collected over a 9 year period in MESA to conduct GWAS on depressive symptoms in four race/ethnicities. We also contrast different approaches of incorporating the repeated measures into the GWAS: (1) analyzing a single time-point measure (baseline), (2) averaging measures over time, and (3) conducting a repeated measures outcome analyses. Finally, we jointly analyze repeated measures GWAS results from MESA and the Health and Retirement Study (HRS) in an overall meta-analysis for European Americans and African Americans to increase our power. To our knowledge, there have been no GWAS of repeated measures of depressive symptoms measured over time in individuals of multiple race/ethnicities.

3.2 Methods

3.2.1 Discovery sample

MESA is a longitudinal study supported by NHLBI with the overall goal of identifying risk factors for subclinical atherosclerosis [118]. The MESA cohort was recruited in 2000-2002 from six Field Centers: Baltimore, MD (Johns Hopkins University Field Center), Chicago, IL (Northwestern Field Center), Forsyth County, NC (Bowman Gray Field Center), Los Angeles, CA (UCLA Field Center), New York, NY (Columbia Field Center), and St. Paul, MN (University of Minnesota Field Center). MESA participants were 45-84 years of age and free of clinical cardiovascular disease at

baseline. At each site a probability sample of approximately 1,100 participants was selected through a variety of population-based approaches. At baseline the MESA cohort included 6,814 men and women, with 38.5% non-Hispanic whites (EA), 27.8% non-Hispanic African Americans (AA), 22% Hispanics (HA), and 11.8% Chinese (CA). Participants attended a baseline examination (2000-2002) and three additional follow-up examinations approximately 18-24 months apart. At each clinic visit, participants completed a series of demographic, personal history, medical history, access to care, behavioral, and psychosocial questionnaires in English, Spanish, or Chinese. Depressive symptoms were assessed using the Center for Epidemiologic Studies Depression scale (CES-D) at exams 1, 3 and 4. The total number of participants and the corresponding response rates (of participants alive) were: exam 1 (n=6,814), exam 2 (n=6,239, 92%), exam 3 (n=5,946, 89%), exam 4 (n=5,704, 87%). After removing participants with missing genetic data, depressive symptom score, or covariates used for analysis, we had an analysis sample size of 6,335 (EA: 2,514; AA: 1,603; CA: 775; HA: 1,443) individuals. The total number of individuals with one, two or multiple repeated measures can be reviewed in Table 11. Institutional review boards at each site approved study protocol.

3.2.2 Depressive symptom score

Depressive symptom score was assessed using the 20-item CES-D Scale [14]. The CES-D was developed by the Center for Epidemiologic Studies for use in general population surveys [14, 19]. The CES-D has an excellent internal consistency (Cronbach's alpha = 0.90) [14], and is designed to assess depressive symptoms at a specific period in time (over the past week). The outcome measure for this analysis is a

sum of the 20 items, ranging from 0 to 60. If more than 5 items were missing, the CES-D score was not calculated. If 1-5 items were missing, the scores were summed for completed items, dividing the sum by the number of questions answered and then multiplying by 20. There were 5,178 participants with three measures of CES-D, 507 with two measures, and 650 with only baseline CES-D measures, for a total of 6,335 participants with 17,198 observations. The CES-D scores were log-transformed to improve normality.

Anti-depressant use was defined as taking any or multiple of the following medications: Selective Serotonin Reuptake Inhibitors (SSRIs), Monoamine Oxidase Inhibitor (MAOI), Tricyclic anti-depressant, and/or Non-tricyclic anti-depressant other than MAOI. Anti-depressant use was assessed at each exam and corrected CES-D scores were estimated for each exam. A total of 7.6%, 7.9% and 8.1% of persons were on anti-depressant medications at exams 1, 3 and 4, respectively. We corrected for anti-depressant use with methods previously described [120]. Briefly, assuming that the depression score is lower in treated than in untreated participants, and that participants with high depression scores, on average, respond less to their medication than persons with lower depression scores, we used a nonparametric imputation algorithm to adjust for the treatment effect. Separately for men and women and within each ethnicity separately, we replaced CES-D score for a person using anti-depressants with the mean depressive symptom score of all persons using anti-depressants with greater or equal CES-D scores. This method is based on an algorithm previously used to adjust blood pressure for persons on antihypertensive medication [119]. We chose not to exclude participants taking anti-depressant medication as they often are individuals with depression or higher

depressive symptom scores and thus add value to genetic studies. To improve normality consideration for the outcomes, baseline, averaged and repeated measures CES-D (adding one point to all values) were log-transformed after adjustment for anti-depressant use.

3.2.3 Genotyping

MESA is a participating study in the SNP Health Association Resource (SHARe) Project. About one million SNPs were genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0. The IMPUTE 2.1.0 program was used in conjunction with HapMap Phase I and II reference panels (CEU+YRI+CHB+JPT, release 22 - NCBI Build 36 for African-, Chinese- and Hispanic-American participants; CEU, release 24 - NCBI Build 36 for European Americans) to increase the number of available SNPs to approximately 2.5 million markers. We accounted for population substructure by including the top four ethnicity-specific principal components (estimated from genome-wide data) as adjustment covariates in all analyses, as proposed previously by MESA investigators and elsewhere [121, 122].

3.2.4 Joint Sample

We used the Health and Retirement Study (HRS) as a joint sample to be combined with MESA GWAS results in a meta-analysis [149]. These two studies have comparable participants, and similar measures of phenotype. The HRS surveys a representative sample of more than 26,000 Americans over the age of 50 every two years starting in 1992. HRS data includes information on depressive symptoms measured with a short form of the CES-D, the CES-D8. The CES-D8 includes a subset of eight items from the full 20-item CES-D [14]. The depression score for each participant was composed of the total number of affirmative depression answers. The HRS depression

symptom score ranges from 0 to 8. Participants missing two or more of the eight items were excluded from the analyses. The Institutional Review Board at the University of Michigan, where the study was conducted, approved study protocol before data collection. The depressive symptom phenotype for MESA was constructed form the full 20 items available while the HRS depressive symptom phenotype was calculated from the 8 available items.

Over 12,000 HRS participants were genotyped for about 2.5 million SNPs using the Illumina Human Omni-2.5 Quad beadchip. Genotypes were imputed for European Americans and African Americans using MACH software (HapMap Phase II, release #22, CEU panel for European Americans and CEU+YRI panel for African Americans). We accounted for population substructure by including the top four ethnicity-specific principal components (estimated from genome-wide data) as adjustment covariates in all analyses. There were 10,163 HRS participants after removing those with missing outcome, covariate or genetic information. A total of 507 had only one measure of CES-D8, 34 had only two measures, and 9,982 had three or more CES-D8 measures, for a total of 72,273 observations.

3.2.5 Genome-wide association analysis

We contrasted GWAS results using different approaches to incorporate the time-varying phenotypic data: using a single (baseline) measure, taking the average across exams, or conducting a repeated measures analysis that accounts for correlation of responses within individuals.

Baseline and averaged GWA studies were analyzed using a one-step linear regression approach, adjusting for age, sex, site (in MESA) and the first four genome-wide principal components, stratified by race in PLINK v.1.07 [123, 124]. The analytic approach and included covariates are consistent with previous GWAS [119, 120]. Each SNP was analyzed separately, using SNP dosages, in an additive genetic model.

Below are the linear models used in the baseline:

$$E(y_i) = \alpha + \boldsymbol{\beta X}_i + \gamma SNP_i$$

and averaged GWA analysis:

$$E(\bar{y}_{i\cdot}) = \alpha + \boldsymbol{\beta X}_i + \gamma SNP_i$$

Where y_i is the log-transformed depressive symptom measure (baseline CES-D) and $\bar{y}_{i\cdot}$ is the averaged CES-D over all available time points for individual *i*, $\boldsymbol{X}$ is a vector of covariates: age at baseline, sex, site at baseline (for MESA), the top four ethnic-specific principal component loadings for individual *i*, and *SNP* is the dosage values for a particular SNP for individual *i*.

For the repeated measures, we used generalized estimating equations (GEE) to account for within-individual correlations between repeated CES-D measures [150]. Advantages of the repeated measures analysis include improved characterization of a time-varying phenotype and greater power than afforded by a single outcome measure. We did not include a time indicator, since there was no significant time effect across exams for MESA participants. Below is the generalized estimating equation model considering age, sex, site and ethnic-specific principal components:

$$g(E(y_{it})) = g(\mu_{it}) = \alpha_i + \boldsymbol{\beta}\boldsymbol{X}_i' + \boldsymbol{\gamma}\boldsymbol{Z}_{it}' + \delta SNP_i$$

$$V(y_{it})) = \phi a_{it}^{-1} v(\mu_{it})$$

Throughout the dissertation: $R_{tt'} = corr(y_{it}, y_{it'}) = \rho$

For a given depressive symptom measure y (log-transformed within each exam), y_{it} is the t[th] measure (i.e., depressive symptom score measure at exam 1, 3, or 4) for participant *i*. The identity link function is used to describe the variance of y_{it} as a function of the mean, where μ_{it} is the marginal mean of y_{it}. The vector **X** represents time-invariant covariates including sex and top four principal components for individual *i*, measured at baseline. Time-varying covariates, represented by **Z** are age and site for individual *i* at time *t.* Within the 'geepack' package in the R software, we used an exchangeable (compound symmetric) correlation structure, represented as $\boldsymbol{R}_{tt'} = \rho$.[151, 152].

3.2.6 Comparison of p-values across phenotype approach

To examine whether p-values from GWAS in MESA were consistent in rank across the three analysis approaches (baseline, averaged across exams, repeated measures), we calculated Spearman's correlations between the ranks of p-values (for SNP-phenotype associations for baseline versus averaged, baseline versus repeated measures, and averaged versus repeated measures) for the set of SNPs within ethnic group. Spearman's correlation is defined as:

$$r_s = \frac{\sum_{l=1}^{L}(x_l - \bar{x})(y_l - \bar{y})}{\sqrt{\sum_{l=1}^{L}(x_l - \bar{x})^2 \sum_{l=1}^{L}(y_l - \bar{y})^2}}$$

Where x is the rank for individual SNP *l* for a given approach *j*, and y is the rank for individual SNP *l* for a given approach *k*, where $j \neq k$. Rank ties or value duplicates are assigned a rank equal to the average of their positions in the ascending order of the values. We would expect that the test statistics corresponding to the averaged versus repeated measures approach would be highly correlated due to the definition of these two phenotypes.

3.2.7 Meta-analysis

To increase statistical power to detect SNP association, we performed a fixed-effects meta-analysis combining results across all four ethnicities within the MESA study for each of the three phenotype definitions (baseline, averaged, repeated measures), weighting by sample size. In order to further investigate consistency of associations across different studies we also conducted a meta-analysis for European Americans and African Americans (separately) across the MESA and HRS studies for the repeated measures phenotype. We use only the African American and European American samples due to the availability of a large enough sample size for these two ethnicities in HRS. For the analysis that includes both MESA and HRS, the repeated measures phenotype was selected to allow for maximum power. All meta-analyses were performed using METAL [153].

3.3 Results

3.3.1 Descriptive statistics

Descriptive statistics for the MESA and HRS samples are presented in Table 12. The MESA analysis sample includes 6,335 individuals, of which 48% are male, mean age

at baseline is 62.2 years and approximately 40%, 25%, 12%, and 23% are of European-, African-, Chinese-, and Hispanic-American self-reported ethnicity, respectively.

In MESA, the mean baseline depressive symptom score ranged from 6.3 (standard deviation (SD): 6.6) in the Chinese subsample to 9.9 (SD: 9.22) in the Hispanic subsample out of a possible score of 60. CES-D scores in this sample tended to increase slightly over time so that average scores across visits tended to be higher than baseline scores for all ethnicities, except in the Chinese sample which decreased slightly over time. The intraclass correlation (within-person correlation) across all exams for which an individual had a valid CES-D score (up to three time-points) ranged from 0.44 in African Americans to 0.60 in European Americans.

The HRS analysis sample contains 10,163 respondents, with 8,652 European Americans (85%) and 1,511 African Americans (15%). The HRS sample is 41% male with an average age at baseline of 58 years. The CES-D8 depressive symptom score in HRS EA and AA participants increased, though negligibly in EA participants, over time. The intraclass correlation for the HRS participants across exams was 0.48 for the EA participants and 0.51 for the AA participants.

3.3.2 Ethnicity-specific association analysis in MESA

Table 13 shows the number of SNPs, minimum p-value of the adjusted association between SNP dosage and outcome, and the genomic-control inflation factor, lambda, for each ethnicity in MESA and HRS. Quantile-Quantile (QQ) plots and Manhattan plots for all GWA analyses is shown in Appendix 14-Appendix 20) The inflation factor, the extent to which the chi-square statistic is inflated due to confounding

by ethnicity [154], is very close to 1.0 for all analyses, indicating that the potential confounding effect of population structure is adequately adjusted. One SNP reached genome-wide significance in the Hispanic subset in the baseline CES-D approach in the intronic region of the *MUC13* gene (rs1127233, 3q22.1, p-value = 2.73×10^{-8}). This gene has previously been linked to cancer pathogenesis (e.g. [155-164]) but has not been implicated in any psychiatric disorders. There were no other genome-wide significant hits in any of the ethnicities for any of the modeling approaches. There were, however, unique (LD $R^2 < 80\%$) genome-wide suggestive ($5 \times 10^{-8} <$ p-value $\leq 5 \times 10^{-6}$) hits in each ethnicity for each of the modeling approaches: baseline CES-D (EA n=9; AA n=7; CA n=1; HA n=10), averaged CES-D (EA n=6; AA n=9; CA n=2; HA n=4), and repeated measures CES-D (EA n=11; AA n=11; CA n=4; HA n=11) (Table 13). For all ethnicities the majority of the p-values decreased in size from the averaged to the repeated measures CES-D analysis (EA: 50.7% 95%CI (50.6, 50.8), AA: 51.1 (51.0, 51.1), CA: 51.7 (51.6, 51.7), HA: 50.6 (50.6, 50.7). Additional results (presented in Table 14) show the number of SNPs at certain thresholds of α (1×10^{-6}, 1×10^{-5}, 1×10^{-4}, and 1×10^{-3}) for the baseline and averaged approaches. In general, more SNPs were implicated at lower p-values in the averaged approach than in the baseline approach. This, in combination with an increase in the number of SNPs at the genome-wide suggestive level from the baseline to the repeated measures analysis, indicates greater power with the repeated measures analysis.

3.3.3 Sensitivity Analysis

In order to examine whether correction for anti-depressant use influenced results, sensitivity analyses were conducted by excluding participants using anti-depressants. These analyses were performed on the top 10 SNPs associated with depressive symptoms

as ranked by p-value (i.e. the 10 SNPs with the smallest p-values and with ethnicity-specific minor allele frequencies greater than 5% and linkage disequilibrium (LD) $R^2 <$ 80%) in each ethnicity for analyses based on the repeated measures. Results were consistent with the original analyses.

3.3.4 Comparison of results across approaches

To compare association results between the different versions of the CES-D scores, we plotted the p-values from each pair of SNPs for the baseline CES-D score compared to the averaged CES-D score phenotype (Figure 19), the baseline CES-D score compared to the repeated measures CES-D score (Figure 20), and the averaged CES-D score to the repeated measures CES-D score (Figure 21) within each of the four ethnicities in MESA. Each panel shows the $-\log_{10}$ p-value from the two approaches in comparison with an x = y line overlaid, for each of the four ethnicities. The x = y line represents perfect concordance between p-values in the two approaches. For all four ethnicities, the Spearman's rank correlations between the baseline versus averaged CES-D phenotype and between the baseline and repeated measures CES-D phenotypes ranged between 0.46 and 0.57. The correlations between p-values for the averaged versus repeated measures CES-D phenotype were higher. The European sample had the highest correlation (0.92), while the Chinese subset had the lowest (0.85) (Table 15).

3.3.5 Meta-analysis across ethnicities in MESA

The results from the three meta-analyses performed within MESA and across ethnicities for the baseline, averaged and repeated measures CES-D scores are presented in Table 16. For every unique (LD $R^2 < 80\%$) SNP with a p-value $<1x10^{-6}$, we present the chromosome, rs number, base pair location, coded allele, coded allele frequency, Z-score,

meta-p-value, direction of effect, and closet gene within ±50kB of the SNP. The meta-analysis only included SNPs with ethnicity-specific minor allele frequency (MAF) > 0.05. The MAF was calculated within ethnicity using only participants from the MESA study. Thirteen SNPs reached the genome-wide suggestive threshold in these meta-analyses (baseline n=3; averaged n=5, repeated measures n=3). The smallest p-value we observed was in the repeated measures meta-analysis on chromosome 2, (rs41379347, 2q32.2, p-value = 1.81×10^{-7}). This SNP was only present in the Chinese- and Hispanic-American subsamples (with MAF > 0.05). This SNP is in the intronic region of the *STAT1* gene, IFN-γ transcription factor signal transducer and activator of transcription 1, previously implicated as a tumor suppressor [165, 166]. This SNP has not been previously linked to depressive symptoms.

3.3.6 Joint-analysis across studies

Results from the joint-analyses for the European and African Americans, separately, across the MESA and HRS studies are presented in Table 17. While no SNP reached the genome-wide level, eight SNPs (EA n=3; AA n=5) satisfied the suggestive threshold for significance. In European Americans, the smallest observed p-value was on chromosome 4 (rs6842756, 4q35.1, p-value = 6.54×10^{-7}) located within the *ENPP6* gene, which is expressed primarily in the kidney and brain and has not been implicated in any disorders or diseases [167]. In African Americans, the smallest observed p-value was on chromosome 20 (rs2426733, p-value = 2.07×10^{-6}) located downstream of the *RBM38* (20q13.31) oncogene. *RBM38* encodes an RNA binding protein found to regulate *MDM2* (12q14.3-q15) gene expression through mRNA stability [168, 169], but has not been

identified in genetic studies of depressive symptoms or any other psychiatric disorders [165, 167].

3.3.7 Consistency with previous GWAS on depressive symptom scores

There has been one published GWAS conducted on depressive symptom scores [120]. This GWAS found one genome-wide significant hit in overall meta-analysis of 51,258 European-ancestry individuals (rs161645, 5q21, $p = 4.78x10^{-8}$). In our baseline CES-D phenotype meta-analysis across ethnicities within MESA, this SNP had a p-value of 0.067, in the averaged CES-D phenotype GWAS a p-value of 0.006, and in the repeated measures GWAS a p-value of 0.008. The overall direction of effect was consistent with the published GWAS for EA, AA, and HA, though the direction of effect was reversed for CA. The SNP was present in all four MESA ethnicities. In meta-analyses performed across the MESA and HRS studies (for European and African Americans), the p-values for this SNP were 0.951 and 0.113 respectively. The direction of effect in HRS EA was opposite that of MESA EA, though the HRS-specific SNP association was not significant (p-value = 0.48).

3.4 Discussion

This study uses genome-wide association studies of depressive symptoms in a longitudinal framework and across ethnicities to find common variants for depressive symptoms. We include a joint-analysis sample for European and African Americans that, when combined with the MESA sample, composed of 16,498 individuals. We found one genome-wide significant SNP in Hispanic Americans in the baseline, ethnicity-specific,

CES-D approach (rs1270666, 2q32.2, p-value = 2.73×10^{-8}). This is a novel finding in the depressive symptom genetic literature. Though power to detect genetic variants of depression has been shown to increase when assessing depression quantitatively — as opposed to using a dichotomous definition or cutoff point [24] — we did not find any variants that reached genome-wide significant levels in the European-, African- or Chinese-American, ethnicity-specific GWAS, across ethnicity meta-analysis for any ethnicities, or across study meta-analysis for the European and African Americans. However, we did find several novel variants at a genome-wide suggestive level, particularly in the repeated measures analysis. This increase in the number of SNPs found at the genome-wide suggestive level is a reflection of the increase in the power to detect genetic variants using a repeated measures method.

This is the first genome-wide association study, to the authors' knowledge, to investigate depressive symptoms in a longitudinal setting across four different ethnicities. A previously published GWAS on depressive symptoms identified a SNP (rs161645) associated with a large sample of European-ancestry participants measured at a single time point. It is important to note that European Americans from MESA were used in the discovery sample for the previously published GWAS. Our analysis provides support for the association of this SNP with depressive symptoms not only in MESA European, but also in African, Chinese, and Hispanic Americans.

Our analysis suggests that there may be benefits to using a repeated measures analysis in GWAS of phenotypes that may change over time. Longitudinal data is increasingly available from prospective cohorts. A more complete characterization of longitudinal phenotypes provides a powerful platform for analyzing genetic associations

of complex traits. Though we did find evidence of increased power (the number of independent genome-wide suggestive regions and the percent of SNPs decreasing in p-value from averaged to repeated measures depressive symptom score - Table 13), the computational time and computing resources to analyze longitudinal data using a repeat measures approach can be large. Baseline models ran in SNPTEST approximately two to four hours per ethnicity for all 22 chromosomes on four nodes compared to the repeated measures models running at roughly three days for one chromosome, for one ethnicity, on one node. However, the benefits of using repeated measures outweigh the drawback in time to perform the analysis.

The MDD GWAS literature to date includes nine GWAS of MDD, with only one genome-wide significant result [113]. We did not find a significant association with depressive symptoms for the SNP that has reached genome-wide significance in MDD GWAS, nor did we find SNPs within previously hypothesized candidate genes that met criteria for genome-wide or genome-suggestive significance. One potentially important reason for this is that despite the CES-D correlating strongly with depression and having been used in hundreds of studies, the CES-D is not a diagnostic tool like the Composite International Diagnostic Interview (CIDI). The CES-D only measures depressive symptoms over the past week. The MESA study exams were spaced approximately 12 – 24 months apart (the HRS surveys 24 months apart). It is possible that failure to capture changes in depressive symptoms between the assessments introduced measurement error in the phenotype. Nevertheless, the use of multiple measures is a major improvement over studies of depressive symptoms measured at a single point in time.

We included only common variants (those with ethnicity-specific MAF > 5%) in our analysis. One reason we may not have found any significant genetic variants of depressive symptoms is that we did not investigate rare variants or copy number variants that were not part of the genotyping panel or imputation used in these studies. New methods for analyzing rare variants or SNP sets, such as Sequence Kernel Association Testing (SKAT), are being developed and applied and may help to further elucidate genetic predictors of depressive symptoms at a gene-level and across ethnicities [3]. Individual SNPs and variants differ in frequency across ethnicities leading to differences in power to detect genetic effects. However, genes themselves do not differ across ethnicities and form a more natural unit of analysis and inference. Additionally, it is possible that multiple SNPs with small effects, working in concert, could affect individual susceptibility to depression and depressive symptoms [170]. Further, no interactions (gene-gene or gene-environment) were evaluated in these analyses. Interactions could play an important role in revealing the pathogenesis of depression and depressive symptoms.

Combining genetic information across multiple ethnicities can result in false-positive findings from admixture within genetically distinct populations. In our analyses, we used ethnicity-specific principal components to account for admixture within each ethnic group and filtered initial GWAS results by ethnicity-specific minor alleles. The meta-analysis software accounts for both magnitude and direction of effect when combining information across studies (in this case different ethnicities) which is especially appropriate when studies contain differences in ethnicity, phenotype distribution, gender or constraints in sharing of individual level data [153].

Identifying genes that are associated with depression has tremendous potential to transform our understanding and treatment of depression. Utilizing longitudinal measures in GWA studies for depressive symptoms allows researchers to get a better picture of depression over the life-course. Though this study did not find any gene variants that reached genome-wide significance in the repeated measures approach, it provides a first step in examining depressive symptoms in different longitudinal settings and also across multiple ethnicities.

3.5 Acknowledgements

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Table 11 Number of participants with one, two or multiple repeated measures, MESA and HRS

Multi-ethnic Study of Atherosclerosis

Ethnicity	Sample size	One measure	Two measures	Three measures	Total observations
EA	2514	183 (7.3%)	158 (6.3%)	2173 (86.4%)	7018
CA	775	84 (10.8%)	73 (9.4%)	618 (79.7%)	2084
AA	1603	209 (13%)	133 (8.3%)	1261 (78.7%)	4258
HA	1443	174 (12.1%)	143 (9.9%)	1126 (78%)	3838
Total	6335	650 (10.3%)	507 (8%)	5178 (81.7%)	17198

Health and Retirement Survey

Ethnicity	Sample size	One measure	Two measures	Three+ measures	Total observations
EA	8652	27 (0.3%)	107 (1.2%)	8518 (98.5%)	62073
AA	1511	7 (0.5%)	40 (2.6%)	1464 (96.9%)	10200
Total	10163	34 (0.3%)	147 (1.4%)	9982 (98.2%)	72273

EA: European Americans, AA: African Americans, CA: Chinese Americans, HA: Hispanic Americans

Table 12 Descriptive statistics by ethnicity

	MESA[1] n = 6,335				HRS[2] n = 10,163	
	EA	**AA**	**HA**	**CA**	**EA**	**AA**
	n=2,514	n=1,603	n=1,443	n=775	n=8,652	n=1,511
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Depression score[3]						
Baseline CES-D	8.0 (7.8)	7.6 (7.6)	9.9 (9.2)	6.3 (6.6)	1.2 (1.8)	1.2 (1.8)
Averaged CES-D	8.7 (7.4)	7.8 (6.7)	10.2 (8.5)	6.2 (5.6)	1.2 (1.3)	1.9 (1.6)
Age	62.6 (10.2)	62.2 (10.1)	61.4 (10.3)	62.4 (10.4)	58.4 (8.8)	56.8 (8.2)
Sex (%)						
Male	48	46.4	49.3	49.7	41.1	42.3
Site (%)						
Baltimore, MD	20.1	30.1	0	0	-	-
Chicago, IL	20.9	16.1	0	35.4	-	-
Forsyth County, NC	21.8	26.5	0.2	0	-	-
Los Angeles, CA	5.3	8.9	38.4	64.3	-	-
New York, NY	8.3	18.4	29.9	0.3	-	-
St. Paul, MN	23.6	0	31.5	0	-	-
Anti-depressant Use (%)	12.2	3.8	5.8	2.5	-	-
Intraclass correlation						
Repeated Measures CES-D	59.7	44.1	57.1	57.4	47.7	50.5

[1]Multi-Ethnic Study of Atherosclerosis, [2]Health and Retirement Study, [3]CES-D measured as 20-item sum in MESA and as 8-item sum in HRS. EA: European Americans, AA: African Americans, CA: Chinese Americans, HA: Hispanic Americans

Table 13 Minimum p-value from GWAS of baseline, averaged, and repeated measures of CES-D[1] across ethnicities, MESA[2] and HRS[3]

Study	Ethnicity	# of SNPS	Baseline CES-D score			Averaged CES-D score			Repeated measures CES-D score		
			min p-value	# of unique hits[4]	λ[5]	min p-value	# of unique hits	λ	min p-value	# of unique hits	λ
MESA	**AA**	2559964	2.05E-07	7	1.01	6.64E-07	9	1.00	1.63E-07	11	1.01
	EA	2269552	1.33E-07	9	1.01	8.26E-07	6	1.00	6.04E-07	11	1.01
	CA	1943213	2.48E-06	1	0.99	1.42E-06	2	1.00	2.71E-07	4	1.02
	HA	2285460	3.85E-08	10	1	1.61E-06	4	1.00	9.25E-07	11	1.01
HRS	**AA**	2678868	-		-	-		-	2.07E-06		1.01
	EA	2393700	-		-	-		-	6.54E-07		1.04

[1]Center for Epidemiologic Studies – Depression, [2]Multi-Ethnic Study of Atherosclerosis, [3]Health and Retirement Study, [4]Number of unique (independent) SNPs LD $R^2 < 0.80$, filtered at ethnicity-specific minor allele frequency < 5%, genome-wide suggestive ($5x10^{-8} <$ p-value $\leq 5x10^{-6}$), [5]genomic control lambda. EA: European Americans, AA: African Americans, CA: Chinese Americans, HA: Hispanic Americans

Table 14 Comparison of the number of SNPs significant at four α thresholds for the baseline and averaged approaches

	AA		EA		CA		HA	
	Baseline	**Averaged**	**Baseline**	**Averaged**	**Baseline**	**Averaged**	**Baseline**	**Averaged**
	n_{SNPs} (%[a])	n_{SNPs} (%[a])	n_{SNPs} (%[a])	n_{SNPs} (%[a])	n_{SNPs} (%[a])	n_{SNPs} (%[a])	n_{SNPs} (%[a])	n_{SNPs} (%[a])
p-value < $1x10^{-6}$	2 (0.08)	8 (0.30)	1 (0.04)	5 (0.19)	0 (-)	0 (-)	0 (-)	21 (0.85)
p-value < $1x10^{-5}$	32 (1.29)	19 (0.71)	44 (1.67)	30 (1.25)	18 (1.25)	2 (0.13)	27 (1.04)	116 (4.69)
p-value < $1x10^{-4}$	297 (11.99)	235 (8.82)	263 (9.98)	210 (6.94)	100 (6.94)	90 (5.89)	207 (7.98)	350 (14.14)
p-value < $1x10^{-3}$	2146 (86.64)	2404 (90.17)	2328 (88.32)	2326 (90.47)	1324 (91.82)	1435 (93.98)	2361 (90.98)	1989 (80.33)
Total[b]	2477	2666	2636	2571	1442	1527	2595	2476

[b]Percentage is calculated out of the total number of SNPs with p-values < $1x10^{-3}$

[b]Total=total number of SNPs with p-values < $1x10^{-3}$

AA: African Americans, EA: European Americans, CA: Chinese Americans, HA: Hispanic Americans

Table 15 Spearman's correlation coefficients (r_s) and 95% confidence intervals for paired p-values in Multi-Ethnic Study of Atherosclerosis

		Baseline vs averaged CES-D score r_s, (95% Confidence Interval)	Baseline vs repeated measures CES-D score r_s, (95% Confidence Interval)	Averaged vs repeated measures CES-D score r_s, (95% Confidence Interval)
MESA	**AA**	0.53, (0.53, 0.53)	0.54, (0.54, 0.54)	0.88, (0.88, 0.88)
	EA	0.54, (0.54, 0.54)	0.57, (0.57, 0.57)	0.92, (0.92, 0.92)
	CA	0.48, (0.48, 0.48)	0.46, (0.46, 0.47)	0.85, (0.85, 0.85)
	HA	0.54, (0.54, 0.54)	0.56, (0.55, 0.56)	0.88, (0.88, 0.88)

AA: African Americans, EA: European Americans, CA: Chinese Americans, HA: Hispanic Americans

Table 16 Meta-analysis results[1] across ethnicities in MESA[2] (p-values < $1x10^{-5}$) for each depressive symptom approach

Approach	CHR	SNP	Location	Coded Allele	Coded Allele Frequency	Z-score	P-value	Direction[3]	Closest Gene[4] within ±50kb
Baseline									
	8	rs2440212	97270629	A	0.66	4.47	7.73E-06	++++	(GDF6)
	9	rs13440434	131953827	A	0.87	-4.50	6.79E-06	----	(GPR107)
	10	rs7087469	54339854	A	0.13	4.76	1.93E-06	++?+	-
	13	rs9560521	89457392	A	0.13	4.69	2.69E-06	++++	(LINC00559)
	16	rs8046816	71863525	A	0.47	4.53	5.92E-06	++++	-
	20	rs17215529	3923402	A	0.85	4.79	1.66E-06	++?+	RNF24
Averaged									
	1	rs3100865	2795967	T	0.49	4.44	9.02E-06	++++	-
	2	SNP_A-1966287	191577187	T	0.89	-4.58	4.57E-06	??--	STAT1
	2	rs7602149	114357038	T	0.84	-4.57	4.78E-06	--?-	LOC728055
	2	rs13001068	182706602	A	0.92	4.50	6.95E-06	?+?+	(PDE1A)
	7	rs697521	16730681	T	0.13	-4.74	2.12E-06	--?-	BZW2
	8	rs7350109	60753909	A	0.81	-4.50	6.88E-06	--?-	-
	11	rs1448128	121291660	C	0.24	-4.58	4.61E-06	----	-
	22	rs5760767	23696411	T	0.50	4.58	4.62E-06	++++	(TMEM211)
Repeated measures									
	1	rs11590206	145665933	A	0.16	-4.72	2.33E-06	----	(GJA5)
	2	SNP_A-1966287	191577187	T	0.89	-5.22	1.81E-07	??++	STAT1
	2	rs7602149	114357038	T	0.84	-4.62	3.83E-06	++?+	LOC728055
	4	rs13139186	96637940	T	0.90	-4.48	7.44E-06	----	UNC5C
	4	rs233976	104823918	A	0.21	4.47	7.75E-06	?+++	TACR3
	7	rs11771332	86539742	A	0.81	-4.48	7.45E-06	?-?-	(KIAA1324L)
	9	rs2211185	1332721	T	0.77	4.55	5.42E-06	++++	-
	18	rs2728505	21474070	A	0.55	-4.47	7.84E-06	----	-
	22	rs5760767	23696411	T	0.51	4.54	5.68E-06	----	(bA9F11.1)

[1]filtered at ethnicity-specific minor allele frequency of 0.05, where the SNP was present in at least two ethnicities, LD R^2 < 80%, and heterogeneity p-value ≥ 0.1 [2]Multi-Ethnic Study of Atherosclerosis; [3]Order corresponding to direction positions: African, European, Chinese, Hispanic American; [4]parentheses indicate location outside of gene

Table 17 Meta-analysis results[1] between MESA[2] and HRS[3] (p-values < 1x10^{-5}) for each depressive symptom approach within ethnicity

Race	CHR	SNP	Location	Coded Allele	Coded Allele Frequency	Z-score	P-value	Direction[4]	Closest Gene[5] within ±50kb
African American									
	1	rs10776776	114384683	T	0.55	4.73	2.30E-06	++	(SYT6)
	1	rs1417303	235193008	T	0.59	-4.43	9.46E-06	--	LOC440737
	2	rs4629180	101454802	A	0.83	-4.51	6.41E-06	--	(LOC731220)
	2	rs6711630	126534599	T	0.93	4.58	4.70E-06	++	
	7	rs10249133	12514004	T	0.39	-4.47	7.67E-06	--	(LOC100133035)
	8	rs17067630	3661853	A	0.85	4.70	2.57E-06	++	CSMD1
	11	rs11036016	40661316	A	0.80	4.68	2.94E-06	++	LRRC4C
	15	rs4551976	49264445	T	0.63	-4.45	8.48E-06	--	(CYP19A1)
	16	rs365962	85267450	C	0.69	-4.53	5.83E-06	--	(LOC101928614)
	20	rs2426733	55454729	A	0.40	-4.75	2.07E-06	--	(RBM38)
European American									
	1	rs12031875	71357685	A	0.82	4.81	1.54E-06	++	ZRANB2-AS2
	4	rs6842756	185341452	A	0.92	4.98	6.54E-07	++	ENPP6
	6	rs6941340	16145531	T	0.48	-4.47	7.95E-06	--	
	9	rs11794102	111772109	A	0.91	4.54	5.70E-06	++	PALM2-AKAP2
	13	rs6492314	110267411	C	0.28	-4.75	2.00E-06	--	
	16	rs12921740	20219533	T	0.51	-4.55	5.44E-06	--	(GP2)
	18	rs2612547	41290709	A	0.83	4.47	7.94E-06	++	SLC14A2

[1]filtered at ethnicity-specific minor allele frequency of 0.05, where the SNP was present in at least two ethnicities, LD R^2 < 80%, and heterogeneity p-value ≥ 0.1; [2]Multi-Ethnic Study of Atherosclerosis; [3]Health and Retirement Study [4]Order corresponding to direction positions: African, European, Chinese, Hispanic American; [5]parentheses indicate location outside of gene

Figure 19 Comparison of p-values for genome-wide association studies for baseline CES-D score compared to averaged CES-D score

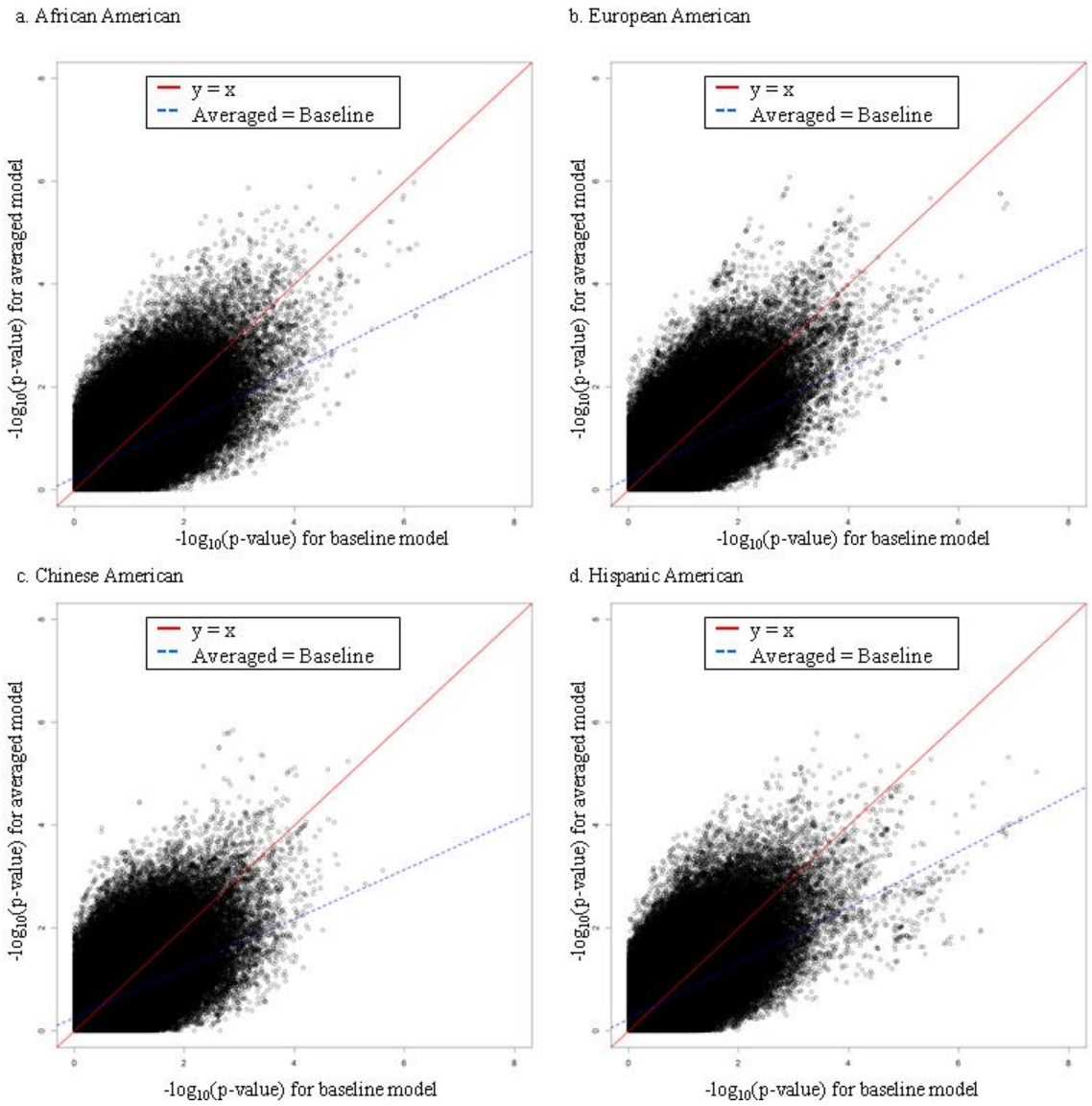


Figure 20 Comparison of p-values for genome-wide association studies for baseline CES-D score compared to repeated measures CES-D score

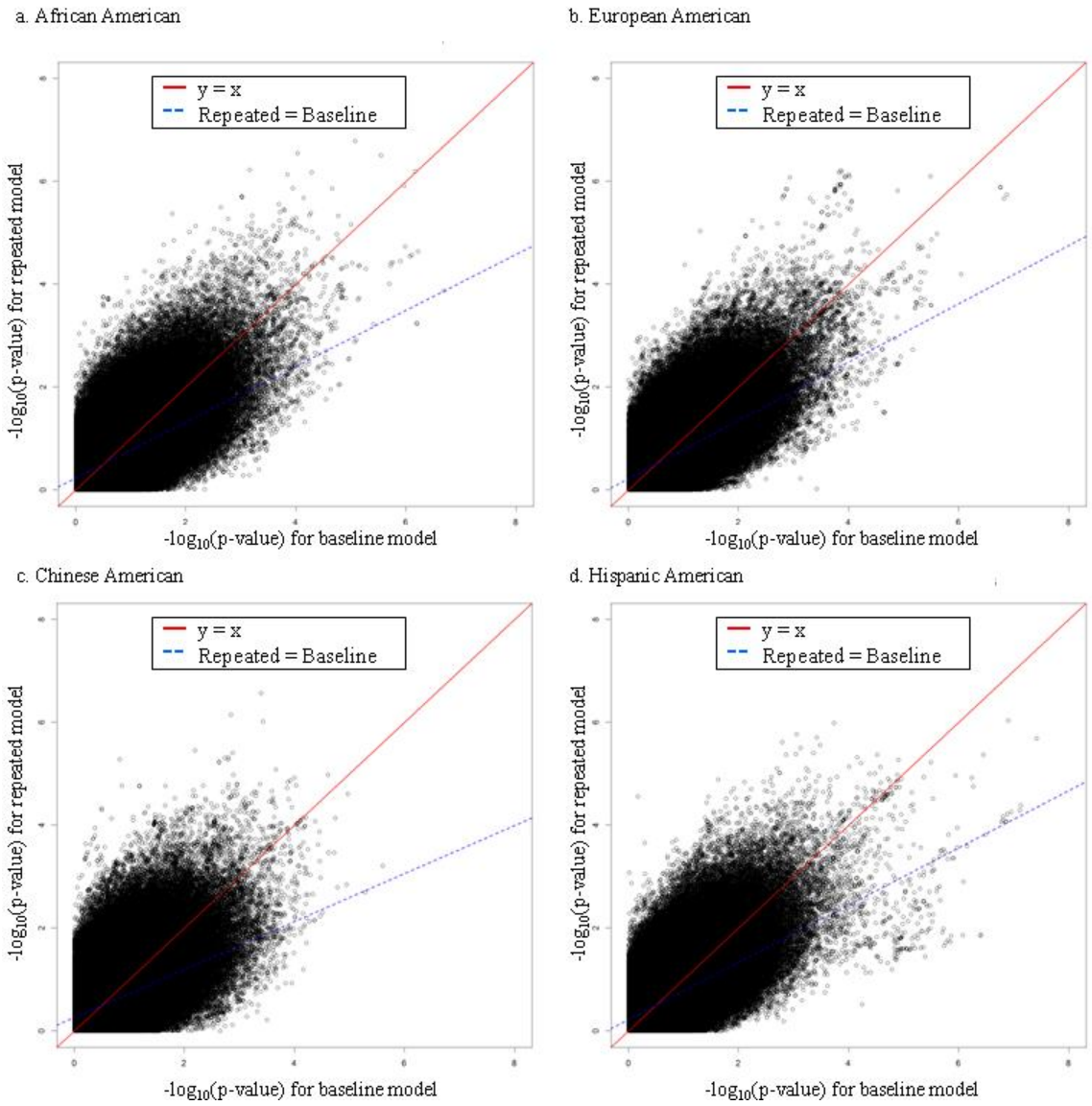
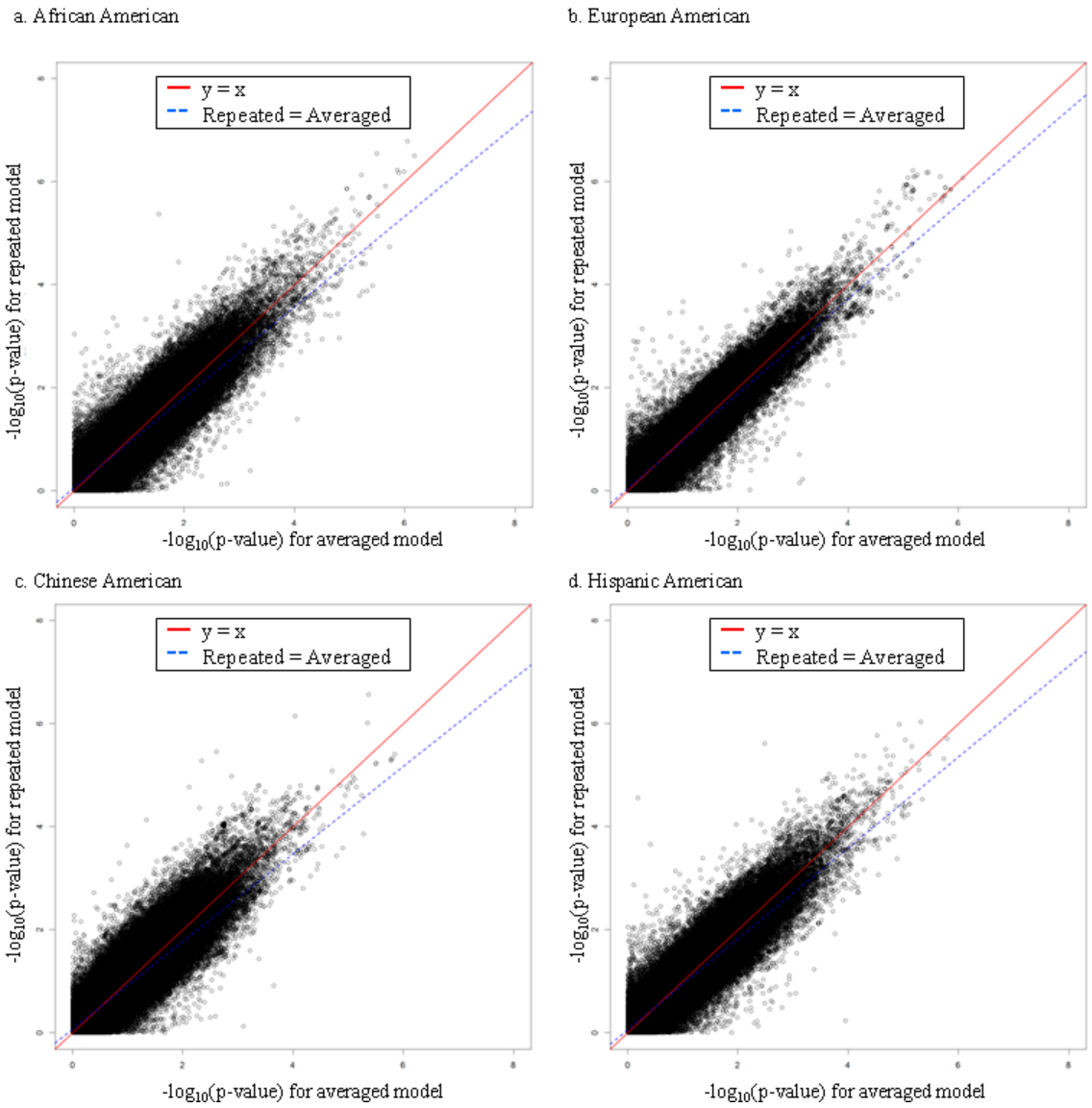


Figure 21 Comparison of p-values for genome-wide association studies for averaged CES-D score compared to repeated measures CES-D score



CHAPTER IV

Associations of genetic regions with depressive symptom scores across ethnic group groups in the Multi-Ethnic Study of Atherosclerosis

4.1 Introduction

Multiple genes are posited to be involved in disorders of highly complex pathophysiology such as mental health disorders. While examining single nucleotide polymorphisms (SNP) through genome-wide association studies (GWAS) is an important first step in identifying genetic risk factors for depressive symptoms, SNP-set based analyses may help us better understand the association between genetic variants and complex phenotypes by identifying genetic regions that are associated with the phenotype across different ethnicities).[171] Because relevant variability in a given genetic region may be indexed by different SNPs in different ethnicities, the failure to perform gene-region analyses may result in underestimates of the effects of genetic variability on the phenotype. For example, there have been a number of conditions (e.g., bipolar disorder, coronary artery disease, hypertension, Parkinson's, amyotrophic lateral sclerosis, Crohn's disease, rheumatoid arthritis, types I and II diabetes, and age-related eye disease) for which analyses of genetic regions identified important genetic predictors whereas traditional SNP analyses did not.[172] The authors of this study identified genes in a single cohort, and confirmed via meta-analysis for multiple cohorts, suggesting that these regions may be more replicable than SNP-based analyses.[172]

Though we have been studying mental health disorders for decades and have characterized the basic epidemiology of depression and depressive symptoms, we have yet to discover a proverbial genetic "smoking gun" through GWA studies of SNPs. Further, we have not been able to identify genetic variants that are associated with depressive symptoms across multiple ethnicities, likely due to different ancestry-based patterns in population stratification and differences in linkage disequilibrium (LD) patterns.[173-175] This results in different "tag SNPs" that are associated with the causal variant(s) appearing in different ethnicities and could lead to what appear to be inconsistent (or non-replicated) SNPs across ethnicities.

I have established that a depressive symptom phenotype over time in a repeated measures approach is beneficial over a single-time point (baseline) measure in GWAS in terms of power. However, well-developed and validated methods of analyzing SNP sets using repeated measures have yet to be implemented in genetic association studies. Therefore, in this chapter, results from averaged depressive symptom scores GWAS are used to take our level of inference from SNPs to sets of SNPs to help identify genetic regions across ethnicities that may be associated with depressive symptoms.

4.2 Methods

4.2.1 Previous GWAS

This study is based off of analysis performed on data from the Multi-Ethnic Study of Atherosclerosis. This study and methods are described extensively in chapters II and III. Briefly, MESA is a longitudinal study supported by NHLBI consisting of individuals from six field centers.[118] All MESA Classic cohort members who provided DNA

samples and were included in the MESA SHARe project are included in this analysis. These analyses were performed in African Americans (AA), European Americans (EA), Chinese Americans (CA), and Hispanic Americans (HA).

The outcome of interest, depressive symptom score, was assessed using the 20-item CES-D Scale[14], adjusted for anti-depressant use and averaged over all exams for which the measure was administered. The ethnicity-specific GWA studies for averaged depressive symptom score were conducted using a linear model, adjusted for age at baseline, sex, site at baseline and the top four ethnicity-specific principal components. SNPs were analyzed as dosages in the SNP-based GWAS using an additive genetic model.

4.2.2 Covariates

Age, sex and study site were assessed at the MESA baseline exam. There were a total of 6,335 MESA participants included in the averaged depressive symptom GWAS (AA 25%, EA 40%, CA 12%, HA 23%). Average age (standard deviation) for the AA, EA, CA, and HA sub-samples was 62.2 (10.1), 62.6 (10.2), 61.4 (10.3), and 62.4 (10.4) years, respectively. Slightly less than half of each ethnicity was male (AA 48.0%, EA 46.4%, HA 49.3%, CA 49.7%). Participants were ascertained from six study sites (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles, CA; New York, NY; and St. Paul, MN).[118] Ethnicity-specific principal components were used to adjust for population stratification.

Adult socioeconomic position (ASEP) was included as an additional covariate to assess any residual confounding over the adjustment for ancestry through the inclusion of principal components. Since several measures of ASEP were available (measuring

different dimensions of socioeconomic position), indicators were summarized into an ASEP score. The methods are based on previous work and combine information on income, education, and wealth (ownership of a home, car, land/property or investments).[129, 130] Income was defined in four categories (<$25,000, $25,000–39,999, $40,000–74,999, or +$75,000). At the baseline examination, highest level of education completed was reported and for these analyses operationalized into four categories (completed high school or less, some college but no degree/technical school certificate, associate or bachelor's degree, or graduate/professional degree). The four wealth indexes included whether the participant: (1) had investments such as stocks, bonds, mutual funds, retirement investments, or other investments (yes/no), (2) owned their home (yes/no) (3) owned a car (yes/no) and (4) owned land or another property that was not their primary residence (yes/no). To create the summary score for ASEP, the individual measures for income, education and wealth were summed (income variable (0 – 3, low to high), education (0 – 3, low to high), and for each wealth indicator, a single point was added). The ASEP score ranged from 0 – 10, with higher scores indicating greater ASEP.

4.2.3 Region selection

Regions for analysis were selected by first ranking the 5,000 SNPs with the lowest p-values from the averaged depressive symptom GWAS (filtered at ethnicity-specific minor allele frequency (MAF) > 0.05) within each ethnicity. Once those SNPs were identified, the union of the SNPs (n_{SNP} = 19,932) was obtained, and each SNP was analyzed in a fixed-effects meta-analysis across the four ethnicities using METAL, weighted by sample size.[153] The small amount of overlap is not surprising due to

differences in LD structures across the ethnicities with different tag SNPs for the causal variant(s) emerging in each ethnicity, and that there were almost 2.5 million imputed or genotyped SNPs analyzed within each ethnicity. The sheer number of analyzed SNPs reduces the probability that the same SNP would be in the top 5,000 in two ethnicities. From the meta-analysis results, SNPs were retained if they had MAF > 0.05 in more than one ethnicity (n_{SNP} = 18,645). SNPs were ranked by lowest p-value (by meta-analysis p-value: $P_{(1)} \leq P_{(2)} \leq \ldots \leq P_{(nSNP)}$) and the top 100 meta-analysis SNPs ($P_{(1)} - P_{(100)}$) were identified.

Starting with the SNP with the lowest meta-analysis p-value ($P_{(1)}$ – referred to as the index SNP), a SNP set region was defined including all SNPs (not only SNPs in the meta-analysis) within a 20 kilobase (kb) region up and downstream of the index SNP (eliminating any SNPs in the meta-analysis top 100 in this region from being an index SNP of a second region). The 40kb total region was selected to conservatively capture the average size of a linkage disequilibrium (LD) block. In EA and AA populations, average LD, calculated by r^2, declines to approximately 0.15 - 0.25 at a distance of 40kb.[176] We continued this process until all regions were identified from the top 100 SNPs from meta-analysis ($n_{regions}$ = 47). It is possible that regions overlap slightly if the index SNP of $region_i$ and the index SNP of $region_j$ ($i \neq j$) are more than 20kb but less than 40kb away from each other. This occurred five times (regions [3, 4], [5, 6], [7, 8], [27, 28, and 29], [41, 42]).

4.2.4 Sequence Kernel Association Testing (SKAT)

SKAT was performed for each SNP set region for each ethnicity separately. SKAT analysis results in a Q score for each region. The Q statistic is composed of a

mixture of chi-squared distributions under the null hypotheses that can be evaluated explicitly and used as a reference distribution to compute the P-values. Genetic main effects are tested by:

$$\bar{y}_{i\cdot} = \alpha_0 + \boldsymbol{\alpha}'\boldsymbol{X_i} + \boldsymbol{\beta}'\boldsymbol{G_i} + \epsilon_i$$

Where $\bar{y}_{i\cdot}$ is the log-transformed, averaged depressive symptom score corresponding to individual *i*, α_0 is an intercept term, $\boldsymbol{X_i}$ is a vector of non-genetic covariates (age, sex, study site, PC1 – 4, and ASEP), $\boldsymbol{G_i}$ is a matrix of best-call genotypes (0 = no copies of the coded allele, 1 = one copy of the coded allele, 2 = two copies of the coded allele). The coded allele is the same for all ethnicities. Measurement error ϵ_i follows any distribution with mean zero and variance σ^2. The vector of regression coefficients for the covariates is represented by $\boldsymbol{\alpha}$, and $\boldsymbol{\beta}$ is a vector of regression coefficients for the *p* observed gene variants in the region. A primary assumption of SKAT is that each β_j, j = 1, …, p follows an arbitrary distribution with mean zero and variance $w_j\,\tau$. The weights, w_j, are specified as the MAF in this analysis. Testing H_0: $\tau = 0$ is equivalent to testing H_0: $\boldsymbol{\beta} = 0$. Since this analysis is only concerned with the effects of common SNP variants, not the effects of rare variants or epistatic effects, the analysis is implemented using a linear kernel. Results with significant p-values indicate that there is at least one non-zero beta in the region. The variance-component score statistic is

$$Q = (\overline{\boldsymbol{y}} - \hat{\mu})'\boldsymbol{K}(\overline{\boldsymbol{y}} - \hat{\mu})$$

where **K** = **GWG'**, $\hat{\mu}$ is the predicted mean of $\overline{\boldsymbol{y}}_{\boldsymbol{i}}$ under the null model.4.2.5 Meta-analysis

MetaSKAT allows for the meta-analysis of SNP set-level results across cohorts, in this case ethnicities.[177] To allow for heterogeneity across studies, MetaSKAT assumes effect sizes of markers in different studies are independent and follow a common distribution. The meta-analysis SKAT test statistic is:

$$Q_{het-meta-SKAT} = \sum_{j=1}^{m} \sum_{k=1}^{K} w_{kj}^2 S_{kj}^2$$

Where *j* is a specific variant and *m* is the total number of variants in a region, *K* is the number of cohorts (ethnicities), w_{kj}^2 is the marker-specific weight (a function of MAF of SNP *j*) and S_{kj}^2 is the score statistic of the j^{th} variant in linear regression model. This test assumes the effect sizes of markers in different studies are independent and follow a common distribution. Individual-level genotype data were used, with an unweighted kernel (weights.beta = c(1,1)), and allowing for ethnicity-specific MAFs.

4.3 Results

The information about each region is presented in Table 18. The index SNP, chromosome, region start and stop position (kb), coded allele, the ethnicity from which the SNP was discovered, MAF, minor allele (MA), and p-value for the discovery ethnicity, and the p-value from the averaged CES-D meta-analysis are described. There were nine index SNPs originally from the African-American GWAS, 17 index SNPs from the European-American GWAS, four index SNPs from the Chinese-American GWAS, and 15 SNPs from the Hispanic GWAS. One index SNP (index SNP 22) was in the top 5,000 SNPs for both African Americans and Hispanic Americans.

Out of the 47 regions, 21 had no genes within ±100kb of the index SNP.[178] There were 14 regions that lay within (or partially within) a gene, seven regions which were in a gene and also had other genes within ±100kb of the index SNP, and five regions that were not in genes but had genes within ±100kb of the index SNP. Twenty-one regions had no genes within ±100kb of the index SNP.

4.3.1 SKAT

SKAT and MetaSKAT results are presented in Table 19. This table includes ethnicity-specific p-values and the number of markers used in analysis (after non-varying SNPs and SNPs with MAF < 0.05 were eliminated) in each region, as well as the across-ethnicity MetaSKAT p-values. LocusZoom[178] plots for each region is located in Appendix 21 – Appendix 67. Plots show p-values from the averaged CES-D GWAS plotted against chromosomal position for each ethnicity, for each region, with an overlay of the recombination rate in cM/Mb.

4.3.2 MetaSKAT

At an α of 0.05 in the ethnicity-specific SKAT analysis adjusted for age, sex, site, and ASEP there were twelve regions significantly ($\alpha = 0.05$) associated with averaged depressive symptoms in the AA sub-sample, eighteen regions in the EA sub-sample, six regions in the CA sub-sample, and eleven regions in the HA sub-sample. After Bonferroni correction for the number of regions ($\alpha_{Bonferroni} = 0.001$), one region (region 43) in both the AA and CA sub-samples, six regions (regions 1, 17, 19, 26 – 28) in the EA sub-sample and two regions (regions 7, 8) in the HA sub-sample remained significant

There were no genes within ±100kb of the index SNP for region 1; regions 8, 17, 19 and 43 fell within the genes PPA2, GPLD1, and MEGF11 respectively. These genes are discussed later in this chapter. Regions 26 – 28 are overlapping regions and do not have any established genes within ±100kb of the index SNP for any of the three regions. Regions seven and eight are overlapping regions near ABCB11 and partially in G6PC2.

In the MetaSKAT analysis adjusted for age, sex, site, and ASEP, 29 out of 47 regions were significant at an α of 0.05. Four regions remained significant after Bonferroni adjustment ($\alpha_{Bonferroni} = 0.001$): region 19 (located within *GPLD1*, 6p22.1), and regions 26 – 28 (chromosome 8:60733909kb-60850808kb) and two regions, region 17 (located within *PPA2*, 4q24) and region 43 (located within *MEGF11*, 15q22.31) were approaching significance (p-value = 1.40×10^{-3}, and 1.18×10^{-3}, respectively).

4.4 Discussion

In this analysis we have taken results from a GWAS on averaged depressive symptom score from four different ethnicities, determined the top SNP sets from the GWAS across ethnicities, and examined the SNP-set associations with averaged depressive symptom score. The SNP-set association analysis using SKAT resulted in nine total significant regions at an $\alpha_{Bonferroni}$ of 0.001, one in both the HA and CA, six in the EA and two in the HA subsample adjusting for age, sex, site, and ASEP. MetaSKAT analysis resulted in four regions (three of which neighbored each other) that were significant after Bonferroni adjustment (region 19, p-value 1.71×10^{-4}; region 26, p-value 1.17×10^{-4}; region 27, p-value 9.69×10^{-5}; region 28, p-value 7.47×10^{-4}) and two regions that were marginally significant (region 17, p-value = 1.40×10^{-3}; region 43, p-

value 1.18 x 10^{-3}). MetaSKAT p-values of all regions, adjusted for age, sex, site, and ASEP, ranged from 9.7 x 10^{-5} to 6.6 x 10^{-1}.

4.4.1 Region 17

One region (17) on chromosome 4 had been previously associated with psychiatric phenotypes, though not for depressive symptoms specifically. Though only marginally significant at the Bonferroni level when adjusted for age, sex, site, and ASEP, region 17 is contained completely within the *PPA2* (pyrophosphatase (inorganic) 2) gene.[179] Two SNPs in *PPA2* have been reported to reach genome-wide significance (p-value 3.68 x 10^{-7}, 5.05 x 10^{-7}) in GWAS for clinician-reported illness severity of schizophrenia.[180] Additionally, a gene (*DKK2*) in the chr4q22-q32 area was identified based on systematic gene-based screening to be associated with quantitative trait of alcohol dependence symptom counts.[181] This region may play a part in mental health psychopathologies. Significance in this region is driven primarily by the EA sub-sample, which is shown in Figure 22 by the large number of SNPs with small p-values in the EA ethnicity. This region was marginally significant (p-value = 0.0014) at the $\alpha_{Bonferroni}$ of < 0.001 after adjusting for ASEP, suggesting that the relationship between the joint SNP effect in this region and averaged depressive symptom scores is partially mitigated by adult socioeconomic position – which will be investigated further in the subsequent chapter.

4.4.2 Region 19

Region 19 is contained completely within the *GPLD1* (glycosylphosphatidylinositol specific phospholipase D1) gene on chromosome 6. Within this gene rs1883415 and rs9467160 have both been associated with the liver enzyme

alkaline phosphatase levels (6.0×10^{-26}, 1.0×10^{-11})[182, 183] This gene, or SNPs within this gene, has not been found to be associated with depressive symptoms or any other psychiatric disorder. Region 19 shows a different pattern of p-values than in region 18 (Figure 23). While the strongest signal is in the EA ethnicity, there is a cluster of low p-values in the AA ethnicity near position 24.57Mb. This region remained significant at the $\alpha_{Bonferroni}$ of < 0.001 after adjusting for ASEP.

4.4.3 Regions 26 – 28

These regions are within a gene desert with no genes within ± 250kb of either side of the region. This set of regions includes a strong overall signal of an association with depressive symptoms, particularly in the EA ethnicity, though there are also marked signals in the CA and HA sub-samples as well (Figure 24). Within each ethnicity, the strongest signals in each region are coming from different SNPs (e.g. the smallest p-value in EA is not from the same SNP that has the strongest signal in AA). These three regions all remained significant at the $\alpha_{Bonferroni} = 0.001$ after adjusting for ASEP.

4.4.2 Region 43

This region is located in the *MEGF11* gene (multiple EGF-like-domains 11, 15q22.31).[167] Strong evidence was seen in both the AA and CA ethnicity-specific SKAT analysis (AA p-value = 1.70×10^{-4}, CA p-value = 3.59×10^{-4}). Variants within this gene have been cited as predictors of hemorrhagic stroke and hypertension in Japanese individuals,[184, 185] but this region is novel in psychiatric disorders. There is strong evidence of an association with averaged depressive symptom scores in both the AA and CA subgroups (Figure 25).

For the regions which were not located in genes, the functionality of the region was investigated through the Encyclopedia of DNA Elements (ENCODE). ENCODE aims to build a database of functional elements in the human genome, including protein and RNA level elements, and regulatory elements that control cells and circumstances in which a gene is active.[186, 187] Because this region is in a gene desert, a preliminary bioinformatic analysis may provide information on functional elements related to enhancer or transcription factor binding in this region. These analyses (Figure 26) reveal that this region may overlap with an H3K4Me1 and H3K27Ac histone mark (a feature often located near active regulatory elements), several DNase1 hypersensitivity clusters (a chromatin accessibility feature common to cis-regulatory sequences), and transcription factor binding sites. All of this evidence is suggestive of potential functional consequences of genetic variation in this particular region – suggesting that further functional characterization is warranted.

Unlike a previously published GWAS/SNP-set analysis[171] – we did not take our top SNPs from our ethnicity-specific GWAS. Rather we selected the top SNPs from a meta-analysis across the four ethnicities, since our goal was to find regions associated with depressive symptom phenotype across multiple ethnicities. This is the reason we may not have seen our strongest signal in a region as that region's index SNP. It is also apparent that there may be effects in only a subset of the ethnicities (e.g. in EA and HA only, in CA, HA, and AA only, etc.) as opposed to across all four of our examined ethnicities. Future research should consider all combinations of ethnicities in SNP-set analysis to elucidate regions that are associated with phenotypes under study.

Using MetaSKAT permitted heterogeneous effects across the ethnicities and a reduction in the number of statistical tests performed over individual SNP analysis. MetaSKAT allows for the summary of SNP heterogeneity, in terms of direction of effect, into a single statistic. That is, a SNP-set could have significant positive effects in one ethnicity and significant negative effects in a second ethnicity that could result in a null overall effect of the SNP-set using other methods, whereas MetaSKAT would indicate that the region was a significant predictor of the outcome. This method allowed us to discover genetic signals from a set of SNPs that were not apparent when the SNP-level GWAS was performed. Like previously published MetaSKAT analysis on complex traits,[171] this method better reflects the biology of the trait because truly associated genes likely have variants with differing direction and size of effect in different ethnicities. Our results produced an important insight into the genetic association of depressive symptoms across multiple ethnicities: there is evidence that different SNPs from different ethnicities may be implicating genetic regions that are consistent across ethnicities. Our findings (particularly region 19 (Figure 23) and regions 26-28 (Figure 24)) provide justification for moving out of performing solely individual SNP-based GWAS and into adding regional/gene-level analysis when examining a phenotype across multiple ethnicities.

Though regions were chosen using a fairly conservative genetic distance of ±40kb, regions of true association could be larger or smaller than our selected size. We detected regions that overlapped, which may imply larger regions ought to be created from these abutting regions. Future research should determine biologically relevant regions while still incorporating the SNP-based GWAS information, or possibly use other

approaches (moving windows, LD block refinement, gene-regions, etc.) to elucidate genetic regions.

No genetic studies to date have examined depressive symptoms at a genetic region level, let alone across multiple ethnicities. This chapter represents novel methods and findings that advance our ability to examine the variations in multiple regions and their associations with depressive symptoms not only within ethnicities but across them as well. These methods can be extended to any complex phenotype.

4.5 Acknowledgements

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Table 18 Region information for the 47 regions

				Discovery Ethnicity				
Region	**Index SNP**	**CHR:Region start/stop position (bp)**	**Coded Allele**	**Discovery Ethnicity**	**MAF**	**MA**	**p-value**	**Meta p-value, Averaged CES-D**
1	rs3100865	1:2775967-2815967	T	EA	0.29	T	3.95E-05	9.02E-06
2	rs7550557	1:216321556-216361556	A	CA	0.05	G	1.07E-03	3.92E-05
3	rs12711789	2:114307098-114347098	A	HA	0.08	A	1.53E-03	1.86E-05
4	rs7602149	2:114337038-114377038	T	HA	0.10	T	1.58E-04	4.78E-06
5	rs6710525	2:114392038-114432038	A	HA	0.08	G	1.35E-03	3.05E-05
6	rs13425176	2:114413390-114453390	A	HA	0.08	C	1.24E-03	2.68E-05
7	rs519887	2:169469131-169509131	T	HA	0.46	C	8.61E-05	3.00E-05
8	rs853772	2:169502901-169542901	T	HA	0.48	T	9.92E-05	4.48E-05
9	rs12692904	2:169992063-170032063	T	EA	0.23	C	1.88E-03	4.06E-05
10	rs13001068	2:182686602-182726602	A	HA	0.06	G	1.06E-04	6.95E-06
11	rs1569108	2:183196291-183236291	T	AA	0.46	C	1.97E-03	5.01E-05
12	SNP_A-1966287	2:191557187-191597187	T	HA	0.11	T	2.97E-05	4.57E-06
13	rs4389282	2:192565881-192605881	T	EA	0.08	T	1.10E-03	4.30E-05
14	rs6802476	3:151302625-151342625	C	HA	0.09	G	1.50E-04	5.01E-05
15	rs3796972	4:104755240-104795240	A	EA	0.32	C	2.02E-06	4.89E-06
16	rs233976	4:104803918-104843918	A	EA	0.17	A	1.90E-04	1.34E-05
17	rs2726516	4:106545655-106585655	A	EA	0.43	A	1.21E-04	3.58E-05
18	rs13130595	4:177364585-177404585	T	CA	0.25	C	2.52E-03	3.93E-05
19	rs9467173	6:24547252-24587252	T	EA	0.46	T	1.02E-03	2.71E-05
20	rs4626500	7:5353108-5393108	A	AA	0.11	A	1.46E-03	1.30E-05
21	rs697521	7:16710681-16750681	T	AA/HA	0.18/0.09	G	1.01E-03/6.09E-04	2.12E-06
22	rs10234941	7:42452470-42492470	T	AA	0.14	T	1.44E-03	1.33E-05

23	rs11771332	7:86519742-86559742	A	HA	0.11	T	1.54E-04	2.52E-05
24	rs13271255	8:5383703-5423703	T	EA	0.11	C	1.39E-03	2.98E-05
25	rs4273841	8:59034140-59074140	C	HA	0.18	G	3.27E-04	1.45E-05
26	rs7350109	8:60733909-60773909	A	EA	0.25	T	6.85E-06	6.88E-06
27	rs4360284	8:60771439-60811439	C	EA	0.26	G	1.25E-05	6.89E-06
28	rs9643512	8:60810808-60850808	T	EA	0.26	T	8.53E-05	2.41E-05
29	rs1377249	8:78208511-78248511	A	AA	0.24	C	1.22E-03	1.97E-05
30	rs17148529	10:10685682-10725682	T	AA	0.14	T	4.94E-04	2.83E-05
31	rs17620681	10:54883832-54923832	T	EA	0.22	C	1.91E-03	1.83E-05
32	rs1159612	10:85299038-85339038	T	AA	0.11	T	1.13E-03	2.87E-05
33	rs11015985	10:129649308-129689308	T	EA	0.21	C	8.33E-04	1.21E-05
34	rs1448128	11:121271660-121311660	C	EA	0.19	C	3.74E-05	4.61E-06
35	rs1863838	12:9183291-9223291	T	AA	0.23	G	3.14E-05	4.84E-05
36	rs776896	12:39714697-39754697	A	HA	0.17	A	1.16E-03	3.20E-05
37	rs2229774	12:51871812-51911812	A	EA	0.07	A	3.30E-04	1.04E-05
38	rs1765856	13:33802221-33842221	A	EA	0.28	G	9.06E-04	2.45E-05
39	rs9560521	13:89437392-89477392	A	EA	0.09	A	1.03E-03	1.92E-05
40	rs9323096	14:43747537-43787537	A	CA	0.09	A	2.98E-03	4.92E-05
41	rs10149069	14:43786179-43826179	A	CA	0.09	G	1.58E-03	2.22E-05
42	rs3784589	15:29062006-29102006	A	EA	0.06	A	2.59E-04	3.89E-05
43	rs12148583	15:64262685-64302685	A	CA	0.47	T	9.93E-06	1.66E-05
44	rs729650	15:77126365-77166365	T	AA	0.11	C	3.68E-04	4.51E-05
45	rs8046816	16:71843525-71883525	A	AA	0.43	C	5.16E-04	3.83E-05
46	rs2728505	18:21454070-21494070	A	HA	0.40	C	5.76E-04	2.27E-05
47	rs4140486	22:23660087-23700087	A	HA	0.20	G	1.73E-03	6.28E-06

CHR: chromosome, bp: basepairs, MAF: minor allele frequency, MA: minor allele, CES-D: Center for Epidemiologic Sciences – Depression score, AA: African American, EA: European American, CA: Chinese American, HA: Hispanic American

Table 19 SKAT and MetaSKAT, fully adjusted

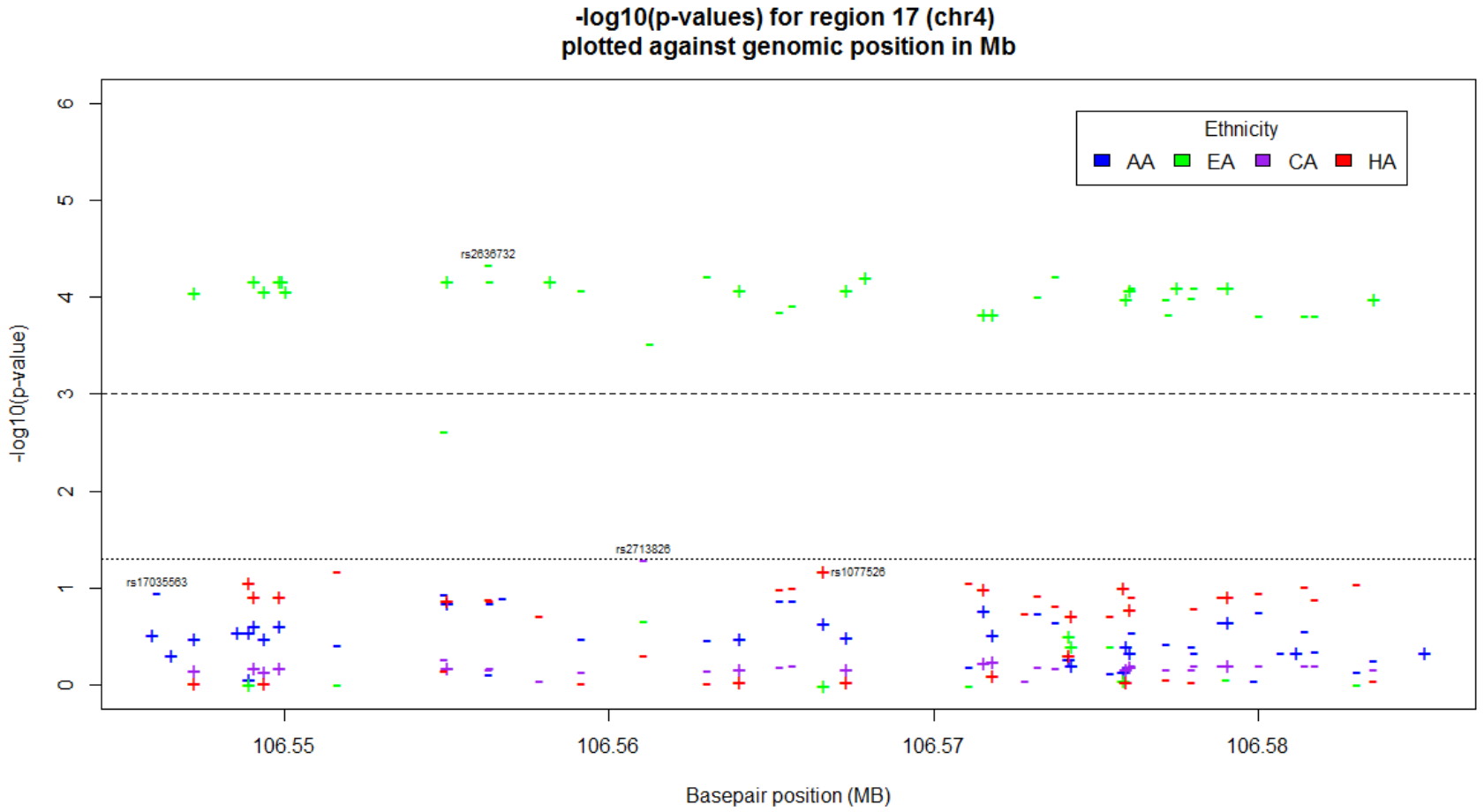
		SKAT(AA)		SKAT(EA)		SKAT(CA)		SKAT(HA)		MetaSKAT	Genes within ±100kb of index SNP[a]
Region	**Chr**	**p-value**	**n_{SNP}**	**p-value**	**n_{SNP}**	**p-value**	**n_{SNP}**	**p-value**	**n_{SNP}**	**p-value**	
1	1	8.80E-01	18	1.60E-04	19	6.55E-01	20	1.56E-01	22	7.71E-03	-
2	1	1.98E-01	47	1.93E-01	43	9.86E-02	41	7.43E-01	46	1.96E-01	-
3	2	2.28E-01	53	7.66E-03	41	1.34E-01	40	6.61E-02	53	4.27E-02	(ACTR3)
4	2	3.62E-01	43	8.31E-02	35	1.69E-01	33	9.29E-02	43	6.16E-03	ACTR3
5	2	4.47E-01	38	2.37E-02	32	1.33E-01	32	1.50E-01	38	4.14E-02	ACTR3
6	2	3.11E-01	50	1.66E-02	44	7.50E-02	41	1.90E-01	50	1.58E-02	ACTR3
7	2	1.15E-01	76	1.33E-01	63	5.60E-01	56	5.95E-05	75	4.07E-03	(ABCB11)
8	2	2.22E-01	56	2.70E-01	52	8.09E-01	45	2.93E-04	55	1.35E-02	G6PC2, (ABCB11)
9	2	1.16E-01	55	7.59E-03	59	1.77E-02	49	3.44E-01	56	3.41E-03	(BBS5)
10	2	9.34E-01	72	5.36E-02	63	1.70E-01	57	2.13E-02	71	3.36E-02	(PPP1R1C, PDE1A)
11	2	8.09E-03	58	1.66E-02	57	9.38E-01	49	1.08E-01	55	2.08E-03	-
12	2	6.09E-01	45	8.56E-01	38	3.51E-01	35	3.40E-03	42	2.11E-01	STAT1, (STAT4)
13	2	4.04E-01	20	9.69E-02	20	1.24E-01	18	4.84E-01	25	1.72E-01	TMEFF2
14	3	1.73E-01	93	1.07E-01	89	5.73E-01	89	3.44E-02	94	4.42E-02	-
15	3	7.40E-01	34	7.65E-03	33	6.92E-01	30	7.25E-02	36	5.16E-01	TACR3
16	4	6.60E-01	42	1.21E-01	30	6.57E-01	30	1.89E-01	42	1.89E-01	TACR3
17	4	7.47E-01	57	2.31E-04	54	6.79E-01	45	2.70E-01	57	1.40E-03	PPA2, (EEF1A1P9)
18	4	4.66E-02	73	2.44E-01	67	1.07E-02	60	2.15E-01	77	3.40E-01	ASB5, (SPATA4)
19	4	2.50E-02	84	2.88E-04	77	1.34E-01	79	9.36E-01	85	1.71E-04	GPLD1, (MRS2, ALDH5A1)
20	6	5.03E-02	9	4.43E-01	12	1.41E-01	8	3.01E-02	11	8.19E-02	TNRC18
21	7	3.07E-01	58	3.00E-02	70	5.11E-01	54	2.24E-02	73	8.12E-03	(BZW2, TSPAN13)
22	7	1.42E-02	75	1.48E-01	77	5.84E-01	54	7.67E-01	74	6.61E-02	-
23	7	2.90E-02	48	1.12E-01	49	7.75E-01	42	2.03E-01	47	2.76E-02	KIA13242
24	7	8.76E-03	129	1.52E-01	120	4.21E-01	97	8.59E-01	127	6.95E-02	-
25	8	4.09E-01	34	5.92E-01	23	3.25E-01	21	4.88E-02	34	3.25E-01	FAM110B
26	8	5.31E-02	35	1.62E-04	18	9.04E-02	28	1.15E-01	36	1.17E-04	-
27	8	3.71E-01	41	1.26E-04	25	1.31E-01	30	1.62E-01	42	9.69E-05	-

28	8	5.98E-01	54	5.96E-04	45	3.03E-01	45	4.76E-01	54	7.47E-04	-
29	8	4.54E-02	65	9.25E-02	60	1.87E-01	56	2.84E-01	65	4.31E-02	-
30	8	1.17E-02	79	6.54E-01	74	9.12E-01	68	4.25E-01	80	1.39E-01	-
31	10	8.64E-01	70	4.27E-01	59	4.83E-01	31	8.55E-01	72	6.60E-01	-
32	10	2.56E-01	69	3.07E-01	71	5.51E-01	65	4.97E-02	69	1.63E-01	-
33	10	1.04E-01	47	2.07E-02	57	8.17E-01	43	4.29E-01	56	3.25E-02	PTPRE
34	10	4.40E-03	52	3.02E-02	45	2.55E-01	44	1.11E-01	52	3.23E-03	-
35	11	5.38E-02	51	2.72E-01	47	5.44E-01	41	3.26E-01	50	1.44E-01	P2P
36	12	3.36E-01	65	5.20E-01	44	6.21E-01	60	2.34E-01	64	5.28E-01	CNTN1
37	12	6.30E-01	23	8.44E-02	23	6.80E-01	18	4.79E-01	24	2.77E-01	ITGB7, RARG, (CSAD, ZNF740, MFSD5)
38	12	4.81E-01	94	3.37E-02	85	2.78E-01	78	2.52E-01	94	4.35E-02	-
39	13	3.67E-01	51	2.38E-01	49	9.42E-01	37	4.38E-01	50	3.63E-01	-
40	13	3.86E-01	31	5.08E-02	31	5.32E-03	27	1.57E-01	32	1.02E-02	-
41	14	8.43E-01	54	4.46E-02	56	4.47E-03	47	2.23E-01	53	3.07E-02	-
42	14	8.85E-03	33	4.27E-02	32	7.42E-01	29	5.01E-01	34	1.72E-02	TRPM1, MTMR10, (MIR211)
43	15	1.70E-04	65	3.40E-01	68.00	3.59E-04	63	5.08E-01	68	1.18E-03	MEGF11
44	15	3.63E-02	35	6.88E-02	32	3.48E-01	30	7.49E-01	37	3.00E-02	RASGRF1
45	16	1.52E-01	48	3.43E-01	42	2.23E-01	31	2.66E-01	46	2.23E-01	-
46	18	7.47E-02	52	1.95E-01	44	7.33E-01	44	2.06E-02	53	3.74E-02	-
47	22	7.34E-01	28	1.10E-01	26	1.08E-02	24	1.09E-02	31	2.31E-02	TMEM211, (SGSM1)

Models adjusted for age, sex, site, and adult socioeconomic position

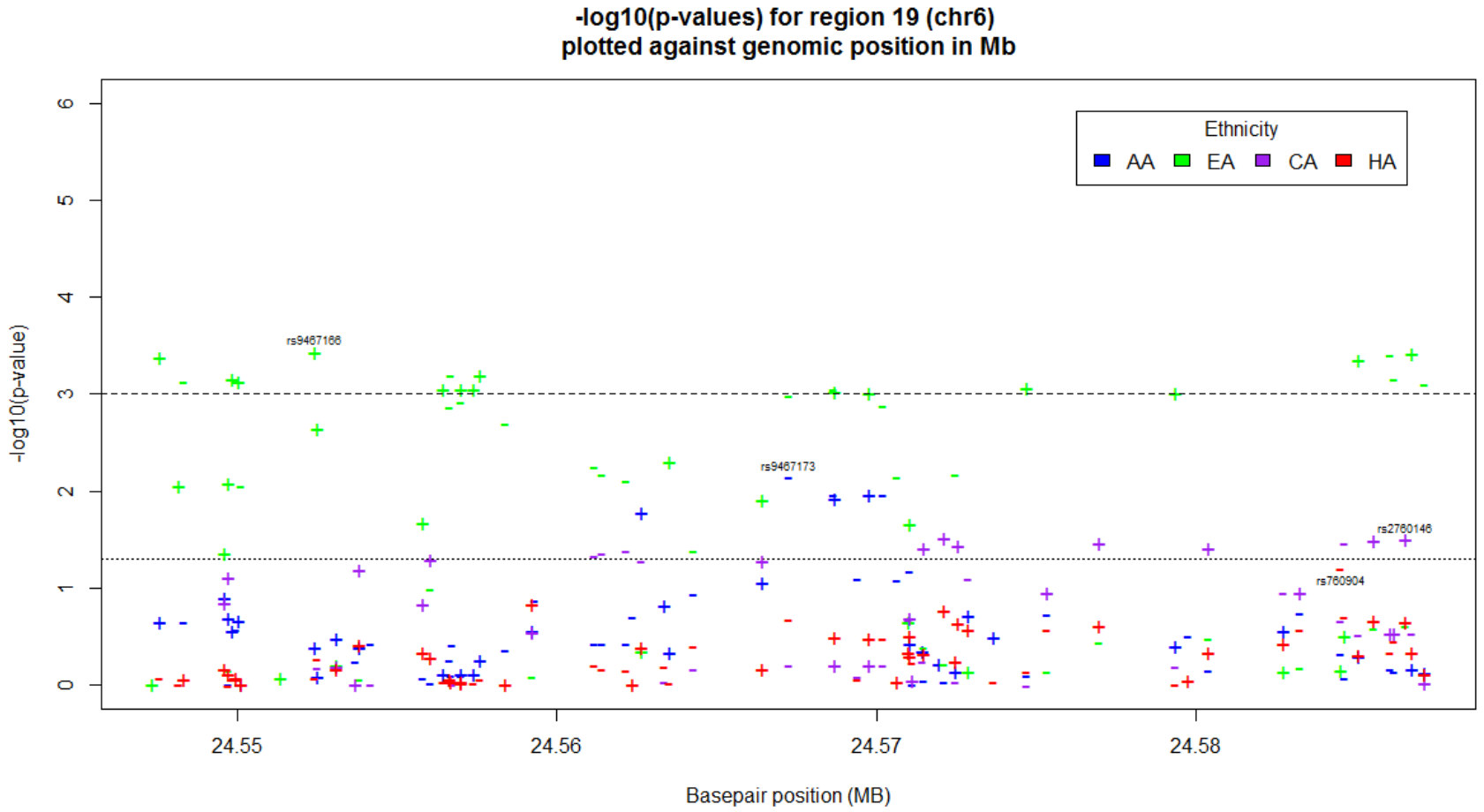
SKAT: Sequence Kernel Association Test, Chr: chromosome, n: number of SNPs in analysis, AA: African American, EA: European American, CA: Chinese American, HA: Hispanic American, highlighted p-values are less than 0.05. [a]Parentheses indicate the region is not within the gene listed

Figure 22 -log$_{10}$(p-values) for region 17 (chr4) plotted against genomic position in Mb



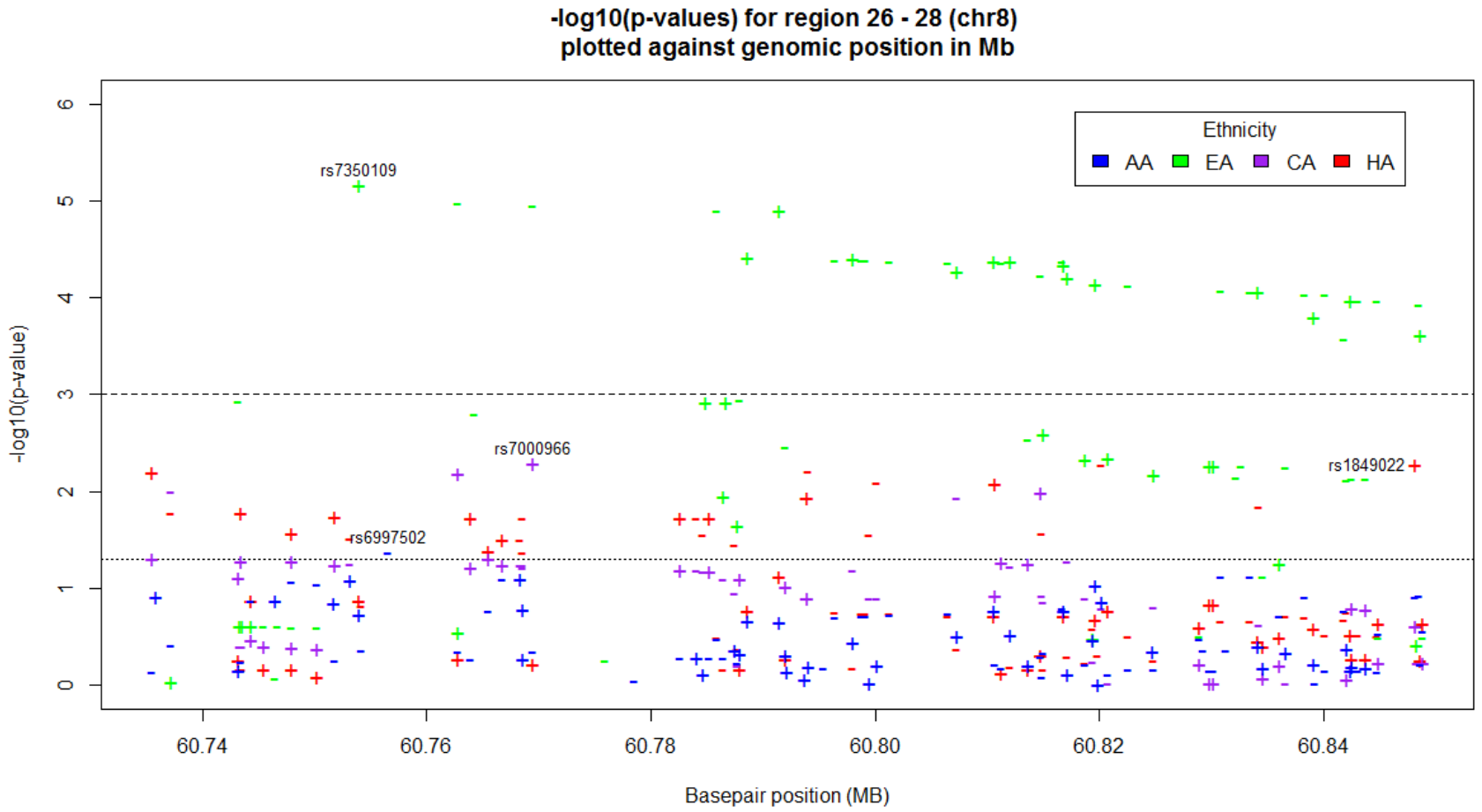
Top SNP in each ethnicity is identified by rs number. AA: African American, EA: European American, CA: Chinese American, HA: Hispanic American. +: positive effect for coded allele, -: negative effect for coded allele. --- indicates p-value = 0.001, ····· indicates p-value = 0.05

Figure 23 -log$_{10}$(p-values) for region 19 (chr6) plotted against genomic position in Mb



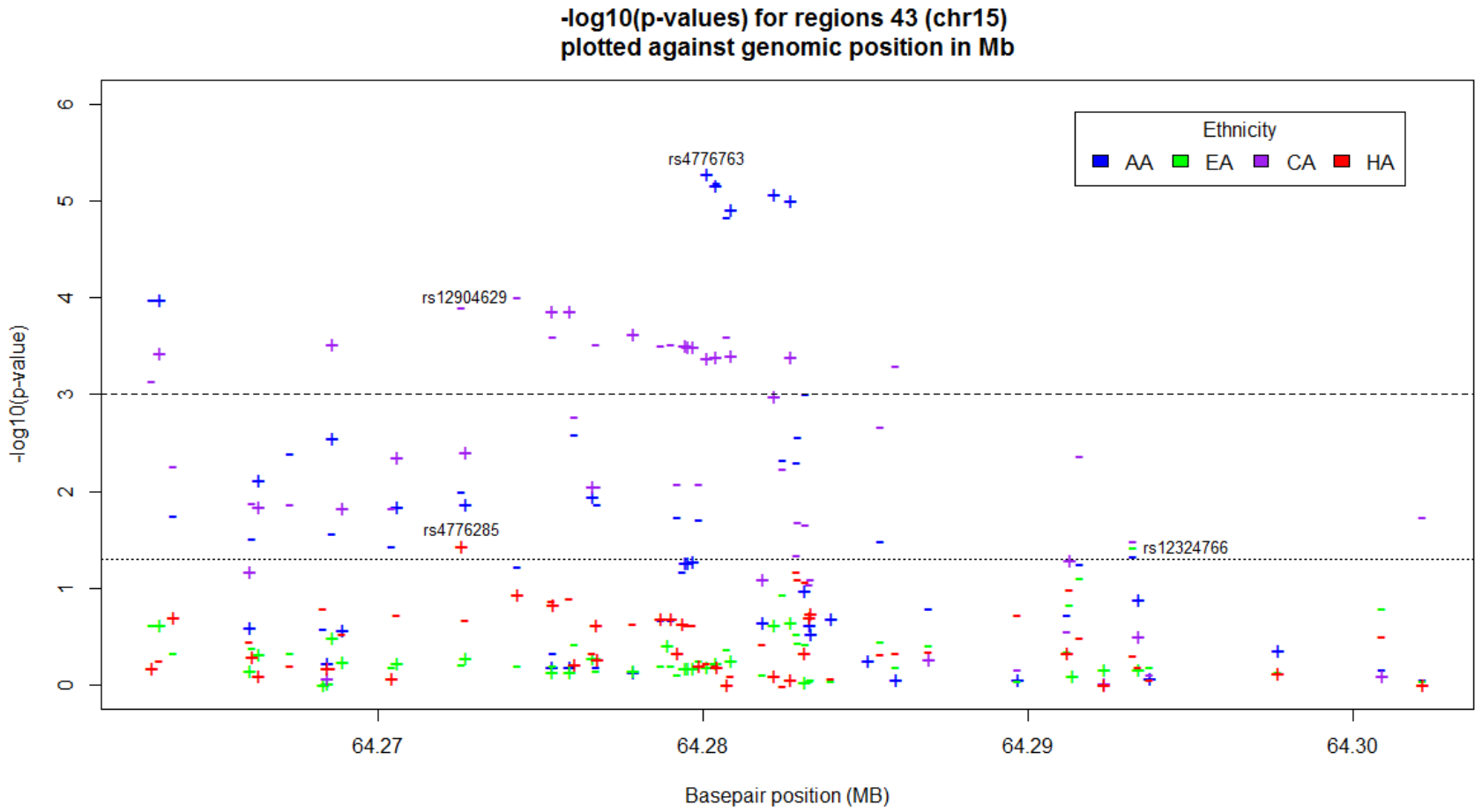
Top SNP in each ethnicity is identified by rs number. AA: African American, EA: European American, CA: Chinese American, HA: Hispanic American. +: positive effect for coded allele, -: negative effect for coded allele. --- indicates p-value = 0.001, ····· indicates p-value = 0.05

Figure 24 -log$_{10}$(p-values) for regions 26-28 (chr8) plotted against genomic position in Mb



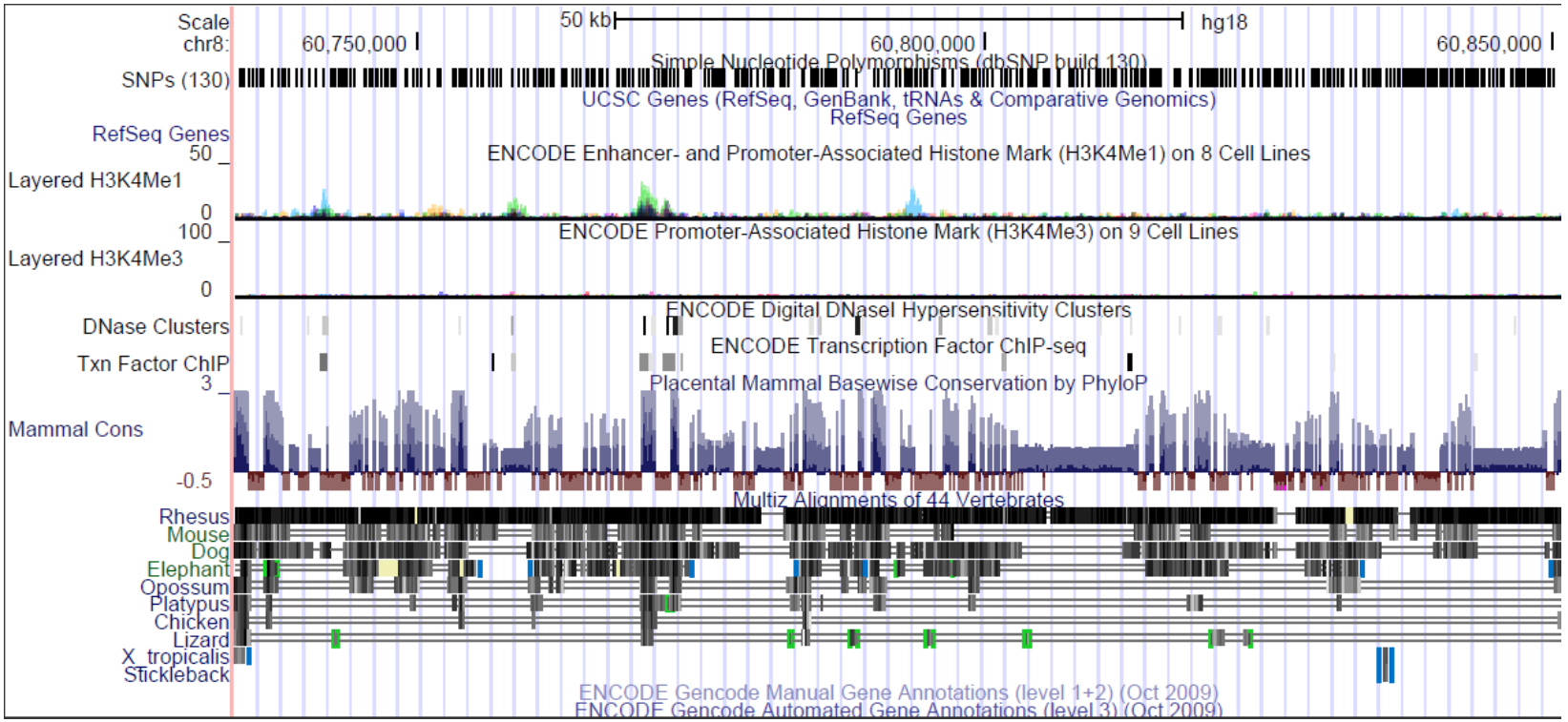
Top SNP in each ethnicity is identified by rs number. AA: African American, EA: European American, CA: Chinese American, HA: Hispanic American. +: positive effect for coded allele, -: negative effect for coded allele. --- indicates p-value = 0.001, ····· indicates p-value = 0.05

Figure 25 -log$_{10}$(p-values) for regions 43 (chr15) plotted against genomic position in Mb



Top SNP in each ethnicity is identified by rs number. AA: African American, EA: European American, CA: Chinese American, HA: Hispanic American. +: positive effect for coded allele, -: negative effect for coded allele. --- indicates p-value = 0.001, ····· indicates p-value = 0.05

Figure 26 Bioinformatic analysis of Regions 26 - 28 using the UCSC Genome Browser and ENCODE database



CHAPTER V

V. Interactions of individual- and neighborhood-level social environment with genetic factors in the prediction of depressive symptoms in the Multi-Ethnic Study of Atherosclerosis

5.1 Introduction

While both genetic and environmental factors have been associated with depressive illnesses when considered in isolation, complex diseases such as these seldom fit into single-factor models. It is likely that characteristics of both internal and external environments play a role in modifying genetic associations with depressive symptoms. Further, it is plausible that the interactions between genetic factors and these internal and external environmental factors may impact disease development. Gene by environment (G x E) interactions associated with disease development or morbidity have previously been found in both physical and mental disorders, specifically for depressive outcomes with genes characterizing serotonin transportation.[95, 117, 188-190] The serotonin transporter gene (*5-HTT*) has been found to interact with environments such as stressful life events, proportion of individuals receiving public assistance (county-level), infant mortality rates and county-level crime rates, demonstrating that both individual (e.g. stressful life events) and external neighborhood (e.g. county-level crime rates) factors convey different risks of depression for different genotypes.[95, 117, 188-190] It has been suggested that G x E interactions have broadly been shown to be more replicable

than single-nucleotide polymorphism (SNP)-disease or gene-disease associations.[111, 191]

A major limitation of prior work on gene by environment interactions in depressive illness has been the type and quality of the social environment measures available. The ability to replicate findings has been shown to differ when environment measures are defined objectively versus subjectively. In the context of the serotonin transporter gene, a review found that studies involving objective measures assessing neighborhood stress replicated G x E interactions either fully or in part, whereas studies relying on self-reported measures often did not replicate.[192] Depression has been connected to several biological pathways including metabolic pathways (e.g. [193]), inflammatory pathways (e.g. [194]), and neurobiological pathways (e.g. [195]). These pathways may be activated by external environments – such as chronic burden or neighborhood stressors (e.g. [196]) There is need to further investigate G x E interactions using larger samples with improved psychosocial and environment measures. This work examines gene-environment interactions with depressive symptoms at both individual- and neighborhood-level environments, and accounting for depressive symptoms over time.

Using quantitative depressive symptom scores measured with the Center for Epidemiologic Studies – Depression scale (CES-D), we investigated interactions between variability in selected genetic regions and both individual-level and neighborhood-level measures of social environments. The genetic regions for investigation were identified based on prior GWAS and SNP-set analyses (chapters III and IV). Environments were defined three separate ways: as individual-level social factors ((1) social support (SS) or

(2) chronic burden (CB)), and (3) separately as neighborhood-level social factors (an index score combining neighborhood social cohesion, perceived neighborhood safety, and neighborhood aesthetic quality).

5.2 Methods

5.2.1 Outcome

The outcome of interest, depressive symptom score, was assessed using the 20-item CES-D Scale[14], appropriately log-transformed to improve normality. CES-D score was then adjusted for anti-depressant use[119, 120], and averaged over all exams for which the measure was administered. This outcome was selected because it was the most powerful approach to capturing the longitudinal nature of depressive symptoms while still having methods available to analyze G x E at the gene level (there are currently no validated methods for analyzing repeated measures of an outcome at the SNP-set level).

5.2.2 Genes

Gene regions are defined using SNP sets identified based on a prior GWAS conducted in all four ethnicities (Chapter III). Methods for selecting SNP regions are described previously (Chapter IV). Briefly, genome-wide association study (GWAS) results, for ethnicity-specific common alleles (minor allele frequency (MAF) $\geq$ 5%), were analyzed across four ethnicities (African (AA), European (EA), Chinese (CA), and Hispanic (HA) Americans) using a fixed effects meta-analysis. Top hits (by p-value) were then filtered using the following criteria: meta-analysis heterogeneity p-value > 0.10, MAF $\geq$ 5%, and the SNP's presence in at least two ethnicities. The top 100 meta-

analysis SNPs were then selected and starting with the SNP with the smallest p-value, a region was created around this index SNP including all SNPs within ±20kb. This distance was selected so that the region captured the average size of a linkage disequilibrium block.[176] If any other SNPs in the top 100 meta-analysis results were in this region, they were eliminated from being the index SNP of another region. This method continued through the list of top 100 SNPs until all possible regions were created. Some overlap is present in regions that had an index SNP_i within ±20kb < index SNP_j < ±40kb, where $i \neq j$. A total of 47 regions were created.

Any region which had a MetaSKAT p-value (adjusted for age, sex, site, top four ethnicity-specific principal components (PC), and adult socioeconomic position (ASEP)) less than 0.20 was included in these analyses (see Chapter IV). The threshold of a MetaSKAT p-value of 0.20 was selected to allow for the possibility of qualitative interaction which could result in a null main effect for the region (for example if the genetic variant is positively associated with CES-D in the presence of the environmental factor but inversely associated in the absence of the factor resulting in an average null effect). A total of 37 regions were included (of 47). Since regions were selected based on lowest p-values from the meta-analysis of individual SNPs and averaged depressive symptom scores across ethnicities, it is not surprising to have such a large percentage of regions with regional genetic effect p-values < 0.20.

5.2.3 Environment

Two individual-level social environments are used in these analyses: chronic burden and emotional social support. These measures represent different dimensions of individual stressors and are thus analyzed in separate models. CB was measured at two

exams in MESA (exams one and three) and is based off of the chronic burden scale developed for the Healthy Women Study.[126] It is an index of affirmative responses to five individual burdens including health (self and others), finances, employment, and relationships that were ongoing for more than six months. Within each exam if a component score was missing, the overall CB for that exam was set to missing. CB was averaged across the two exams for each individual. If either exam was missing, CB was created from the existing measure. If both exams were missing, CB was set to missing. CB was centered at the overall mean. Higher values of CB indicate higher chronic burden (i.e. more burdens).

Emotional social support was available at exams one and three of MESA and is an index rating six questions on a five-point Likert scale (1 = "none of the time" to 5 = "all of the time"). This scale was originally assessed in the Enhancing Recovery in Coronary Heart Disease study.[127] These questions included asking if someone was available to listen, give advice, show love and affection, help with daily chores, provide emotional support, and confide in. Within each exam if a component score was missing, the overall SS for that exam was set to missing. If either exam was missing, SS was created from the existing measure. If both exams were missing, SS was set to missing. SS was centered at the overall mean. Higher scores indicate more social support.

Neighborhood social environment is summarized into a neighborhood index score (NIS) composed of three dimensions: aesthetic quality (AQ), safety (SF), and social cohesion (SC) measured with a 1-mile radius as the definition of neighborhood. A previously published study found that these key features of the environment were significantly associated with depressive symptoms using MESA participants.[1] The

separate neighborhood dimension scales (AQ, SF, SC) pooled information from MESA and the Community Survey (CS) to create crude means for the neighborhood dimensions. The respondent's own answer was not included in the crude mean estimates for the neighborhood, allowing for more objective neighborhood measures than using the MESA participant's perception of neighborhood dimensions alone. The neighborhood level data was linked to the participant's addresses within a 1-mile buffer by matching each participant of the survey within 1 mile based on the latitude/longitude of the address. Responses of "Don't Know" or "Refused" values were set to missing for each of the original variables in each of the surveys. Several questions were reverse coded so that questions reflected better social outcomes with increasing scores.

The NIS was created by averaging the 1-mile means for AQ, SF, and SC across the three exams by neighborhood dimension and then averaging the three exam-specific averages. If any one of the nine variables (AQ exam one, three, and four; SF exam one, three, and four; or SC exam one, three, and four) was missing, then NIS is set to missing. The index score was then mean-centered by the combined-ethnicity mean to aid interpretability. Higher NIS indicates "more positive" overall neighborhood environments, such as a high degree of SF, good AQ, and/or good SC. The index scores range from -1.30 to 0.95, with a mean of 0 and a standard deviation of 0.33 in the combined sample (AA, EA, CA, HA).

5.2.4 Covariates

Covariates include age at baseline exam, sex, study site at baseline exam, top four ethnicity-specific PCs, and ASEP. There were a total of 6,335 MESA participants included in the averaged depressive symptom GWAS (AA 25%, EA 40%, CA 12%, HA

23%). Average age (standard deviation) for the AA, EA, CA, and HA sub-samples was 62.2 (10.1), 62.6 (10.2), 61.4 (10.3), and 62.4 (10.4) years, respectively. Slightly less than half of each ethnicity was male (AA 48.0%, EA 46.4%, HA 49.3%, CA 49.7%). Study site is the field center from which the participant was interviewed and includes: Baltimore MD (Johns Hopkins University Field Center); Chicago, IL (Northwestern Field Center); Forsyth County, NC (Bowman Gray Field Center); Los Angeles, CA (UCLA Field Center); New York, NY (Columbia Field Center); and St. Paul, MN (University of Minnesota Field Center). PCs were calculated using genetic information for each ethnicity separately. ASEP is a summary index of income, education, and wealth. Income (<$25,000, $25,000–39,999, $40,000–74,999, or +$75,000), baseline highest level of education completed (completed high school or less, some college but no degree/technical school certificate, associate or bachelor's degree, or graduate/professional degree), and four wealth indexes (investments (yes/no), home ownership (yes/no), vehicle ownership (yes/no), land/property ownership other than primary residence (yes/no)) were summed (income variable (0 – 3, low to high), education (0 – 3, low to high), a single point for each wealth indicator). The ASEP score ranged from 0 – 10, with higher scores indicating greater ASEP.

5.2.5 GESAT

The GESAT is a variance component score test. For GESAT, the interaction model is:

$$E(\bar{y}_{i\cdot}) = \alpha_0 + \boldsymbol{\alpha_1}'\boldsymbol{X}_i + \boldsymbol{\alpha_2}'\boldsymbol{G}_i + \boldsymbol{\alpha_3}'\boldsymbol{E}_i + \boldsymbol{\beta}_j'\boldsymbol{S}$$

Where $\bar{y}_{i\cdot}$ is the log-transformed (depressive symptom score averaged across exams plus 1) for individual *i*, $\mathbf{X}_i$ is a vector of non-genetic covariates, $\mathbf{E}_i$ is the environmental factor, $\mathbf{G}_i$ is a vector of genetic markers, and $\mathbf{S}_i$ is a vector of G x E interaction terms. One assumes that each of the β_j's, $j = 1, \ldots, p$, follows an arbitrary distribution with mean zero and common variance τ^2, and that the β_j's are independent. Testing H_0: $\tau^2 = 0$ is equivalent to testing H_0: $\boldsymbol{\beta} = 0$, which tests whether at least one of the interaction terms is non zero. Covariates include age, sex, site, top four ethnicity-specific PCs, and ASEP. The variance-component score statistic for τ is

$$Q = (\bar{\boldsymbol{y}} - \hat{\mu})' \boldsymbol{S}\boldsymbol{S}' (\bar{\boldsymbol{y}} - \hat{\mu})$$

Where **S** is the vector of G x E interactions for each individual and variant in the region, $\hat{\mu}$ is the predicted mean of $\bar{\boldsymbol{y}}$ under the null model.

5.2.6 Single SNP interaction model

GESAT does not provide SNP-by-environment interaction parameter estimates (i.e. magnitude or direction of effect). Gene-level analysis was followed with an individual SNP x environment analysis using generalized estimating equation (GEE) methods to estimate both the magnitude and direction of each SNP-by-environment interaction term in a SNP set when there was evidence of a significant gene-level interaction.

For SNP sets that showed significant evidence of interactions with the environments, we estimated the effect of each SNP within the SNP-set, for each ethnic group separately using GEE methods following the model below:

$$E(\bar{y}_{i\cdot}) = \alpha_i + \boldsymbol{\beta}\boldsymbol{X}_i' + \gamma SNP * Environment_i$$

Where $\bar{y}_{i\cdot}$ is the depressive symptom measure averaged across exams and appropriately log transformed for participant i. Instead of the model-based estimator of variance, we used a sandwich estimator of variance for robustness. MESA encourages the use of sandwich based variance estimation.

After interaction models for individual SNP x environment models were run, the p-values for each SNP across ethnicities were combined using Fisher's method [197]:

$$\chi^2_{2k} \sim -2\sum_{i=1}^{k} \ln(p_i)$$

Where p_i is the p-value for the SNP x E interaction for each ethnicity i, and k is the number of ethnicities. This statistic follows a chi-square distribution with $2k$ degrees of freedom. Only cases where two or more ethnicities contribute to the statistic were included. This method gives an estimate of the overall effect of the SNP x E interaction across ethnicities.

5.3 Results

5.3.1 Gene-level

5.3.1.1 Chronic Burden and Social Support

Results from all regions for the SNP-set x CB interaction are shown in Table 20. Of the 37 regions investigated, seven regions showed significant or marginally significant interactions (significant p-value ≤ 0.05, marginally significant $0.05 <$ p-value ≤ 0.10) with CB. Two of these regions had significant or marginally significant interactions in multiple ethnicities (region 1 chr 1:2775967-2815967: EA p-value = 0.07, CA p-value =

0.04; and region 44 chr 15:77126365-77166365: EA p-value = 0.06, CA p-value = 0.09). Region 1 has no established genes within ±100kb of the index SNP. Region 44 lies within the intronic *RASGRF1* (5q31 ras-specific guanine nucleotide-releasing factor 1) gene.[167] This gene has previously been associated with myopia (near-sightedness) in European and Asian ethnicities,[198, 199] but it is novel in depressive symptoms.

Ten regions had ethnicity-specific significant or marginally significant interactions (Table 20) with SS. Only one region had a significant SNP-set interaction with SS in more than one ethnicity (region 29 8:78208511-78248511: AA p-value = 0.04, EA p-value = 0.04). Region 29 does not have any established genes within ±100kb of the index SNP. Though there may be no genes near these regions, it does not preclude the region from being in a potentially important regulatory area.

5.3.1.2 Neighborhood Index Score

Twelve regions were found to have significant interactions with NIS (Table 21). Region 46 (chr 18:21454070-21494070) had significant (p-value ≤ 0.05) interactions in three ethnicities (AA p-value = 0.04, EA p-value = 0.03, HA p-value = 0.00). Region 46 does not have any established genes within ±100kb of the index SNP.

5.3.2 SNP-level

Instances where there was evidence of a significant or marginally significant region-level interaction (p-value < 0.10) in at least two ethnic groups for any SNP-set x E were considered to provide the strongest evidence of cross-ethnicity region-level interaction effects. For these SNP sets, individual SNP x E interactions were examined to determine which SNPs were driving the region-level associations. The top (lowest

Fisher's combined p-value) SNP x E interaction for each region showing CES-D at quartiles of environment (combined ethnicities) for each genotype (0, 1, or 2 copies of the coded allele) were plotted for each ethnicity.

5.3.2.1 Chronic Burden and Social Support

Within the regions identified in the chronic burden (regions 1, 44) and social support (region 29) environments, 14 SNP x E interactions reached statistical significance ($\alpha_{Bonferroni} = 0.001$ – corrected for the average number of SNPs within a region) in the across-ethnicity analysis, all from region 29.

Plots of the top SNP from each of these regions, for each ethnicity show strong evidence of an additive effect for these SNPs in almost all of the ethnicities (Figure 31-Figure 33). Noting that these graphs do not necessarily provide the most accurate reflection of the individual SNP interaction results (environment was modeled as continuous and plotted as quartiles), plots continue to show a large difference between mean depressive symptoms for the chronic burden quartiles (Figure 31,Figure 32) and the social support quartiles (Figure 33). Among all ethnicities, higher levels of the amount of chronic burden experienced denoted increases in mean depression scores. In European, Chinese, and Hispanic Americans, this increase was steepest for those with no copies of the coded allele. In African Americans, highest mean CES-D scores were seen at the highest level of chronic burden for those with two copies of the coded allele (Figure 31). Similar increases in mean depressive symptoms over the quartiles of chronic burden were observed in region 44 (Figure 32). In this region, having two copies of the coded allele conferred resilience to depressive symptoms in the highest chronic burden quartile for the

AA, CA, and EA populations but this genotype was susceptible to higher mean depressive symptom scores in the HA sub-sample.

In the interaction plots for social support quartiles for the top SNP from the Fisher's method meta-analysis show a protective effect of social support for each genotype (Figure 33). There is some evidence of interaction in the Hispanic American sample. The AA and EA sub-samples showed lower mean CES-D scores with zero copies of the coded allele over all levels of social support compared to one or two copies of the coded allele. Both the CA and HA samples showed similar decreasing patterns of depressive symptoms over the increasing levels of social support; however, no copies of the coded allele exemplified higher mean depressive symptoms compared to one or two copies. One point to note in these plots is that the CA sub-sample consistently shows higher levels of depressive symptoms scores in these environments than the other three ethnicities. This can be misleading, as the Chinese sub-sample had the lowest average depressive symptom scores of any of the four ethnicities. One reason for the increased values in the Chinese sample is that these participants tended to have much more strongly skewed measures of environment. Particularly the CA sample had lower levels of deleterious individual- and neighborhood-level factors as well as a smaller sample size overall. As an example, quartiles of chronic burden in the Chinese sample were (Min: 0, Q1: 0, Med: 0.5, Q3: 1, Max: 4.5) whereas in the combined ethnicity the quartiles were (Min: 0, Q1: 0, Med: 1, Q3: 1.5, Max: 5). The combined ethnicity quartiles were used to create the plots.

5.3.2.2 Neighborhood Index Score

Region 46 had 24 SNP x E interactions that reached significance. The lowest p-values in this region were for rs4800653 (p-value 1.47×10^{-6}) and rs1840444 (p-value 7.65×10^{-6}). A complete listing of the ethnicity-specific sample sizes and p-values, and Fisher's chi-square, degrees of freedom, and p-value for cross-ethnicity comparison for each region can be found in Appendix 68 - Appendix 71.

The interaction figures for rs4800653 for each ethnicity reveal evidence of a statistical interaction (Figure 34). In of all the ethnicities, having more copies of the coded allele is associated with higher mean depressive symptom averages in areas with low NIS scores (indicating less safe, less cohesive, and/or less aesthetically pleasing neighborhoods). Conversely, having more copies of the coded allele (while deleterious in low NIS areas) is protective in areas with high NIS scores (indicating safer, more cohesive, and/or aesthetically pleasing neighborhoods).

5.4 Discussion

These analyses used novel methods (GESAT[200]) to elucidate SNP set x social environment interactions associated with depressive symptoms, averaged across exams. Using three different environments, two at the individual-level (chronic burden and social support) and one at the neighborhood-level (neighborhood index score), four genetic regions had significant G x E associations with depressive symptoms. Investigating these associations at the SNP level and combining across ethnicity provided striking evidence of multiple SNP x E interactions within the social support and neighborhood index score environments.

In these analyses we assumed an additive effect for each SNP; that is, for every additional copy of the coded allele, the mean response (averaged depressive symptom score) increases (or decreases) linearly. However, it is likely that the additive model may not be the best-fitting model for every variant within a region. Additional testing with different genetic effect assumptions is warranted to better estimate the true genetic effects of these variants on depressive symptoms.

The method used to test interaction effects for genetic marker sets, GESAT, is computationally efficient, robust, and has several advantages over traditional SNP x environment analysis. In particular, this method has been shown through simulation and real data applications to be a more powerful method over others (e.g. weighted sum statistics[201], cohort allelic sum tests[115], or C-alpha test[202]). GESAT allows for covariate adjustment and can test common variants through the use of an unweighted linear kernel. Since our analyses filtered out any rare variants (MAF < 0.05), this option is particularly important. GESAT also does not assume that all variants will produce effects of similar direction and magnitude by allowing the variance of an individual variant to differ from a mean of zero. Finally, GESAT allows for a test of biologically meaningful regions rather than individual SNPs that may vary in distribution across ethnicities due to evolutionary patterns and may not be functional genetic variants.[173-175] Unfortunately, GESAT does not yet allow for testing of phenotypes over time in repeated measures models, accounting for correlation between measures on the same individual. The extension of GESAT to allow for repeated measures modeling would greatly enhance the ability to detect genetic effects for phenotypes that are better characterized over time.

The Encyclopedia of DNA Elements (ENCODE) aims to build a database of functional elements in the human genome, including protein and RNA level elements, and regulatory elements that control cells and circumstances in which a gene is expressed.[186, 187] Because regions 1, 30, and 46 all presented as being in gene deserts (that is, there were no genes within ±100kb of the index SNP), ENCODE was used to determine if potential functional elements exist in these regions.

5.4.1 Region 1

Region 1 (chr1:2,775,967-2,815,967) lies on the q arm of chromosome one. There is preliminary evidence of an overlap with an H3K4Me1 histone mark (regions often located near active regulatory elements), several DNase1 hypersensitivity clusters (a chromatin accessibility feature common to cis-regulatory sequences), and several transcription factor binding sites (Figure 35).[186, 187] Region 1 contains not only SNPs with high conservation rates (rs1563469, phylogenic conservation score (PCS) = 0.843) across 17 species[203, 204], but also SNPs that lie in Short Interspersed Elements (SINE) (rs897620, rs2445620), in DNA repeat elements (rs2842910), long terminal repeat (LTR) (rs2842911), and Long Interspersed Elements (LINE) elements (rs750786, rs897630) (Appendix 72). Genetic conservation describes the amount and distribution of genetic diversity within species and evolutionary diversity among species as well as the retention of variants within and among populations in order to maintain long-term evolutionary potential.[203]

5.4.2 Region 29

ENCODE analysis for region 29 shows potential for several dense DNaseI hypersensitivity clusters as well as transcription factor binding sites based on ChIP-seq

information. Regulatory regions in general and promoters in particular, tend to be DNase sensitive. There does not appear to be noticeable elevations in enhancer- and promoter-associated histone marks (either H3K4Me1 or H3K4Me1) (Figure 36).[186, 187] Region 29 contains two SNPs with a PCS of 1 indicating extremely high cross-species conservation (rs7831215, rs16939439). This region also houses SNPs that lie in SINEs, DNA repeat elements, LTRs, and LINEs (Appendix 72).

5.4.3 Region 46

In region 46, we see a large amount of evidence for functional elements. In particular, there are several large elevations in enhancer- and promoter-associated histone marks (H3K4Me1) in the positions approximately +30kb and to a lesser extent -45kb from the index SNP. These areas also coincide with many dense DNaseI hypersentivity clusters and transcription factor binding sites (Figure 37).[186, 187] Region 46 contains many SNPs that are in SINEs or LTRs. This evidence on a whole is suggestive of potential functional consequences of genetic variation in these particular regions – suggesting that further functional characterization is warranted.[186, 187]

Taken as a whole, bioinformatic evidence from these regions provide indications of a potential regulatory effect of genetic regions involved in G x E interactions related to depressive symptoms. Typically, regulatory areas modulate gene expression in response to developmental, tissue specific or environmental signals. Influences on gene expression from developmental signals may lay down a basis for methylation across the life course and consequently lead to higher (or lower) depressive symptoms later in life. The regulation of tissue-specific signals could possibly set up the brain's ability to

successfully (or unsuccessfully) adapt to chemical stimuli, while these regulatory regions may also influence how the body responds, at a molecular level, to neighborhood stimuli.

This novel work in examining the impact of G x E interactions on depressive symptoms, across multiple gene regions, environment definitions, and ethnicities was possible through innovative gene-environment set association test techniques, and through detailed assessments of individual-level psychosocial environment and objective neighborhood dimensions. These methods permit an examination of genes/SNP sets across ethnicities, where individual SNPs may not replicate across ethnicities due to ethnicity-specific patterns of linkage disequilibrium or differences in allele frequencies across ethnic groups.[173-175] Future work should focus on examining the functional elements in these regions as well as incorporating methods to examine complex diseases over time in repeated measures models.

5.5 Acknowledgements

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Table 20 Gene-environment set associations tests (GESAT) interaction results for chronic burden x SNP-set and social support x SNP-set

	Chronic Burden				Social Support			
Region	**AA**	**EA**	**CA**	**HA**	**AA**	**EA**	**CA**	**HA**
	p	**p**	**p**	**p**	**p**	**p**	**p**	**p**
1	9.91E-01	**6.93E-02**	**3.53E-02**	6.96E-01	3.25E-01	9.65E-01	3.69E-01	7.14E-01
2	7.61E-01	3.18E-01	3.94E-01	7.82E-01	9.22E-01	8.95E-01	6.97E-01	6.86E-01
3	7.64E-01	4.10E-01	8.35E-01	6.98E-01	7.73E-01	5.50E-01	5.00E-01	8.70E-01
4	3.20E-01	6.93E-01	9.20E-01	9.51E-01	3.89E-01	7.61E-01	4.19E-01	9.88E-01
5	1.73E-01	5.58E-01	9.03E-01	9.66E-01	6.87E-01	7.63E-01	4.28E-01	9.67E-01
6	1.95E-01	6.10E-01	8.54E-01	9.50E-01	5.46E-01	7.82E-01	7.40E-01	9.60E-01
7	1.27E-01	3.58E-01	5.32E-01	6.80E-01	3.67E-01	3.73E-01	3.42E-01	**5.34E-02**
8	3.41E-01	6.46E-01	6.10E-01	5.72E-01	1.91E-01	5.73E-01	3.55E-01	**2.83E-02**
9	1.57E-01	1.33E-01	1.43E-01	5.71E-01	2.09E-01	2.51E-01	1.82E-01	6.78E-01
10	4.62E-01	6.41E-01	4.93E-01	5.95E-01	4.75E-01	9.23E-01	7.62E-01	1.74E-01
11	4.69E-01	3.28E-01	3.70E-01	2.40E-01	8.98E-01	8.61E-01	9.95E-01	9.86E-01
13	1.85E-01	3.21E-01	2.63E-01	**1.27E-02**	**6.40E-02**	4.06E-01	2.15E-01	9.78E-01
14	5.52E-01	3.45E-01	**9.77E-02**	8.59E-01	6.63E-01	**6.88E-02**	5.79E-01	9.08E-01
16	2.91E-01	4.50E-01	6.68E-01	3.88E-01	4.27E-01	3.69E-01	--	7.12E-01
17	3.62E-01	--	1.81E-01	5.45E-01	6.37E-01	4.53E-01	2.90E-01	7.30E-01
19	9.54E-01	7.82E-01	5.03E-01	2.86E-01	1.55E-01	**4.13E-02**	8.99E-01	9.91E-01
20	1.75E-01	3.10E-01	6.17E-01	6.15E-01	2.41E-01	2.89E-01	3.12E-01	7.64E-01
21	5.33E-01	8.92E-01	1.24E-01	8.89E-01	9.43E-01	8.37E-01	7.78E-01	8.00E-01
22	5.57E-01	6.42E-01	7.20E-01	1.87E-01	2.69E-01	4.34E-01	8.66E-01	2.73E-01
23	6.51E-01	9.66E-01	2.91E-01	4.12E-01	5.67E-01	4.28E-01	6.56E-01	9.19E-01
24	6.87E-01	4.45E-01	2.29E-01	6.67E-01	8.18E-01	7.81E-01	7.50E-01	5.25E-01
26	7.65E-01	7.75E-01	7.49E-01	4.42E-01	6.06E-01	**5.26E-02**	9.44E-01	2.49E-01
27	6.32E-01	8.78E-01	8.40E-01	4.30E-01	4.82E-01	**8.63E-02**	9.44E-01	2.50E-01
28	5.62E-01	7.60E-01	2.28E-01	4.98E-01	3.37E-01	1.72E-01	7.52E-01	4.59E-01
29	3.81E-01	7.51E-01	5.90E-01	4.25E-01	**4.19E-02**	**4.20E-02**	3.99E-01	9.08E-01
30	6.67E-01	5.84E-01	4.62E-01	2.40E-01	4.98E-01	9.20E-01	9.05E-01	9.62E-01
32	**7.93E-02**	6.63E-01	6.07E-01	9.51E-01	1.68E-01	8.85E-01	5.84E-01	9.72E-01
33	5.12E-01	6.52E-01	4.92E-01	1.74E-01	1.53E-01	7.13E-01	3.16E-01	2.56E-01
34	6.98E-01	1.26E-01	5.31E-01	2.90E-01	9.02E-01	2.18E-01	1.02E-01	5.54E-01
35	1.34E-01	9.77E-01	4.25E-01	7.62E-01	8.64E-01	9.12E-01	3.43E-01	7.91E-01
38	8.42E-01	7.33E-01	**9.18E-02**	5.40E-01	8.92E-01	9.65E-01	4.38E-01	**2.57E-02**
40	8.45E-01	4.14E-01	2.11E-01	2.10E-01	1.04E-01	9.94E-01	6.35E-01	6.47E-01
41	9.65E-01	5.94E-01	5.25E-01	2.28E-01	5.29E-01	9.08E-01	4.44E-01	7.26E-01
42	2.11E-01	4.60E-01	1.89E-01	3.83E-01	2.67E-01	6.96E-01	3.62E-01	7.62E-01
43	6.09E-01	5.35E-01	7.45E-01	1.24E-01	1.10E-01	5.01E-01	8.86E-01	8.45E-01
44	5.95E-01	**6.46E-02**	**9.17E-02**	6.42E-01	5.78E-01	8.73E-01	6.79E-01	**9.88E-02**
46	7.61E-01	7.12E-01	6.51E-01	9.15E-01	7.23E-01	4.94E-01	7.55E-01	7.84E-01

Only regions with significant joint effects were investigated in the interaction analysis.

-- Indicates a model that did not converge.

AA: African American, EA: European Americans, CA: Chinese Americans, HA: Hispanic Americans, p: p-value. P-values ≤ 0.10 are bolded

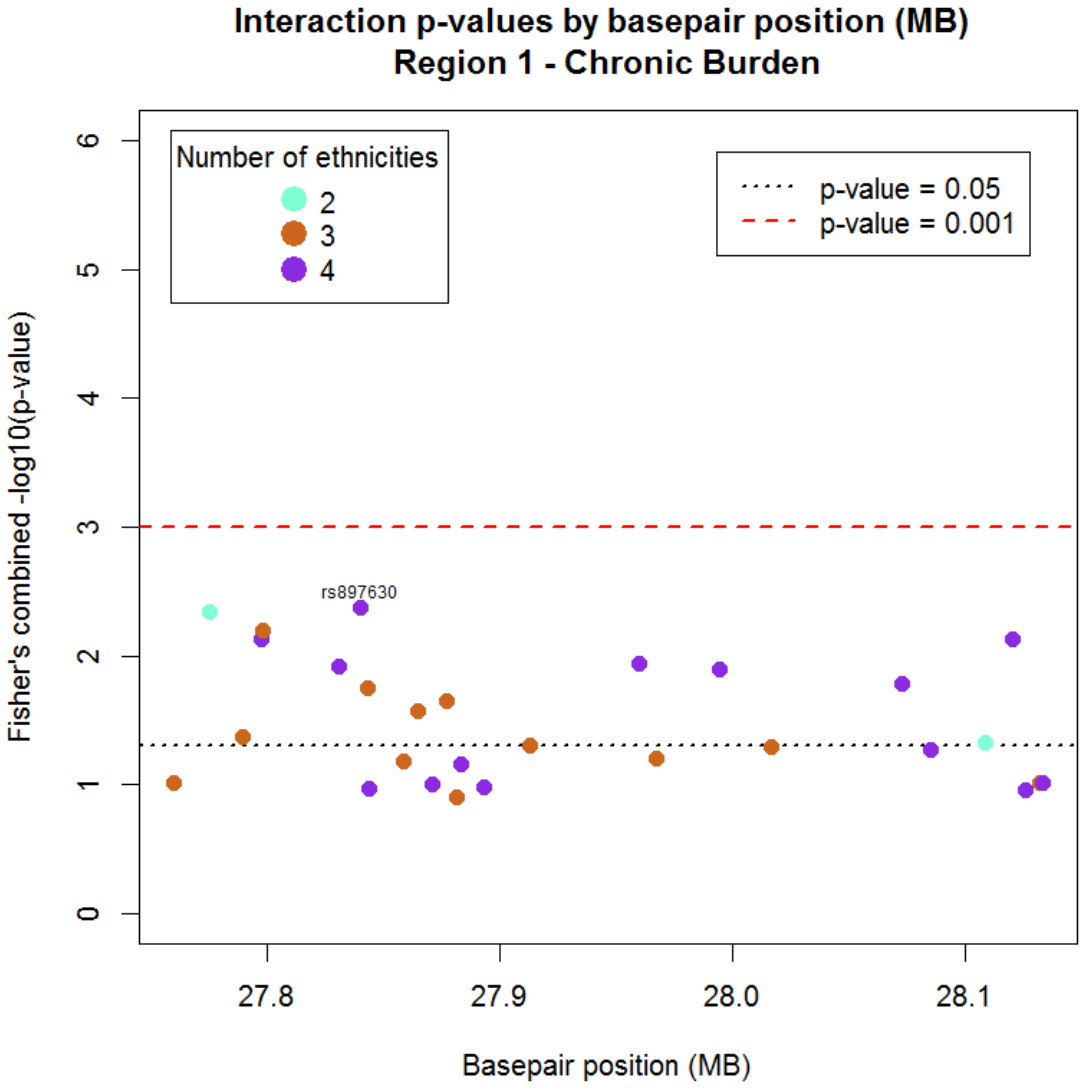
Table 21 Gene-environment set associations tests (GESAT) interaction results for Neighborhood Index Score and SNP-set, by ethnicity

	AA	EA	CA	HA
Region	**p**	**p**	**p**	**p**
1	8.61E-01	4.91E-01	6.07E-01	1.06E-01
2	8.84E-01	5.71E-01	6.81E-01	8.88E-01
3	2.12E-01	8.40E-01	7.56E-01	1.08E-01
4	4.68E-01	6.74E-01	4.66E-01	4.69E-01
5	7.31E-01	3.84E-01	5.36E-01	5.82E-01
6	5.76E-01	3.61E-01	6.07E-01	5.51E-01
7	3.98E-01	6.08E-01	9.34E-01	9.61E-01
8	2.28E-01	4.58E-01	8.49E-01	9.18E-01
9	1.91E-01	1.49E-01	3.62E-01	2.57E-01
10	3.69E-01	2.58E-01	5.55E-01	**7.98E-02**
11	**6.08E-02**	9.63E-01	6.24E-01	5.37E-01
13	4.33E-01	7.36E-01	1.21E-01	4.52E-01
14	8.03E-01	9.52E-01	5.51E-01	3.41E-01
16	9.42E-01	2.40E-01	--	1.77E-01
17	3.99E-01	3.82E-01	1.46E-01	3.82E-01
19	6.44E-01	7.97E-01	**7.83E-02**	4.56E-01
20	**6.18E-02**	3.46E-01	1.02E-01	4.64E-01
21	6.77E-01	5.33E-01	3.22E-01	6.56E-01
22	1.62E-01	9.15E-01	2.06E-01	7.78E-01

	AA	EA	CA	HA
Region	**p**	**p**	**p**	**p**
23	9.59E-01	4.53E-01	**1.98E-02**	3.97E-01
24	3.71E-01	4.25E-01	9.58E-01	3.21E-01
26	9.18E-01	6.75E-01	3.96E-01	2.93E-01
27	8.36E-01	7.32E-01	5.28E-01	**6.11E-02**
28	6.80E-01	9.01E-01	2.29E-01	1.06E-01
29	2.80E-01	2.07E-01	6.14E-01	**6.42E-02**
30	6.50E-01	6.48E-01	8.94E-01	8.76E-01
32	3.55E-01	**9.52E-02**	4.57E-01	4.98E-01
33	9.02E-01	8.81E-01	3.43E-01	1.28E-01
34	1.63E-01	8.34E-01	6.96E-01	**1.41E-02**
35	3.62E-01	7.10E-01	2.39E-01	4.70E-01
38	3.22E-01	2.75E-01	4.65E-01	**5.91E-03**
40	8.77E-01	**8.38E-02**	5.98E-01	5.49E-01
41	5.16E-01	2.84E-01	3.37E-01	3.59E-01
42	3.15E-01	6.07E-01	4.55E-01	2.72E-01
44	7.45E-01	4.43E-01	5.79E-01	8.34E-01
46	6.25E-01	2.05E-01	2.28E-01	8.49E-01
47	**4.23E-02**	**2.57E-02**	4.97E-01	**3.94E-03**

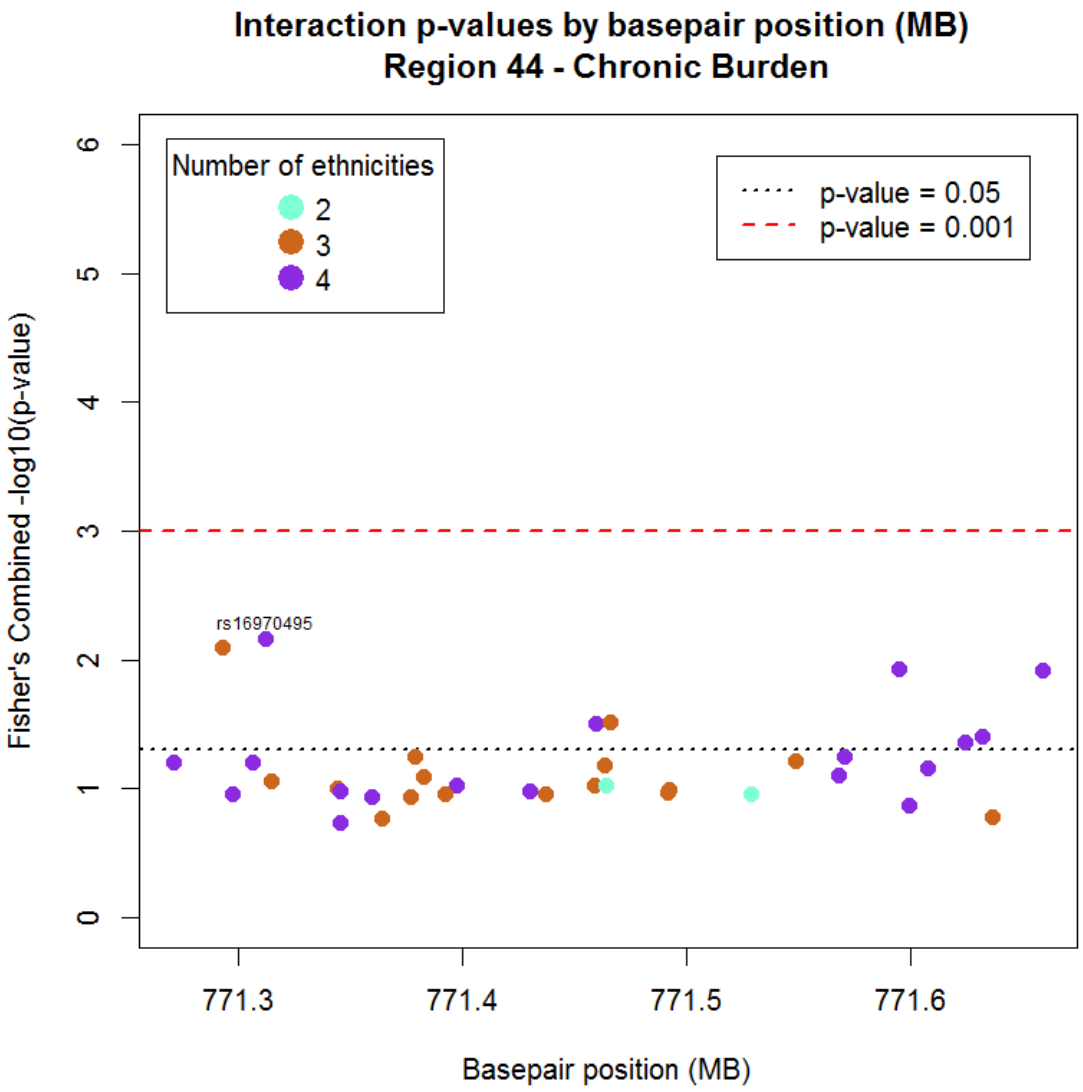
Only regions with significant joint effects were investigated in the interaction analysis. -- Indicates a model that did not converge, AA: African American, EA: European Americans, CA: Chinese Americans, HA: Hispanic Americans. p: p-value, P-values ≤ 0.10 are bolded

Figure 27 Fisher's combined $-\log_{10}$(p-values) for each SNP x environment (chronic burden) interaction in region 1 plotted against genomic position



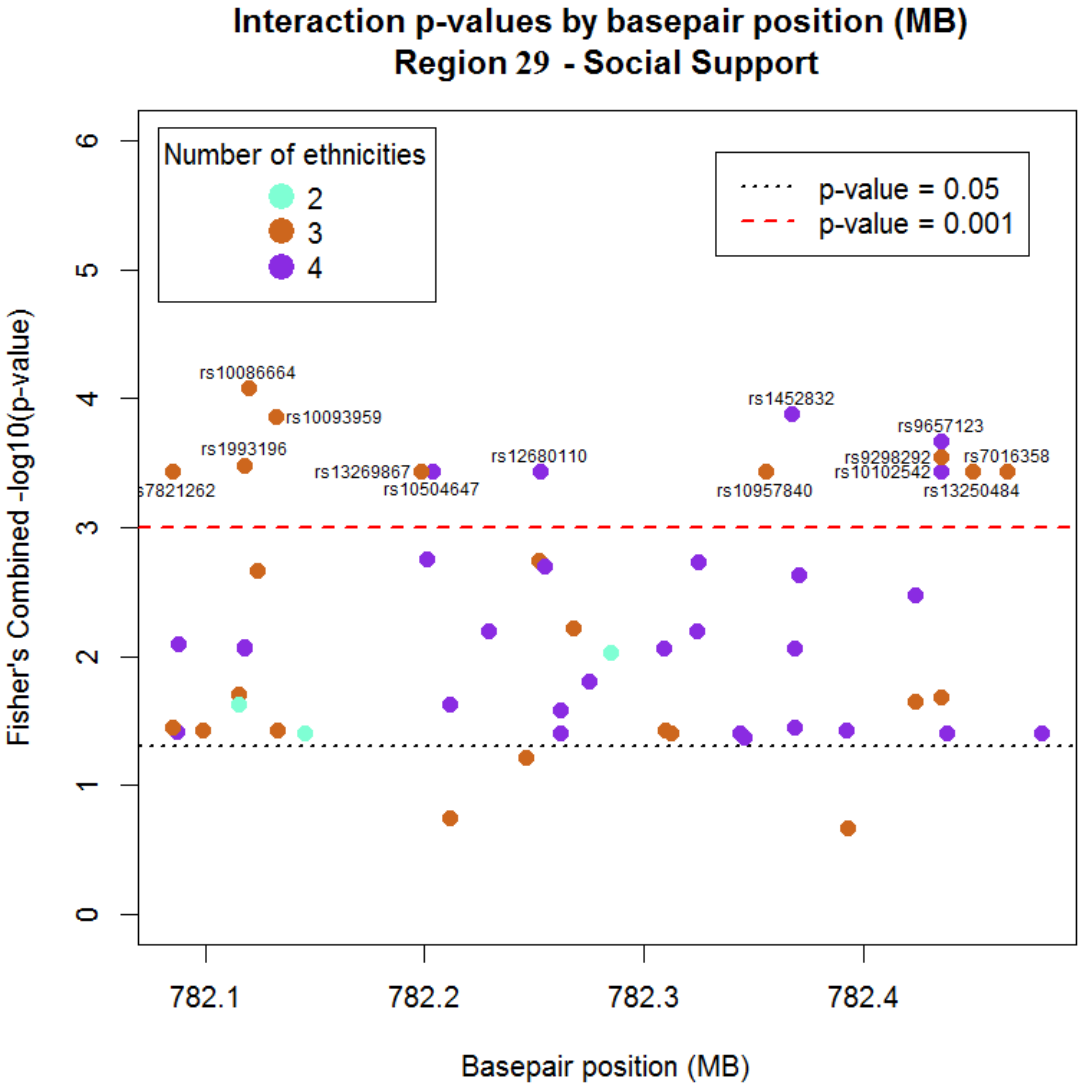
SNPs with Fisher's combined p-value < 0.001 are identified by rs number. Colors indicate the number of ethnicities that were used in calculating the Fisher's combined p-value. Only SNPs with ethnicity-specific minor allele frequency > 5% were included in the Fisher's combined analysis.

Figure 28 Fisher's combined $-\log_{10}$(p-values) for each SNP x environment (chronic burden) interaction in region 44 plotted against genomic position



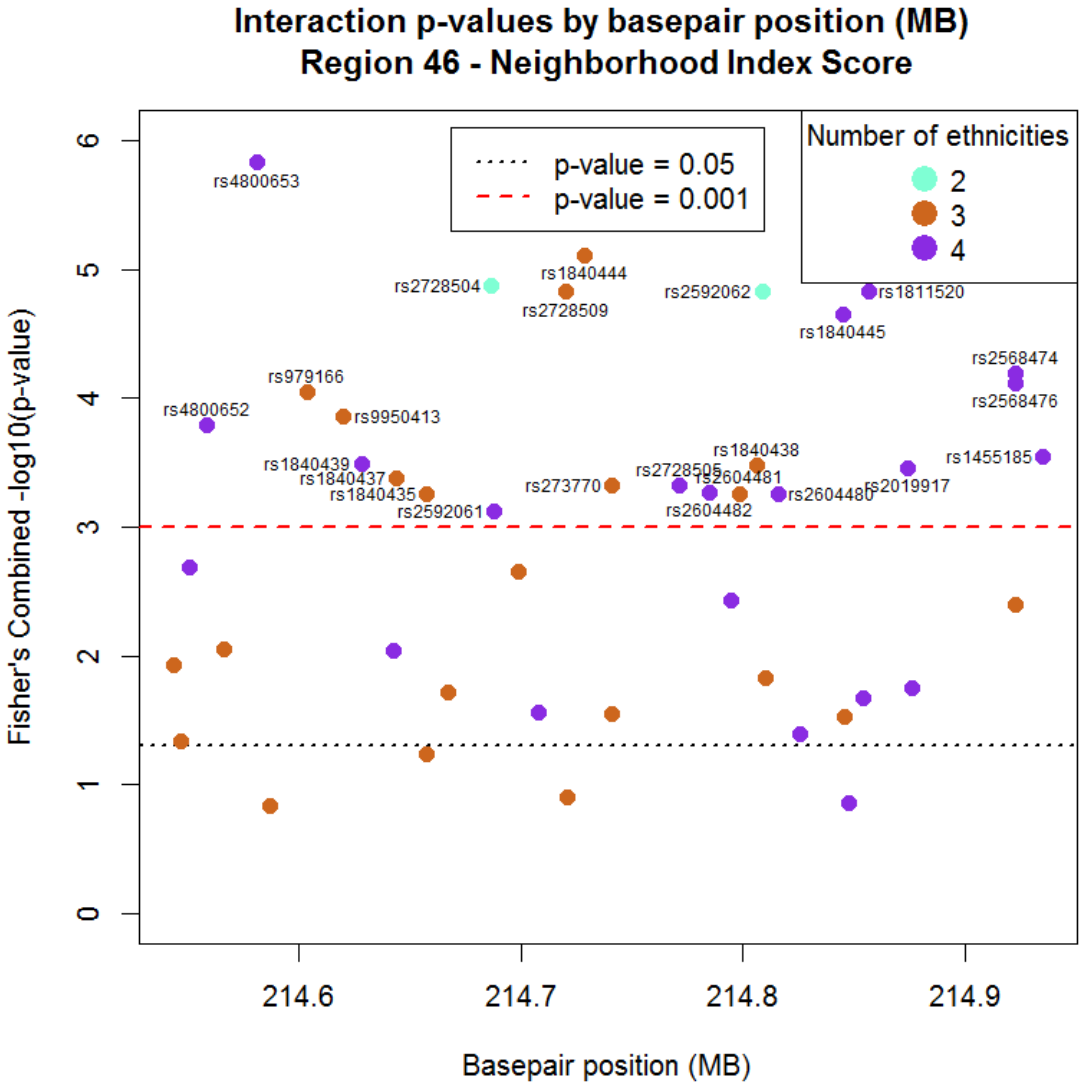
SNPs with Fisher's combined p-value < 0.001 are identified by rs number. Colors indicate the number of ethnicities that were used in calculating the Fisher's combined p-value. Only SNPs with ethnicity-specific minor allele frequency > 5% were included in the Fisher's combined analysis.

Figure 29 Fisher's combined –log10(p-values) for each SNP x environment (social support) interaction in region 29 plotted against genomic position



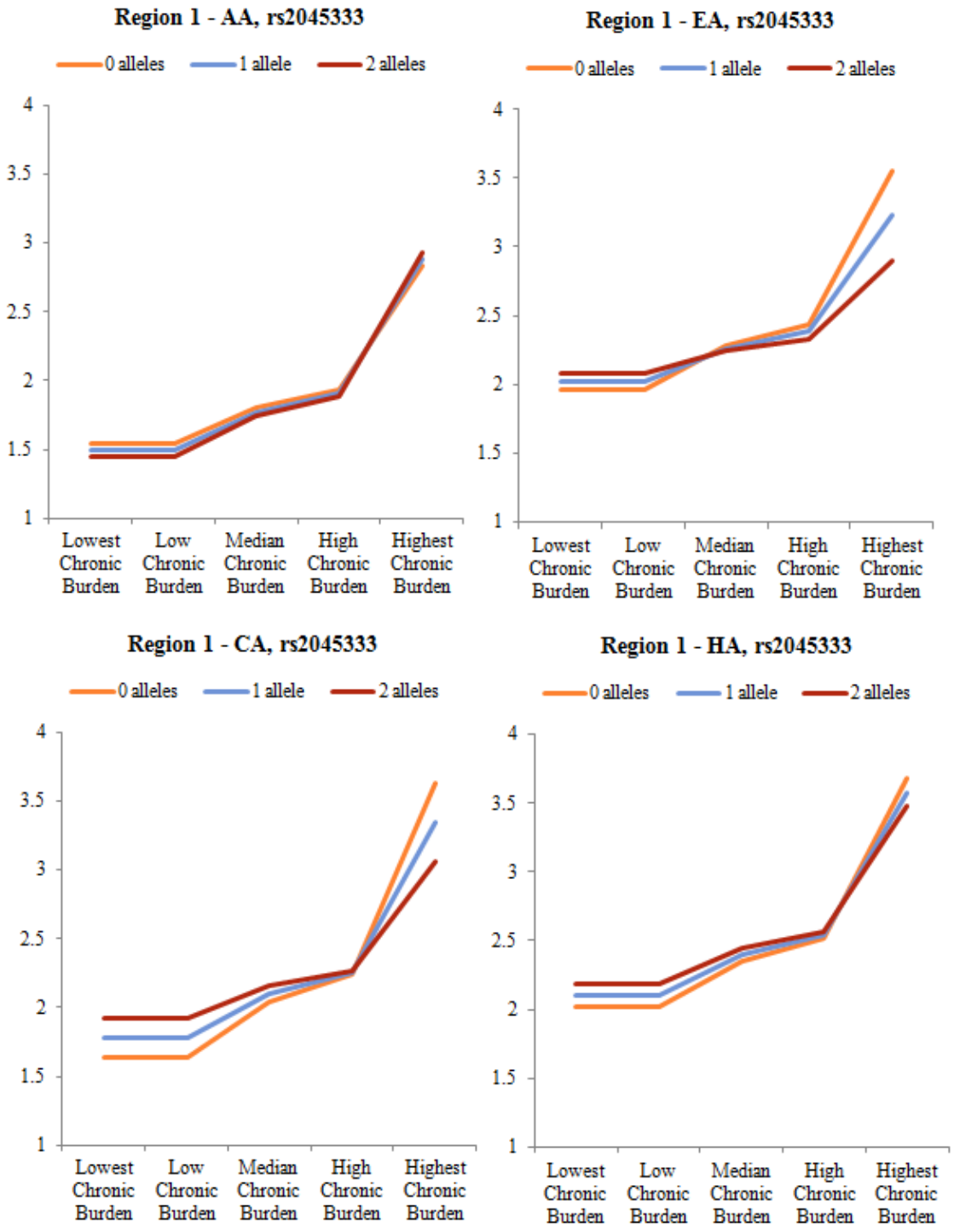
SNPs with Fisher's combined p-value < 0.001 are identified by rs number. Colors indicate the number of ethnicities that were used in calculating the Fisher's combined p-value. Only SNPs with ethnicity-specific minor allele frequency > 5% were included in the Fisher's combined analysis.

Figure 30 Fisher's combined $-\log_{10}$(p-values) for each SNP x environment (neighborhood index score) interaction in region 46 plotted against genomic position



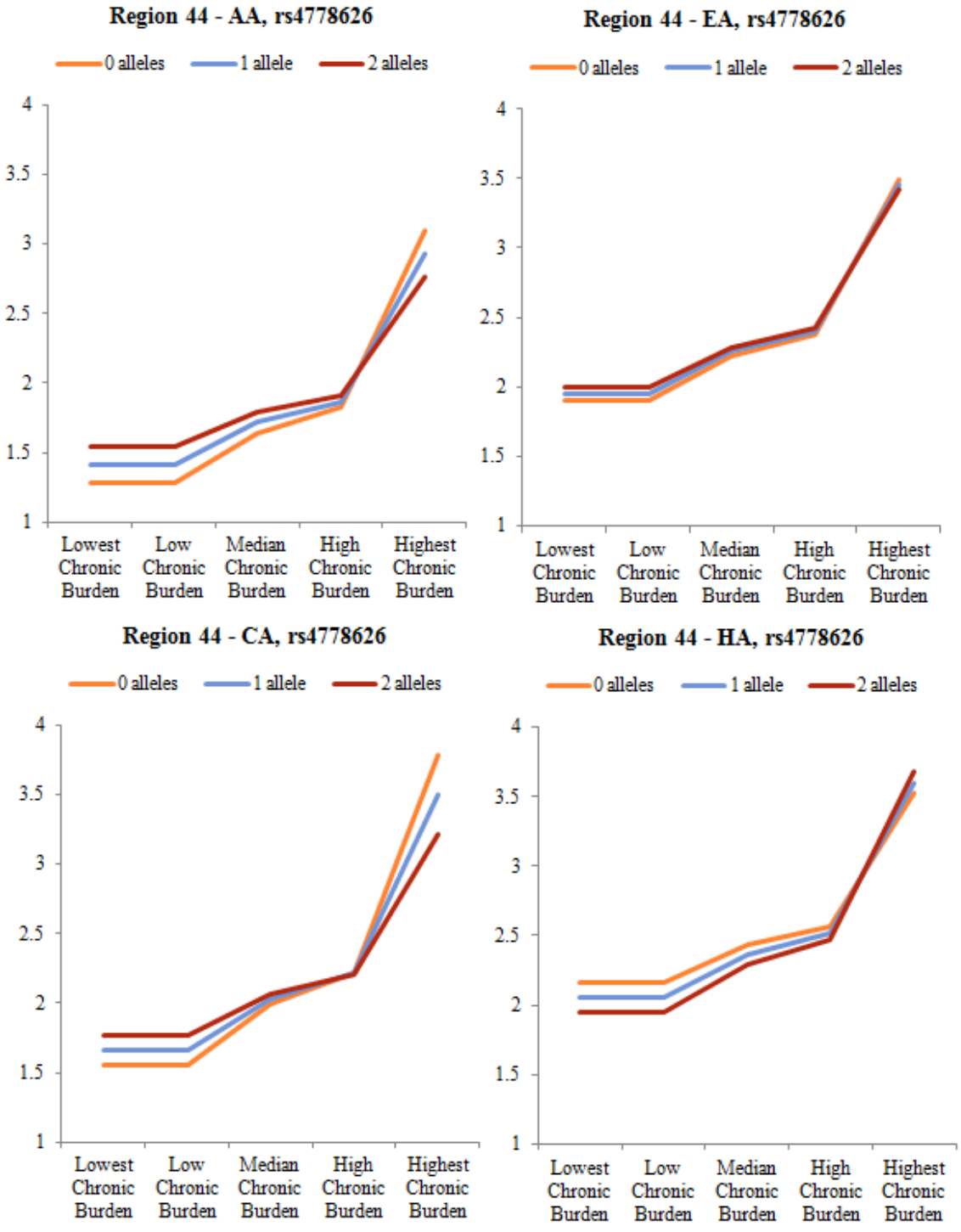
SNPs with Fisher's combined p-value < 0.001 are identified by rs number. Colors indicate the number of ethnicities that were used in calculating the Fisher's combined p-value. Only SNPs with ethnicity-specific minor allele frequency > 5% were included in the Fisher's combined analysis.

Figure 31 Interaction plot for the SNP with the lowest Fisher's combined p-value for region 1



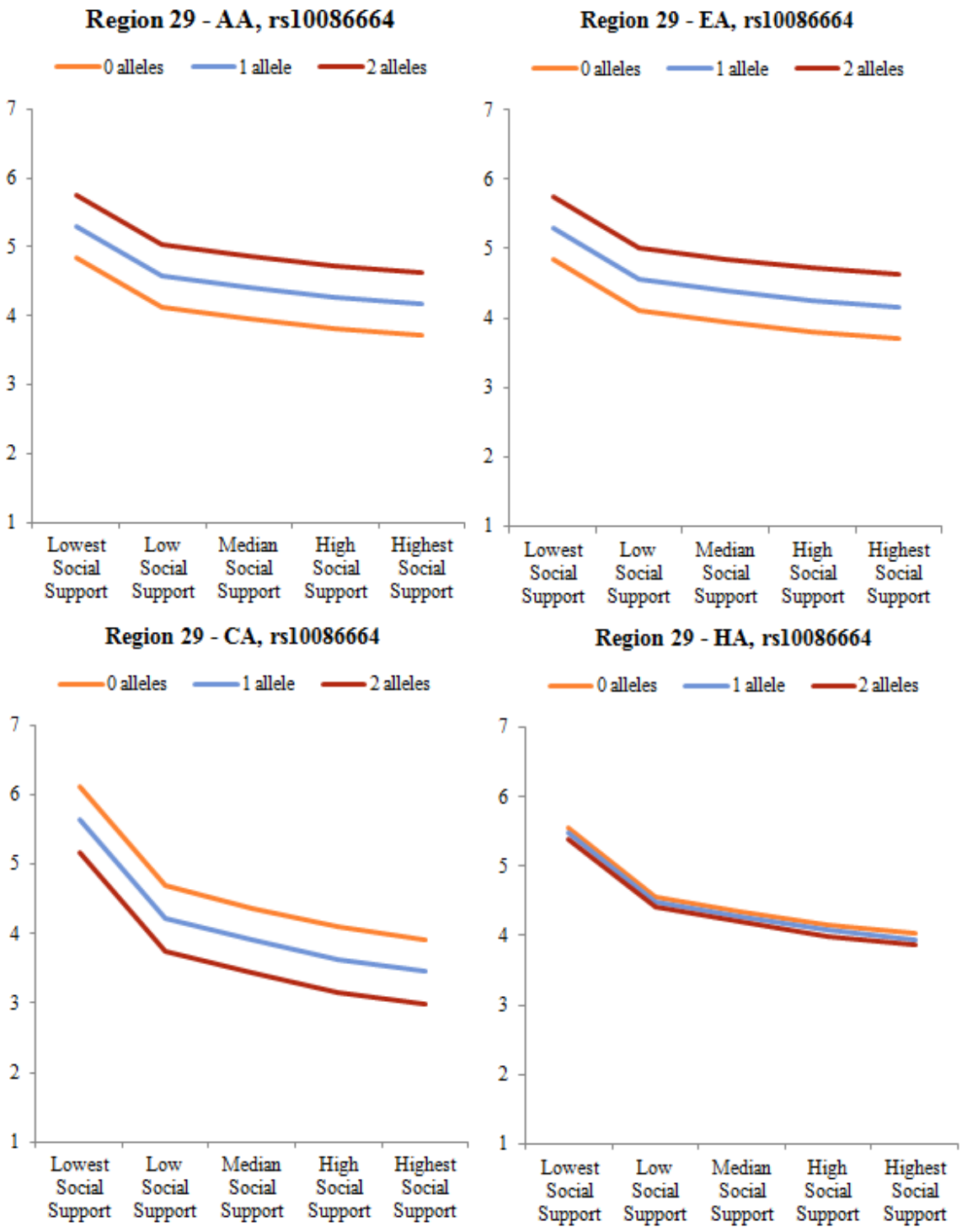
Chronic burden divided into quartiles for plotting purposes, AA: African American, EA: European Americans, HA: Hispanic Americans, Fisher's combined p-value for this region = 4.23×10^{-3}

Figure 32 Interaction plot for the SNP with the lowest Fisher's combined p-value for region 44



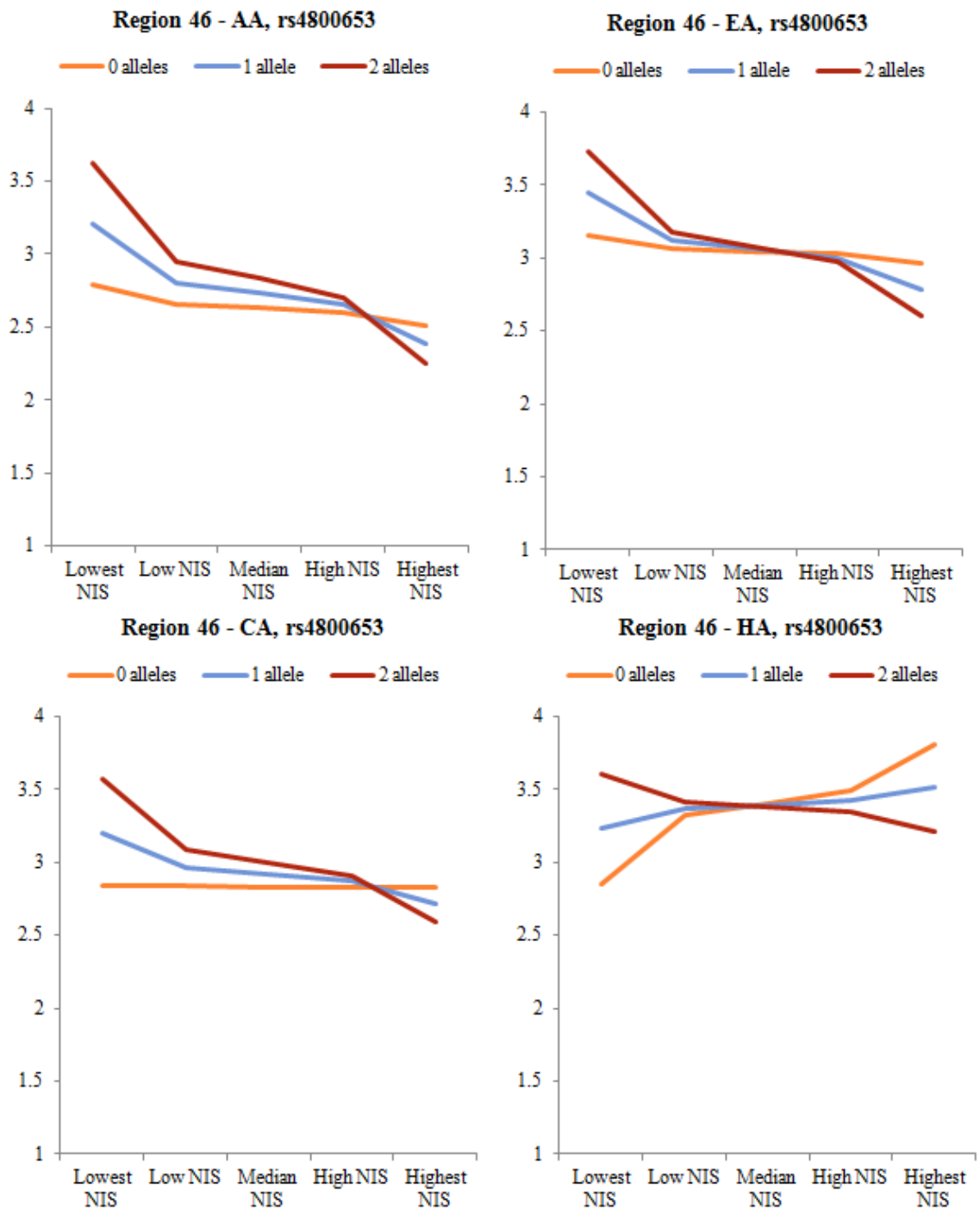
Chronic burden divided into quartiles for plotting purposes, AA: African American, EA: European Americans, HA: Hispanic Americans, Fisher's combined p-value for this region = 6.90×10^{-3}

Figure 33 Interaction plot for the SNP with the lowest Fisher's combined p-value for region 29



Social support divided into quartiles for plotting purposes. AA: African American, EA: European Americans, CA: Chinese Americans, HA: Hispanic Americans, Fisher's combined p-value for this region = 8.27×10^{-5}

Figure 34 Interaction plot for the SNP with the lowest Fisher's combined p-value for region 46



Neighborhood Index Score divided into quartiles for plotting purposes, NIS: Neighborhood Index Score, AA: African American, EA: European Americans, CA: Chinese Americans, HA: Hispanic Americans, Fisher's combined p-value for this region = 1.47×10^{-6}

Figure 35 Encyclopedia of DNA Elements (ENCODE) bioinformatic analysis of region 1 for functional elements in the human genome

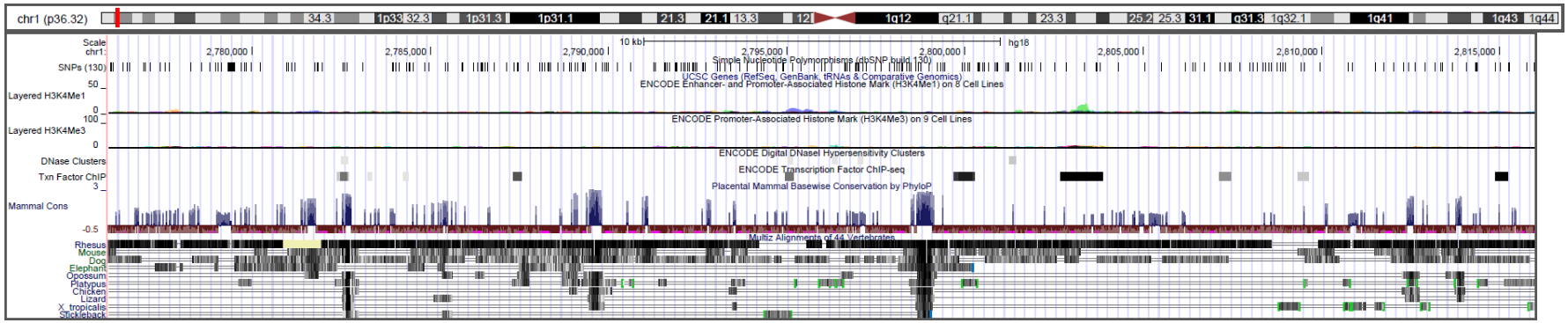


Figure 36 Encyclopedia of DNA Elements (ENCODE) bioinformatic analysis of region 29 for functional elements in the human genome

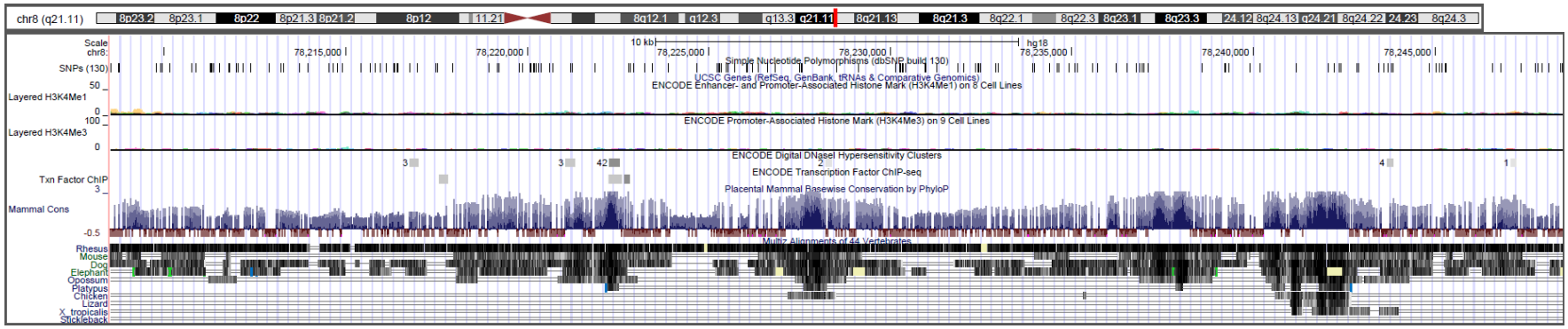
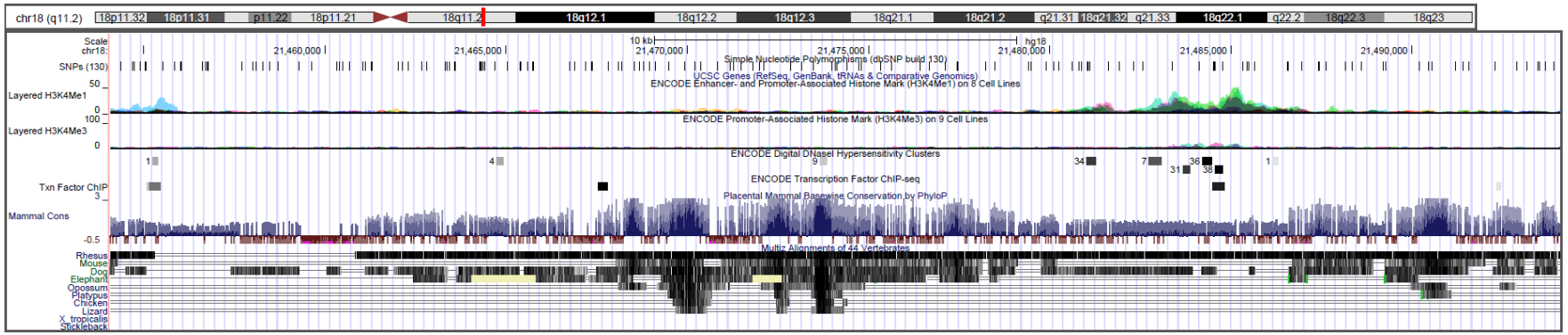


Figure 37 Encyclopedia of DNA Elements (ENCODE) bioinformatic analysis of region 46 for functional elements in the human genome



CHAPTER VI

VI. Conclusion

6.1 Introduction

This dissertation explored genetic and environmental interplay in the context of depressive symptom scores across four genetically distinct ethnicities. This chapter will address strengths and limitations within each analytic chapter, as well as future directions for this research. In chapter III (Comparing genome-wide association studies (GWAS) across different phenotype modeling approaches, and across ethnicities), the goal was to investigate the association of single nucleotide polymorphisms (SNP) and depressive symptom scores in a longitudinal setting to elucidate genetic predictors across four ethnicities (African American (AA), European American (EA), Chinese American (CA), and Hispanic American (HA)). This was accomplished through the use of three different approaches of defining a depressive symptom phenotype. In the analysis investigating ethnicity-specific genetic associations using a baseline measure approach, one SNP in Hispanic Americans was identified at a genome-wide significance level ($\alpha = 5 \times 10^{-8}$). Within each ethnicity, several novel variants were also discovered at a genome-wide suggestive level ($\alpha = 5 \times 10^{-6}$), particularly in the repeated measures approach. In combining p-values across the four ethnicities for the repeated measures approach using meta-analysis, several genome-wide suggestive SNPs were implicated as potential cross-ethnicity genetic predictors of depressive symptoms. Additionally, a SNP previously discovered (and replicated) in European Americans from a prior publication on

depressive symptom GWAS showed evidence of replication across the ethnicities in the Multi-Ethnic Study of Atherosclerosis (MESA).[120] This analysis indicates that a more complete characterization of longitudinal phenotypes provides a powerful platform for analyzing genetic associations of complex traits.

Chapter IV continues the investigation of genetic predictors of depressive symptoms but moves into the framework of SNP-set-level inference. Using the meta-analysis results from the averaged depressive symptom score GWAS across ethnicities, sequence kernel association testing (SKAT) and an extension of SKAT (MetaSKAT) were used to find and meta-analyze SNP-set results across ethnicities. Four SNP-set regions with significant associations for averaged depressive symptoms were discovered across ethnicities. This analysis used innovative techniques to identify genetic signals from a set of SNPs that were not apparent with the individual SNP GWAS. These methods also allowed a combination of genetic associations across ethnicities at the SNP-set level. Importantly, these findings provide justification for moving away from performing solely individual SNP-based GWAS and into the addition of regional/gene-level analysis when examining a phenotype across multiple ethnicities.

Finally, in chapter V, SNP-set by environment interactions using both individual- and neighborhood-level environments were investigated to elucidate modifications of the genetic associations with depressive symptoms by environments. Four genetic regions had significant SNP-set x environment interactions with depressive symptoms (two regions had significant interactions with chronic burden (CB), one region had a significant interaction with social support (SS), and one region with neighborhood index score (NIS)) in multiple ethnicities. In the regions with significant SNP-set by

environment interactions for SS and NIS, combined p-values across ethnicities for individual SNPs showed evidence of additive SNP x environment interactions.

This dissertation represents an important contribution to life sciences in several ways: first, this is the first analyses that incorporates SKAT, MetaSKAT, and GESAT with depressive symptoms; second, through the analysis of common variants as opposed to rare variants in SKAT; third, through the investigation of the association of genetic variants and depressive symptoms across multiple ethnicities; fourth, through a detailed comparison of how longitudinal data can be used to define a mental health phenotype in the context of genetic studies; and finally through the use of both individual- and neighborhood-level interactions with genetic information at both an individual SNP level and a region level. This work contributes to the fields of epidemiology, genetics, and psychiatry.

6.2 Depressive symptoms phenotype

Depressive symptom score measured by the Center for Epidemiologic Studies – Depression (CES-D) is the primary outcome in this dissertation. The CES-D scale assesses several aspects of depression described in the DSM-IV: depressed mood, feelings of guilt and worthlessness, feelings of helplessness and hopelessness, psychomotor retardation, loss of appetite, and sleep disturbance.[7] It was developed by the Center for Epidemiologic Studies for use in general population surveys.[14, 19]

Unfortunately, the CES-D is not a diagnostic interview but a screening measure used to help identify individuals at risk for depression. It may fail to separate depression from generalized anxiety or from depression secondary to other diagnoses. The CES-D also does not address duration and intensity of symptoms, nor does it assess if the

participant's depressive symptoms are a result of bereavement, medication side effects, drugs and alcohol, or physical illness. However, the CES-D scale has high internal consistency, acceptable test-retest stability, excellent validity by clinical and self-report criteria, and substantial evidence of construct validity.[14]

Other diagnostic interviews may provide a better characterization of depressive symptoms (even depression) that would aid in creating a more valid phenotypic measure for analysis. However, these interviews sometimes need to be administered by a clinician or a trained interviewer. The Structural Clinical Interview (SCID) – using DSM-III-R criteria for illness – is a diagnostic instrument that must be administered by a clinical interview. The SCID allows for major axis I diagnoses in modules adapted to assess particular illnesses (e.g. depression) in both current episode (past month) and for lifetime occurrence.[205, 206]

A diagnostic interview that could be administered by a trained interviewer is the Composite International Diagnostic Interview (CIDI). It has been used since its development by the World Health Organization (WHO) in 1990 as an expansion of the Diagnostic Interview Schedule to evaluate mental health with specific modules for Major Depression.[25, 207] The CIDI was primarily developed to be based on the WHO International Classification of Disease (ICD), rather than a diagnosis based on definitions and criteria from the Diagnostic and Statistical Manual (DSM) of Mental Disorders.[208] CIDI diagnoses are significantly related to independent clinical diagnosis, but there is some lack of concordance due partly to the unreliability of clinical interviews.[208] Kessler and Üstün highlight four methodological issues stemming from evaluations of the CIDI: (1) respondents may find some of the CIDI questions confusing due to vaguely

defined terms and multiple clauses, (2) some respondents may not understand tasks implied by the questions, (3) respondents may lack motivation to answer questions accurately due to the potentially embarrassing nature of the questions or stigmatizing experiences, and (4) some respondents may not have the ability to answer questions accurately due to difficulty in recall (e.g. age of onset, number of episodes, etc).[208] One of the major drawbacks of using the CIDI is that, while administration does not require a clinician, it does require a trained interviewer to conduct the assessment.[25]

An alternative interview to the CES-D that can be administered by a lay interviewer is the Beck Depression Inventory (BDI).[209] The BDI is a list of 21 symptoms and attitudes that are rated in terms of intensity. It can be taken in 5 – 10 minutes and is scored by summing the ratings of the 21 items. It was originally designed to be administered by a trained interviewer but is generally self-administered.[209] This may be a more acceptable instrument since it is targeted at depression and can differentiate between depression and anxiety.

Since no reliable biomarker or physiological measure of depression has been established, clinician diagnosis would be the ideal phenotype assessment for these studies, but is not practical due to time and monetary constraints. Accessing electronic medical records for clinical diagnosis of depression may be possible in the future, but currently it is unavailable for MESA participants.

While still using depressive symptoms, perhaps measured by the CES-D, an alternative phenotype may be created using depressive symptom trajectories. Potential overall patterns of depression over the life course have not yet been established, and it is likely that several patterns may exist for individuals with certain attributes (i.e. lifetime

high depression, lifetime low depression, increasing depression over time, decreasing depression over time). Characterizing depressive illness using trajectories may be a pertinent avenue to pursue in future research.

Despite the drawbacks of the CES-D, it has often been used to indicate the prevalence of depression in the literature – however, the CES-D assesses depressive symptoms, which may be a different phenotype than depression. The power to detect genetic variants associated with depression has been shown to increase when assessing depression quantitatively—as opposed to using a dichotomous definition or cutoff point to indicate "depression" —and better captures the phenotypic variations and subclinical depression in those who do not report enough symptoms to be categorized as suffering from depression.[24]

6.3 Genome-wide association studies

Genome-wide association studies are typically designed to identify germline genetic variants associated with the risk of developing human diseases. The method searches the genome for small variants, SNPs that occur more frequently in individuals with a particular disease compared to individuals without the disease. Since this method examines the entire genome, it represents a promising way to study complex diseases which may have many genetic variants that contribute to disease risk. GWAS has been successful in identifying and validating common genetic variants (those with minor allele frequencies (MAF) > 1%) for a variety of human diseases (e.g. type 2 diabetes, coronary heart disease, breast cancer, and asthma).[210-212]

Since the early 2000s, both the Human Genome Project and the International HapMap project have been completed and provide a set of tools that allow researchers to

perform extensive GWAS with these databases that contain references to the human genome sequence as well as a map of human genetic variation both quickly and accurately.[213, 214] Genotype chips, like the Affymetrix Genome-Wide Human SNP Array 6.0 used in MESA, allow for about 906,000 SNPs to be directly typed in a blood sample from an individual. Imputation methods allow for the identification of roughly 2.5 million SNPs total from those (and including those) that are directly genotyped. The genotyping and imputation also include different types of genetic information including rare variants and copy number variants. One reason we may not have found any significant SNPs that were predictive of depressive symptoms in the ethnicity-specific GWAS is that we did not investigate rare variants or copy number variants that were part of the genotyping panel or imputation used in these studies. Imputation is not 100% accurate, nor is direct typing using genotype chips. Because of this, filtering methods are often employed to eliminate SNPs that are genotyped with poor quality. Filtering variants by removing SNPs with SNP-level call rate <95%, individual call rate <95%, and removing monomorphic SNPs allowed for the elimination of SNPs with poor quality from the analyses.

To obtain the most accurate ethnicity-specific imputation, HapMap Phase I and II CEU + YRI + CHB + JPT (release #22 – NCBI Build 36), commonly called the "cosmopolitan panel," was used as the reference panel for the African American, Chinese and Hispanic participants and HapMap Phase I and II – CEU was used as the reference panel (release #24 – NCBI Build 36) for Europeans. These panels are based on populations that were specifically defined. For the YRI (Yoruba), donors were required to have four of four Yoruban grandparents. For the CHB (Han Chinese), donors were

required to have at least three of four Han Chinese grandparents. For the JPT (Japanese), donors were simply told that the aim was to collect samples from persons whose ancestors were from Japan. The criteria used to assign membership in the CEPH population (the CEU panel) have not been specified, except that all donors were residents of Utah. Using ethnicity-specific imputation panels allows us to more accurately match population patterns in SNP frequencies in each ethnicity for imputation – to account for homogeneity within and heterogeneity across ethnicities. The ability to obtain accurate genotype data on multiple ethnic populations allows for the characterization of genetic risk of disease across populations. This is one of the primary strengths of this dissertation. The vast majority of GWAS to date has been performed only in European subsamples which do not allow for comparison across genetically distinct ethnic groups limiting the applicability of study findings beyond European populations.

It is known that allelic effects often differ according to their genetic and environmental context.[215, 216] Quantifying an individual SNP effect is difficult as both genetic and environmental backgrounds of individuals vary greatly.[216] GWAS attempt to reduce genetic heterogeneity by evaluating isolated population groups with higher degrees of genetic homogeneity compared to non-isolated populations (where isolated refers to the lack of out-breeding for many generations).[216] This is done by selecting populations that have similar ancestral backgrounds and analyzing them separately. The four genetically distinct populations in MESA were analyzed separately to address genetic and environmental heterogeneity. That is, that different ethnic groups differ in their environments.

While this dissertation included individuals from four genetically distinct populations, subdivisions within these populations may exhibit different risk profiles of depressive symptoms associated with different genetic variants. For instance, a paper published in 2012 used ~60,000 SNPs selected for minimal linkage disequilibrium to perform population structure analysis on the self-reported Hispanic participants in MESA. The authors showed that the Hispanic sample could be further divided into subgroups with very specific ancestries stemming from Central America, Cuba, the Dominican Republic, Mexico, Puerto Rico, and South America.[217] Though these are important ancestral and cultural differences, the sample sizes of each of these subgroups simply do not allow for powerful analysis using only MESA data. This is generally referred to as population substructure, which is known to be a potential source of confounding in genetic association studies.

Admixture – the joining of two genetically distinct "parent" populations in recent history – will cause confounding of the association between a SNP and the trait of interest if: (1) the allele frequencies of the SNP vary with admixture proportions, (2) the admixture proportions vary among study participants, and (3) the mean value of the trait varies with admixture proportions.[218] Both African American and Hispanic American populations are considered admixed populations due to substantial allelic contributions from European and African ancestors. To account for both population substructure and admixture in each of the four ethnic groups under analysis, principal components calculated within each ethnicity were used as covariates.

Though GWAS have been used for over a decade, most variants identified for diseases (specifically those mentioned above), have had very modest effect sizes, often

explaining less than 1% of the variance of quantitative traits.[219] Because of the small effect sizes, very large sample sizes are required to reach adequate power to detect genetic effects and produce reliable inferences.[220] Most studies alone are underpowered to detect these variants and often collaboration across many studies, involving meta-analysis, are used to increase sample size, and thus power.[212, 219] Preliminary steps have been taken to analyze depressive symptoms in European samples from multiple studies, but in order to harmonize outcomes across studies, only baseline measures were considered.[120] Though this framework is frequently used for common traits with standard measures, it is exceedingly difficult to find studies measuring depressive symptoms using the CES-D in multiple ethnicities, across time.

Once GWAS for depressive symptoms were performed within each ethnicity in MESA, the question became how to compare across ethnicities. Traditional replication – where SNPs with the lowest p-values are "looked up" in an independent sample to determine if the direction of effect is consistent with the current findings and if the replication sample also has a significant effect – has been shown to have less power than a joint analysis method.[221] In a joint analysis, results from two GWAS in two different samples are combined using meta-analysis. There are several different choices of meta-analysis methods that could have been used for these analyses including: p-value meta-analysis, fixed effects, random effects, Bayesian approach and multivariate approaches.[222] Meta-analysis allows us to address the question: are the alleles at a particular marker associated with the disease status across studies (or in this case, ethnic groups). Weighting can improve power and reduce genetic heterogeneous effects. METAL combines information from individual studies (whether separate cohorts or

separate ethnicities) using weights.[153] We utilized the approach in which the direction of effect and p-value observed from each study are converted to Z-scores. Highly negative Z-scores are indicative of small p-values and associations between the coded allele and lower depressive symptom scores while large positive Z-scores are indicative of small p-values and associations between coded alleles and higher depressive symptom scores. The Z-scores are then combined across studies using a weighted sum, where the weights are proportional to the square root of the sample sizes from each study or ethnic subsample.[153, 223]

Ultimately, fixed effects meta-analysis methods using METAL were selected due to the ability of this method to synthesize the effect sizes, and because METAL is easily utilized and implemented. Other methods, such as p-value meta-analysis (i.e. Fisher's combined method), random effects, Bayesian approaches and multivariate approaches introduce too many analytical issues such as not accounting for direction of effects, spuriously large summary effect estimates with selection biases, and computationally intense implementation.[222]

6.4 SNP-set association analyses

Many complex diseases are influenced by the joint effects of genetic variation. A large number of group-wise association tests have been developed recently to evaluate SNP sets and their joint association with disease.[2, 3, 201, 202, 224-229] Group-wise testing has been shown to alleviate problems with intensive computation and multiple testing as well as lead to more stable results and more biologically relevant interpretations.[230-233] In particular, principal component based approaches (PCA),

burden, and variance-component testing (including SKAT) have all been proposed as methods to evaluate the joint effect of SNPs on a disease.[2, 227, 234-236]

PCA is a dimension reduction approach which is often used in genetic analysis to reduce redundant information.[234, 237] The idea is to transform *p* original variables into a set of new predictor variables, *k*, which are made up of a linear combination of the original variables. In using PCA, since many SNPs are in high linkage disequilibrium, the first few eigenvalues are able to capture much of the information in SNP sets. These eigenvalues are then regressed onto the outcome in linear or logistic models.[238]

While PCA may have heavy loadings on important SNPs, meaning that PCA potentially could result in more biologically interpretable findings than other methods, PCA is not without limitations.[238] Latent variables identified in PCA are not necessarily related to the outcome resulting in reduced power for SNP set-based analysis because of the inclusion of SNPs unrelated to the disease.[234] PCA additionally requires a SNP screening step, using all SNPs to create the principal components which may be computationally intensive. Test power from PCA is also affected by the number of PCs included in the analysis.[239]

SKAT has several advantages over other group-wise testing methods. First, SKAT does not assume individual SNPs have similar direction or magnitude of effect within a region and allows for individual variant effects to vary from a mean of zero in either direction. Second, SKAT is a more powerful method, even when sample sizes are small.[3] Importantly, SKAT allows for the adjustment of joint SNP effects by covariates. In addition to the ability to adjust for covariates, SKAT can be extended from its original goal of upweighting rare variants to assessing common variants through an

unweighted linear kernel. Since these analyses did not focus on rare variants, the ability to specify an unweighted linear kernel was particularly important.

MetaSKAT allows our investigation of SNP sets across multiple ethnicities by addressing two particular issues that may limit the ability for an individual SNP to replicate across multiple ethnicities: (1) that there are different underlying patterns of linkage disequilibrium and (2) there are differences in allele frequencies across ethnicities.[174, 175, 177] One drawback of current methods is the inability to assess outcomes in a repeated measures framework, accounting for correlation in the outcome within an individual using robust standard errors. Very recently a method for GEE-based SNP set association tests for continuous and discrete traits in family-based association studies has been published which could plausibly be extended to repeated measures.[240]

One potential limitation of this work is the choice of region size in our SNP set analysis. SNP set regions were chosen based on results from the averaged depressive symptoms phenotype from the meta-analysis across ethnicities using the top 5,000 ethnicity-specific SNPs. The region itself represents all SNPs within ±20 kilobases (kb) of an index SNP from the above analysis – for a total region size of 40kb. While this region represents an *average* LD block,[176] some of the regions created overlapped with other regions. This may be an indication that these particular regions represented a larger LD block than what we would have expected to see. Future research should include conditional analysis to see if the identified regions represent one block with a single "hit" in LD with many SNPs in that region or multiple "hits". Some work has suggested that regions be defined based on LD blocks,[239] while other work has suggested using genes as the region of analysis.[233] Both of these units, the LD block and the gene, may be

more biologically relevant than the regions that were used in these analyses. However, if regions in this analysis were defined as genes (even perhaps as LD blocks), potentially important functional areas, as were identified in chapters four and five, would have been missed. One potential solution is to use a sliding window approach across the entire genome. This approach suffers from several limitations including selection of an appropriate window size and the fact that this method produces myriad tests which suffer from the same multiple testing issues as individual SNP testing.[241] The sliding window approach is not generally valid if it is not known *a priori* that a trend exists and if no correction for multiple testing is applied.[242] Future work may consider investigating all known genes across the genome.

6.5 Gene x environment association analyses

For decades, we have known that failure to assess both genetic and environmental factors together weaken observed associations between true risk factors and disease. Associations from these factors separately combine susceptible and non-susceptible persons and observed associations tend to be shifted toward the null.[243, 244] Identified variants from GWAS have only explained a small proportion of variation in complex diseases. The unexplained variation could be due partly to gene by environment (G x E) interactions, particularly in complex illnesses like depressive symptoms which are thought to have considerable interplay between genetics and the environment.

In epidemiological studies the term ‘interaction’ often has several meanings.[245] *Statistical interaction*: a departure from a pure main effects model with either additive or multiplicative effects for a disease risk or natural or logarithmic effects for a continuous trait,[246] *quantitative interaction*: a statistical interaction in which

effects of one predictor have the same direction at differing levels of another factor but differ in magnitude, *qualitative interaction*: a statistical interaction where effects go in opposite directions at different levels of a second variable,[247, 248] *synergism*: where the burden of disease can be attributed to exposure of two or more factors that is greater than the sum of the risk from each factor alone.[249] In the analyses presented in this dissertation, models are testing for statistical interactions in terms of departure from additivity and synergism. G x E studies in general often suffer from several challenges which are discussed in detail below: exposure assessment, sample size and power, and study design.[246, 250]

6.5.1 Exposure assessment

Few genetic studies have detailed measures of environment, let alone measures of environment at different time points across the life course. Since environmental factors may be multidimensional and vary over time, it is important to be able to capture temporal changes. MESA is unique in that the study has taken great care to assess multiple environments carefully across all exams. Particularly with the MESA neighborhood environments, extreme efforts have been made to produce objective measures of neighborhood factors by using individuals outside of the original MESA survey and synthesizing the results. Misclassification of exposure information in general can be large and could lead to unpredictable biases, especially when exposures differ with respect to disease status, which can ultimately induce spurious interactions.[251] It is also possible that we are not measuring the 'right' environments. Important environmental determinants of disease may be missed because we either do not know exactly what to look for or how to measure the environment correctly when we do. It could also be that

an individual's genetics are too far removed from physiologic or biochemical processes that result from environmental exposures.[244]

6.5.2 Sample size and power

In analyzing G x E interactions, considerable sample sizes are needed to have enough statistical power to detect effects. As a rule of thumb, it has been suggested that detections of interactions require sample sizes at least four times as large as those required for detection of main effects with comparable magnitudes.[252] In GWA studies (essentially the main effect models), sample sizes of tens of thousands of cases are usually required to produce enough power to detect effects based on stringent significance levels and sample sizes in the thousands of cases are typically required for G x E analyses in studies where a few candidate genes are to be studied.[250] Though MESA has several thousand participants, genetic heterogeneity among the four ethnic groups resulted in the decision to analyze these groups separately. Sample sizes within each ethnicity fall short of the *thousands* of individuals recommended for G x E studies. Future research will include efforts to add Health and Retirement Study (HRS) participants while harmonizing environmental measures to enhance the sample size in these analyses.

Other considerations in determining the power to detect effects in G x E studies include the distribution of exposure, allele frequency, and significance level. The inability for some G x E results to replicate may have to do with underpowered discovery or replication samples.[253-255] To counter some of these issues, these analyses eliminated any SNPs within a region that had MAF < 5% (including monomorphic SNPs) from interaction analysis. Though some have suggested that interactions should not be

investigated if there is not a marginal effect[256], a range of interaction effect sizes can be detected even when marginal effects are not detectable.[250] The significance level was set to $\alpha = 0.20$ from the marginal effect for the region to be included in the interaction analysis and $\alpha = 0.05$ for interaction effects. These significance levels were based on previous findings that genetic effects can be apparent solely in groups with relevant environmental exposures – where environmental factors affect only those with a particular susceptibility genotype.[247, 248]

6.5.3 Study design

MESA is a prospective cohort study of unrelated individuals. Choice of the type of study to analyze G x E interactions (as well as main effects) often includes the consideration of the temporal sequence of exposure and disease, control of confounding and other biases, and data quality.[250] Many study designs are available and most have been employed to analyze G x E interactions including classic epidemiologic designs (e.g. cohort, case-control, case-only, randomized trial, crossover trials, etc.), hybrid designs (e.g. nested case-control, case-cohort, two-phase case-control, counter-matching, etc), family-based designs (e.g. case-sibling, case-cousin, case-parent triad, twin studies, etc.), and designs specifically developed for genetic studies (e.g. two-stage genotyping, two-step interaction analysis, DNA pooling).[250]

Cohort designs allow for the comparison of incidents of new cases across groups that are defined by both genes and environments. The particular advantages of using a cohort design for G x E studies is that cohorts are free from most biases and allow for a clear temporal sequence of cause and effect. Though this is true of most cohort designs, the particular instruments selected to assess the phenotype of interest in this analysis

(CES-D scores) and several of the environmental factors (chronic burden and social support) do not allow for such a clear temporal sequence. CES-D is assessed as symptoms occurring in the last seven days, while the scales for chronic burden and social support are measured over varying time frames (e.g. ongoing for more than six months). It is not feasible to define the temporality of cause and effect for our study, so these events are treated more as concurrent occurrences. A primary disadvantage of cohort studies is the need for long follow-up periods which often leads to loss-to-follow-up, particularly in elderly cohorts. However; the response rate (of participants alive) for MESA has been excellent: exam 1 (n=6,814), exam 2 (n=6,239, 92%), exam 3 (n=5,946, 89%), exam 4 (n=5,704, 87%).

Several of the non-traditional designs mentioned above have particular advantages for interaction investigation, most notably family-based association tests. Family-based association tests, including case-parent triads[257], case-sibling designs[258], and designs using family pedigrees[259] avoid bias from population stratification, which is particularly relevant when exploring G x E interactions within different ethnicities. Family-based study designs would allow for the separation of genetic and shared environment contributions to disease risk and allows for assessment of heritability – estimated to be around 30 to 50% for depression based on twin studies.[86] Though there are some advantages to family-based designs, there are several issues that would arise in using these designs to study depressive symptoms and the environments which were analyzed in these analyses. First, family-based association tests are generally less powerful for testing main effects than studies using unrelated individuals.[258] Since the second aim of this dissertation specifically evaluated main effects, the cohort study

design with unrelated individuals was a more powerful choice. Second, family-based studies are more powerful for testing G x E interactions if relatives' exposures are not too highly correlated. It is plausible that recruiting families using neighborhood of residence to define environments may produce exposures that are highly correlated within families. Finally, case-parent triads require that surviving parents are genotyped. This dissertation investigates depressive symptoms in participants who are on average 62.2 years old. It is not likely that all participants would have living parents that could contribute genetic information.

Though there may be some methodological issues that arise when investigating G x E interactions, the MESA cohort, with careful attention to data quality, relatively large samples sizes for multiple ethnicities, and prospective cohort design, makes an ideal sample for these analyses. The MESA cohort information for this dissertation has included four exams spanning 10 years, three of which measured depressive symptoms using the CES-D, as well as objective neighborhood information created by surveying individuals who reside in the same areas as MESA participants. MESA has excellent response rates with minimal loss-to-follow-up.

6.6 Future directions

This work is a novel investigation of the extent to which individual- and neighborhood-level social exposures interact with genetic predispositions to affect levels of depressive symptoms in population-based samples. Though it has extensively used new statistical methods to investigate individual SNPs, SNP sets and SNP set interactions in the context of G x E analysis at both an individual- and neighborhood-level, as well as a continuous phenotype, there is much room to build upon this work.

6.6.1 Repeated measures methods

Extending methods used in this dissertation to allow for repeated measures for an individual in both the SNP set and SNP set x environment analyses will increase the power to detect genetic effects of depressive symptoms. At the time of analysis, no validated methods existed to allow for a repeated measures framework, though several promising methods are either in press or have been recently been published.[240]

6.6.2 Epistasis

Neither SNP x SNP interactions nor SNP set x SNP set interactions were investigated in this dissertation. While epistasis – a phenomenon where the expression of one gene depends on the presence of one or more 'modifier genes'[260] – is an important avenue for investigation of genetic effects, it was simply beyond the scope of the research question for these analyses. If we consider the 37 SNP sets that were investigated in the interaction models, there would be 1,081 two-way interactions and 16,215 three-way interactions. The number of tests can skyrocket quite quickly, involving much computational power. Even when limiting the scope of SNP sets to only those with what were considered marginal main effects (p-value < 0.20), the dimensionality of the interactions is burdensome. Including three (or more) environments only compounds the multiple testing issues. A more comprehensive model for disease with multiple genes and multiple environmental risk factors ought to also consider G x G interactions.[246, 261]

6.6.3 Pathway based analysis: Mediation through epigenetics and other functional mechanisms

One potentially fruitful avenue for future research is to investigate pathway based analysis. This method attempts to understand how intermediate events – such as changes

in gene expression, epigenetic processes, somatic mutations, and interference by small RNAs – may mediate genetic and environmental effects.[262-264] This is incredibly relevant to these analyses since many of our strongest gene-level results did not fall in established gene regions, but rather in areas with potential functional regulatory regions (chapter V). Few studies have embarked on this trajectory,[262] and none – to the author's knowledge – in depressive symptoms. The basic idea of pathway-based analysis methods stems from observations that monozygotic twins, who begin life with identical methylation patters, over the life course are exposed to different environments which may provide mechanisms at the genetic level that result in differing patterns of disease.[265] This type of analysis requires in-depth (and often expensive) measurements of genetic information, such as methylation and expression data, which is slowly gaining popularity in large cohort studies as prices decrease.

6.6.4 Next-generation sequencing and rare variants

Individual SNP analysis has been used to find many disease-associated loci, but region-based analysis has been shown to possess much higher power. In order for the most accurate genetic measures to be analyzed, high quality genotypes must be obtained. Given developments in next generation sequencing technologies and haplotype assembly algorithms – which allow for analysis of very specific genotype patterns and disease risk – we are entering an era where finer and finer genetic information is becoming available. Though not widely implemented (partially due to cost and massive data storage requirements), next generation sequencing methods are making it possible to sequence suspect portions of the genome at an extremely detailed level in subsamples of individuals. Additionally, rare variants – those SNPs that have minor allele frequencies

<1% – are gaining attention as these variants may account for some of the unexplained variance in disease.[250] This sequencing could help to make the analysis of rare variants, in combination with methods designed to up-weight the effects of rare variants so that they are not masked by other variants in a region (e.g. SKAT[2]), more standard practice in genetic association studies.

6.6.5 Consortia efforts

Consortia efforts combine many studies with like samples and similar measures to increase sample sizes. Given the extremely large sample size requirements to obtain adequate power for genetic studies due to multiple testing issues, consortia efforts are almost mandatory in genetic studies. The problem with consortia studies is in finding studies with well-defined and comparable measures. The analyses in this dissertation present a complex problem for consortia, mainly the use of multiple ethnicities and highly detailed measures of environment. While previous consortia efforts for studying depressive symptoms in European ancestry individuals have reached over 50,000 individuals (replication sample size = 51,258), only 34,549 individuals had depressive symptoms assessed with the CES-D. The other individuals used in replication had depressive symptoms defined through other instruments (e.g. Geriatric Depression Scale, Patient Health Questionnaire, Beck Depression Inventory-II, Maastricht Questionnaire). Since depressive symptoms can be assessed using a wide variety of instruments, which are often highly correlated (between 0.77 and 0.86), it may be possible to produce large sample sizes for consortia in these ethnicities if researchers are willing to slightly relax the phenotype definitions. This does; however, bring into question whether these efforts would actually get at the true genetic predictors of depressive symptoms given the

heterogeneity in measurement. Future studies should strive to evaluate both depressive symptoms and environmental factors using consistent measures.

6.6.6 Public health and personal medicine

Depression will soon be second only to cardiac ischemia in terms of disease-related morbidity.[5] Depression is a complex disorder made up of symptoms outlined in the Diagnostic and Statistical Manual – Mental Health.[7] These symptoms are influences not only by clinical, psychosocial and environmental contributors but also by genes. Insight from G x E interactions may have important policy implications for targeted intervention[266], treatment selection[267], and even environmental health standards.[268] Genes and gene regions identified in this study may prove to be parts of important pathways in the development of depression. Ultimately, identifying these pathways could lead to improved pharmacological efforts targeted specifically at certain genetic profiles. Knowledge of a patient's genetic and environmental profiles based on exposure to chronic burden, social support, or neighborhood structure may serve as warning signs to clinicians when presenting in a clinical setting. This may assist health care professionals in identifying depression and identifying strategies to reduce deleterious exposures. Finally, this study provides evidence that living in poorer quality neighborhoods (those with low social cohesion, aesthetic quality, or low perceived safety) put individuals with certain genetic variants at higher risk for more depressive symptoms. Though idealistic, it may be possible to reduce depressive symptoms if public policies for cleaning up neighborhoods, improving relationships across neighbors, and increased law enforcement presence are implemented. Unfortunately, the translation of

scientific understanding about G x E interactions into risk assessment and prevention policies has been limited.[269]

6.7 Concluding remarks

Genetic factors, stressors operating over the life-course, and various aspects of social context (including neighborhood environments) clearly play a role in the etiology of depressive symptoms. This evaluation of the joint effects of these factors as well as their interactions has found preliminary evidence of several genetic regions with implications for depressive symptoms across multiple ethnicities as main effects and also in interactions with environments. Future work should focus on clinically evaluated quantitative outcomes as well as replication efforts not only in European Americans, but in African, Chinese, and Hispanic Americans, too.

APPENDICIES

Appendix 1 Center for Epidemiologic Studies Depression Scale (CES-D) National Institute of Mental Health

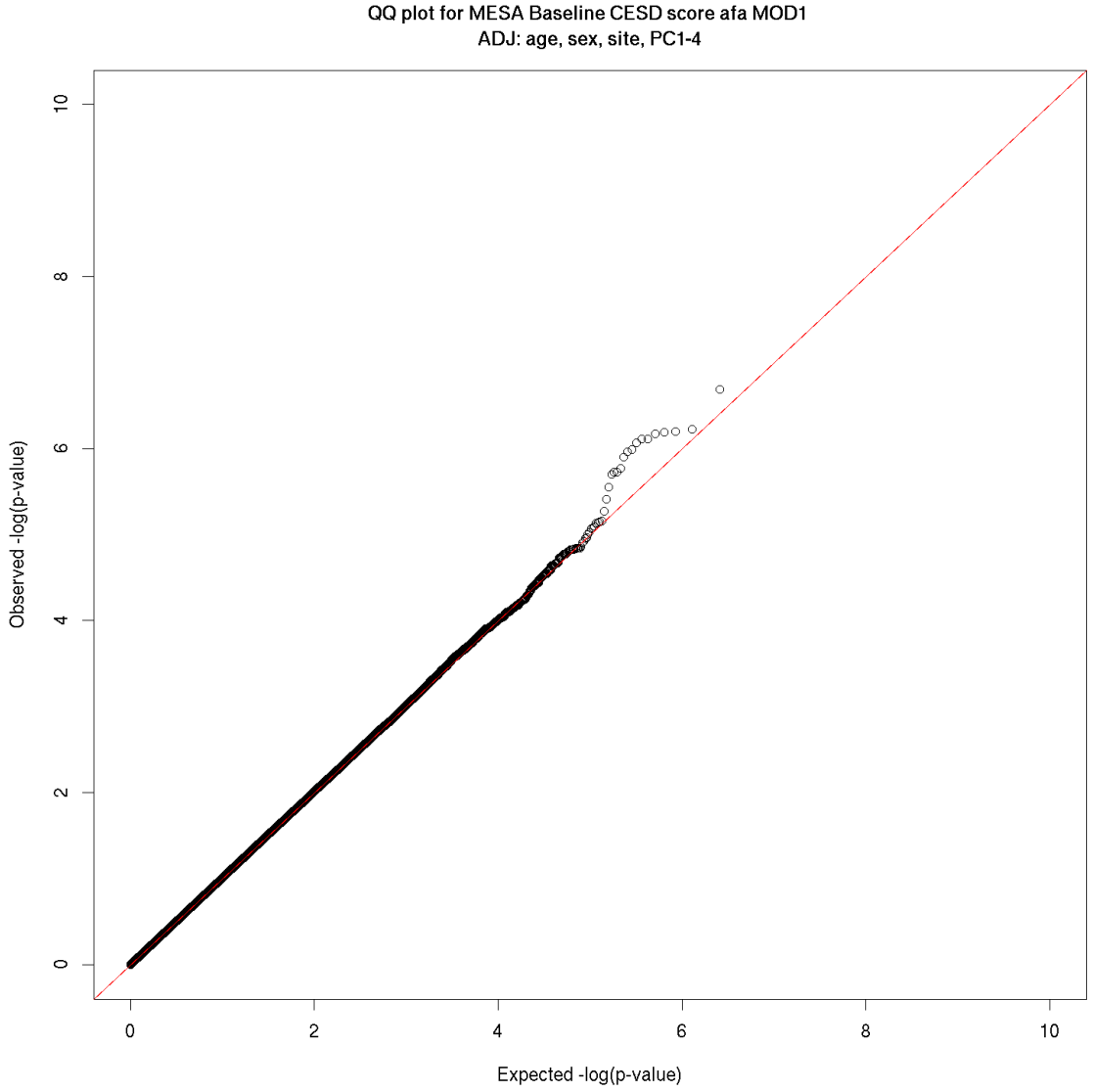
Center for Epidemiologic Studies Depression Scale (CES-D), NIMH

Below is a list of the ways you might have felt or behaved. Please tell me how often you have felt this way during the past week.

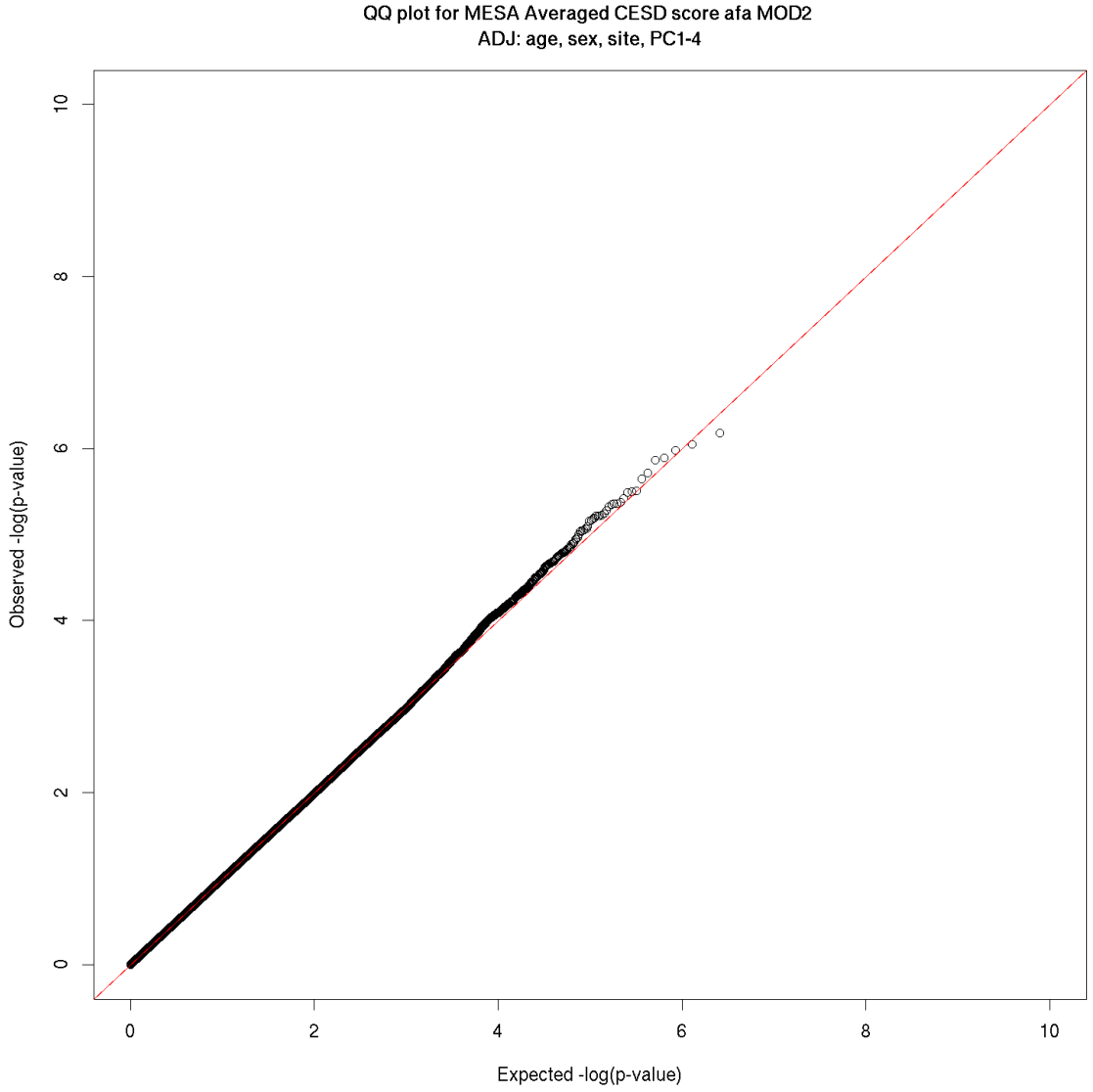
	During the Past Week			
	Rarely or none of the time (less than 1 day)	Some or a little of the time (1-2 days)	Occasionally or a moderate amount of time (3-4 days)	Most or all of the time (5-7 days)
1. I was bothered by things that usually don't bother me.	☐	☐	☐	☐
2. I did not feel like eating; my appetite was poor.	☐	☐	☐	☐
3. I felt that I could not shake off the blues even with help from my family or friends.	☐	☐	☐	☐
4. I felt I was just as good as other people.	☐	☐	☐	☐
5. I had trouble keeping my mind on what I was doing.	☐	☐	☐	☐
6. I felt depressed.	☐	☐	☐	☐
7. I felt that everything I did was an effort.	☐	☐	☐	☐
8. I felt hopeful about the future.	☐	☐	☐	☐
9. I thought my life had been a failure.	☐	☐	☐	☐
10. I felt fearful.	☐	☐	☐	☐
11. My sleep was restless.	☐	☐	☐	☐
12. I was happy.	☐	☐	☐	☐
13. I talked less than usual.	☐	☐	☐	☐
14. I felt lonely.	☐	☐	☐	☐
15. People were unfriendly.	☐	☐	☐	☐
16. I enjoyed life.	☐	☐	☐	☐
17. I had crying spells.	☐	☐	☐	☐
18. I felt sad.	☐	☐	☐	☐
19. I felt that people dislike me.	☐	☐	☐	☐
20. I could not get "going."	☐	☐	☐	☐

SCORING: zero for answers in the first column, 1 for answers in the second column, 2 for answers in the third column, 3 for answers in the fourth column. The scoring of positive items is reversed. Possible range of scores is zero to 60, with the higher scores indicating the presence of more symptomatology.

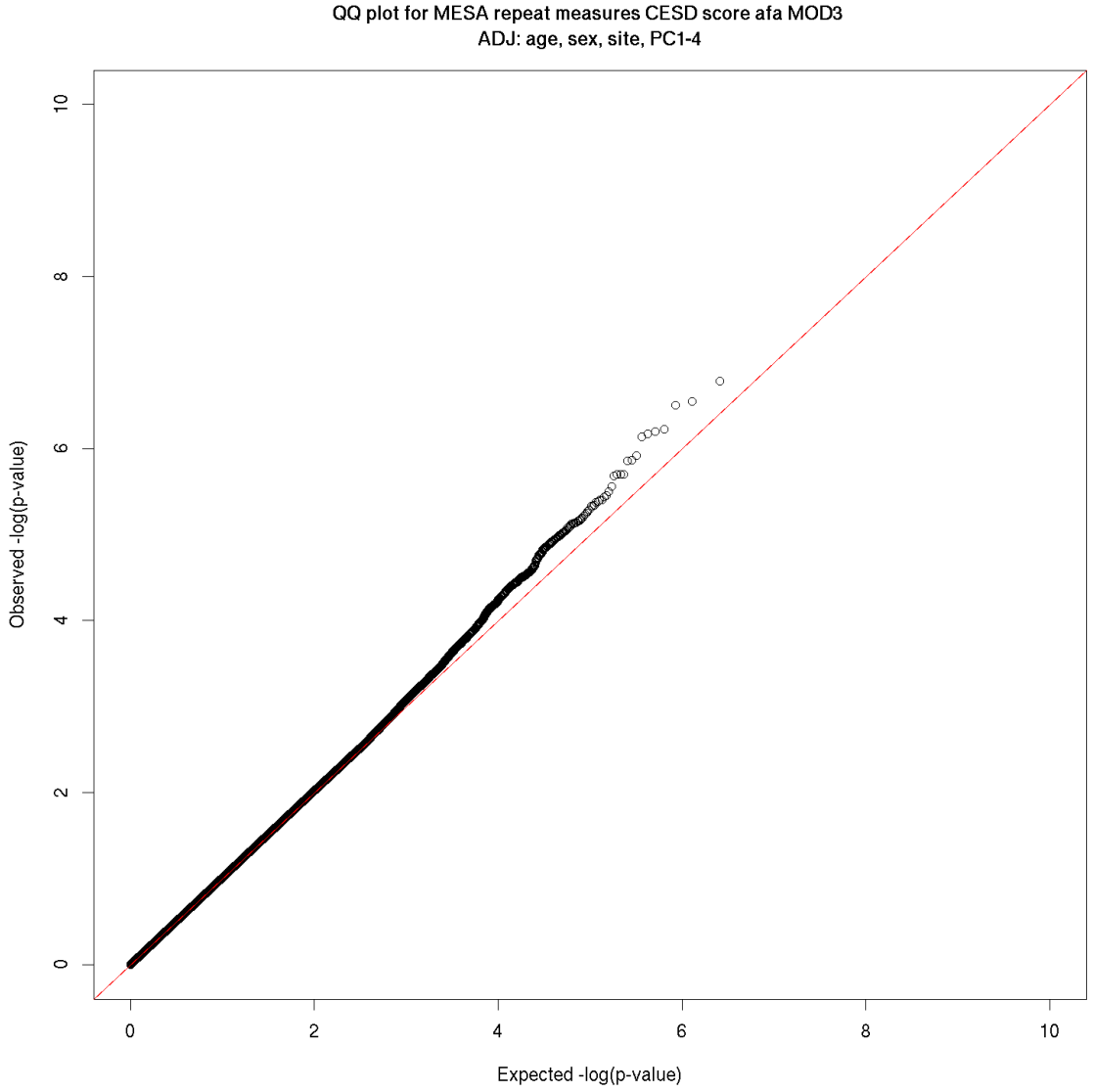
Appendix 2 QQ plot of p-values from SNP association with baseline depressive symptom score from African American GWA analyses adjusted for age, sex, study site and top four principal components, minor allele frequency greater than 5%, Multi-Ethnic Study of Atherosclerosis



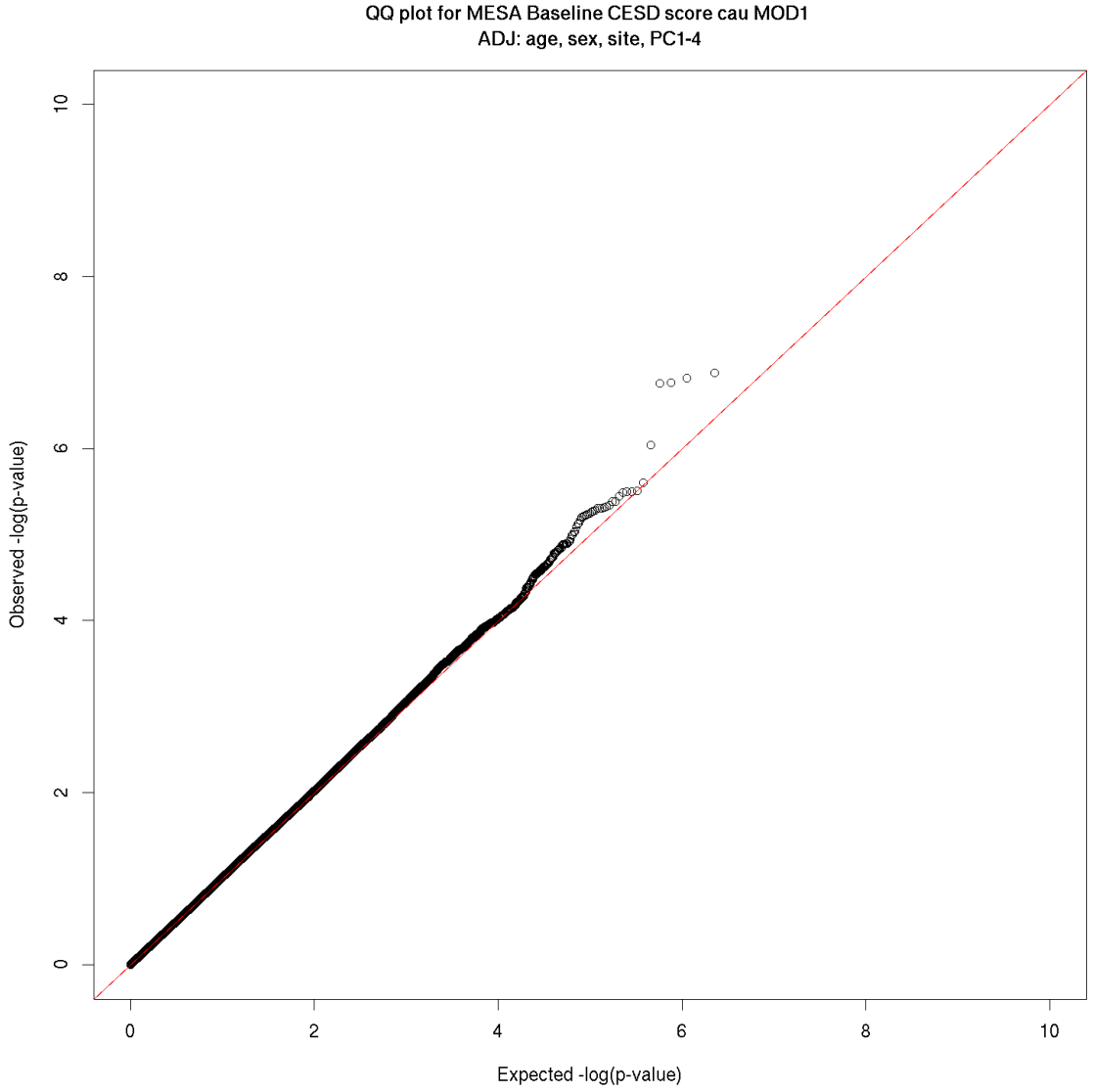
Appendix 3 QQ plot of p-values from SNP association with averaged depressive symptom score from African American GWA analyses adjusted for age, sex, study site and top four principal components, minor allele frequency greater than 5%, Multi-Ethnic Study of Atherosclerosis



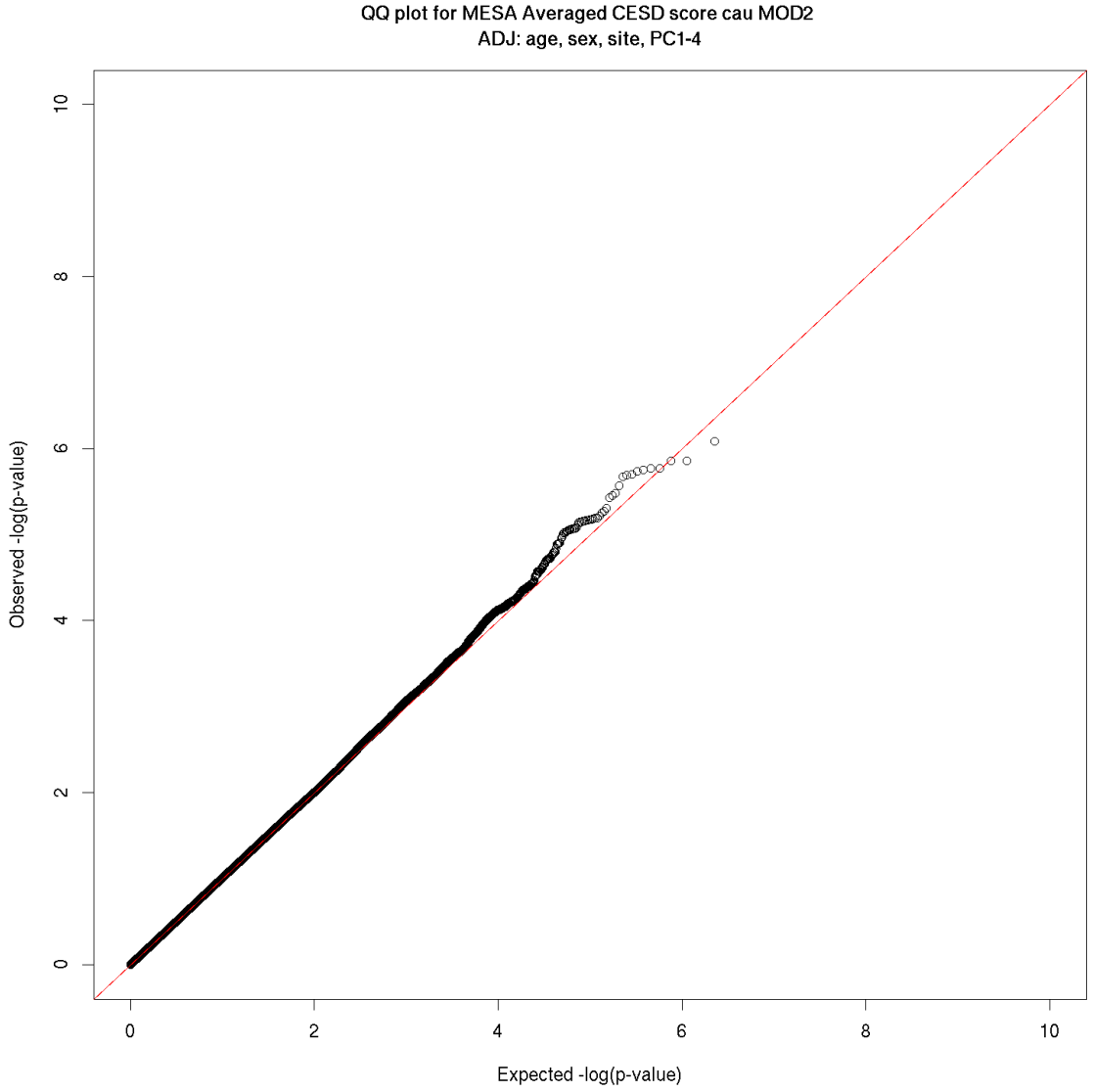
Appendix 4 QQ plot of p-values from SNP association with repeated measures depressive symptom score from African American GWA analyses adjusted for age, sex, study site and top four principal components, minor allele frequency greater than 5%, Multi-Ethnic Study of Atherosclerosis



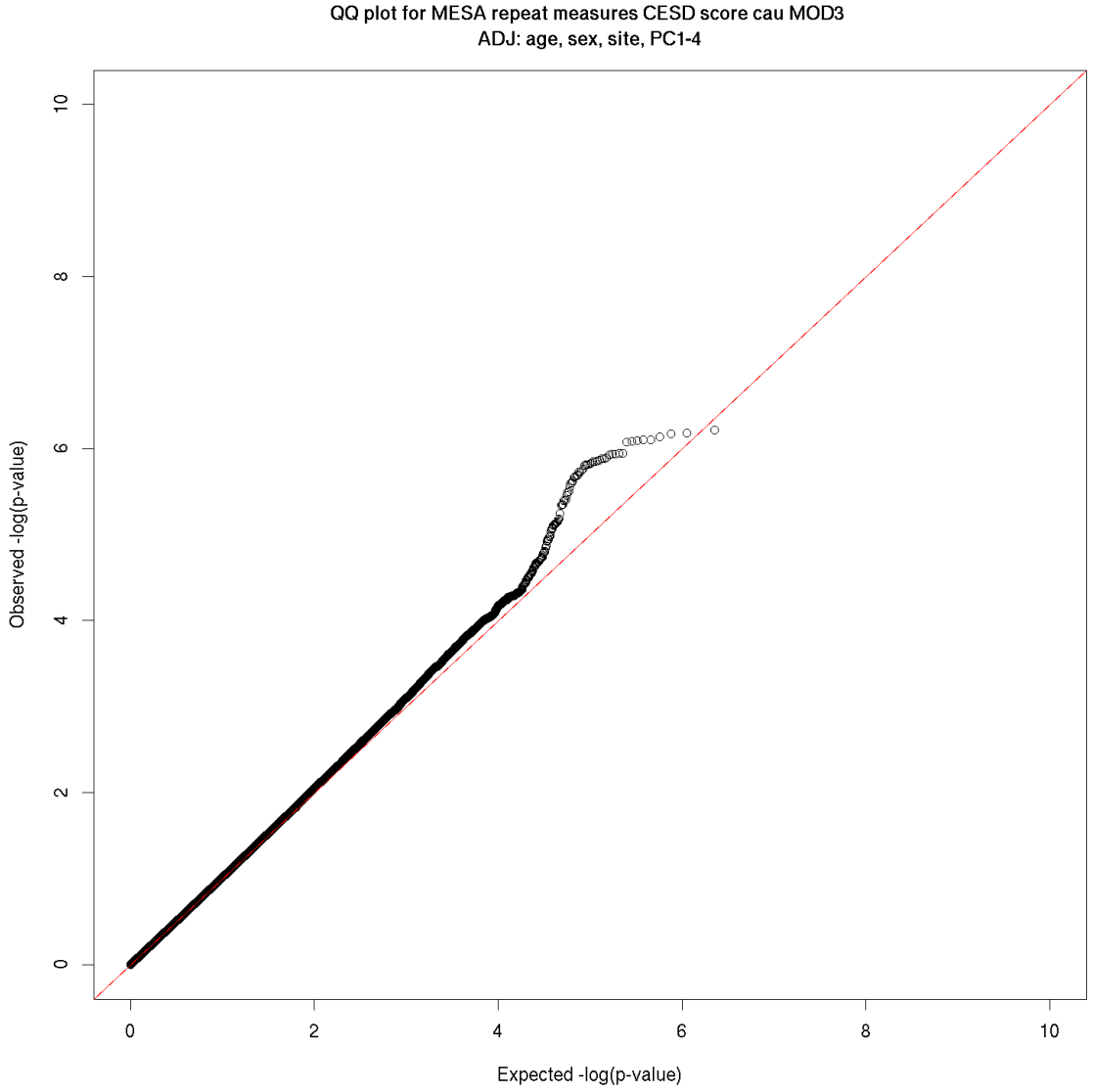
Appendix 5 QQ plot of p-values from SNP association with baseline depressive symptom score from European GWA analyses adjusted for age, sex, study site and top four principal components, minor allele frequency greater than 5%, Multi-Ethnic Study of Atherosclerosis



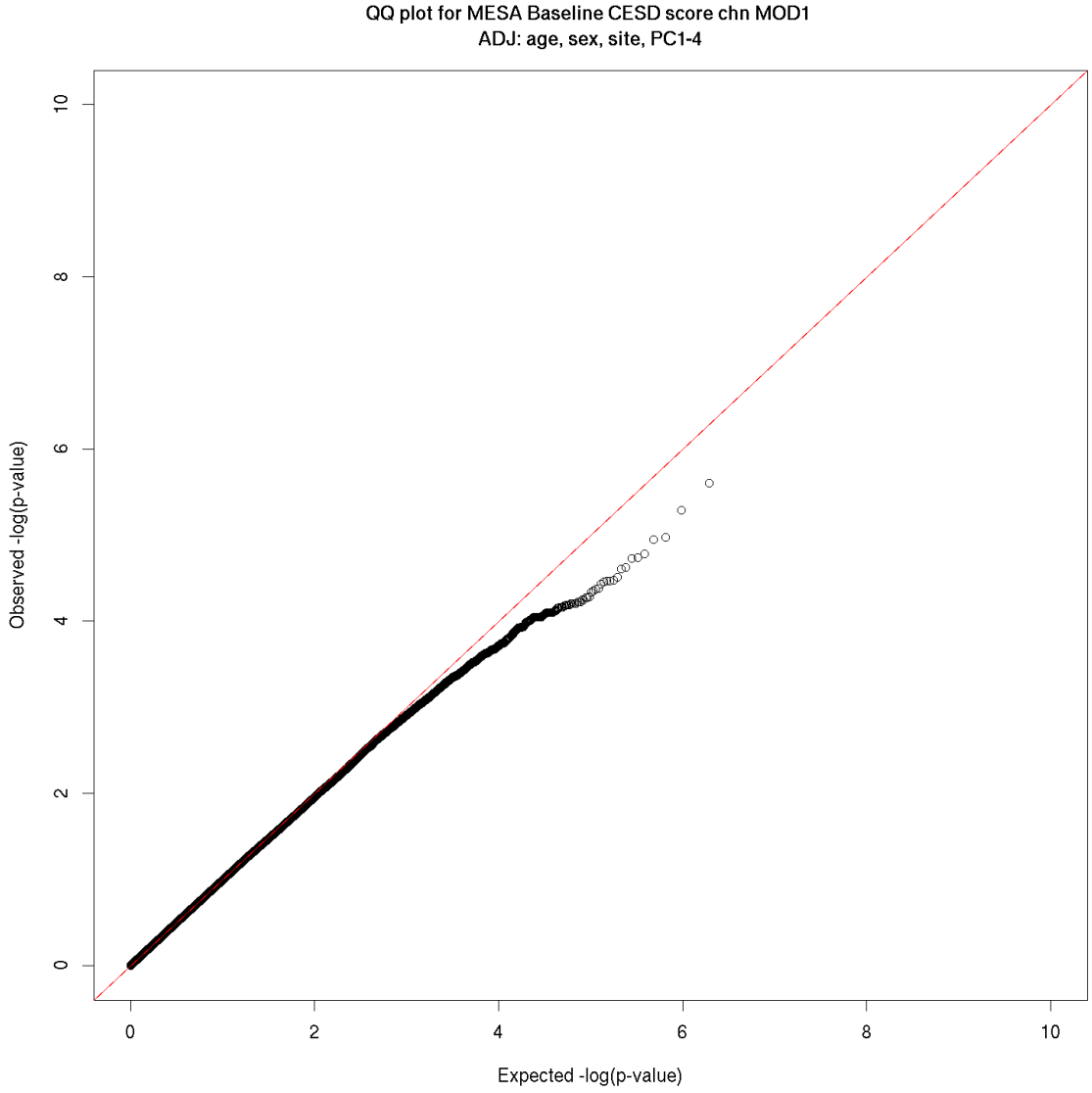
Appendix 6 QQ plot of p-values from SNP association with averaged depressive symptom score from European GWA analyses adjusted for age, sex, study site and top four principal components, minor allele frequency greater than 5%, Multi-Ethnic Study of Atherosclerosis



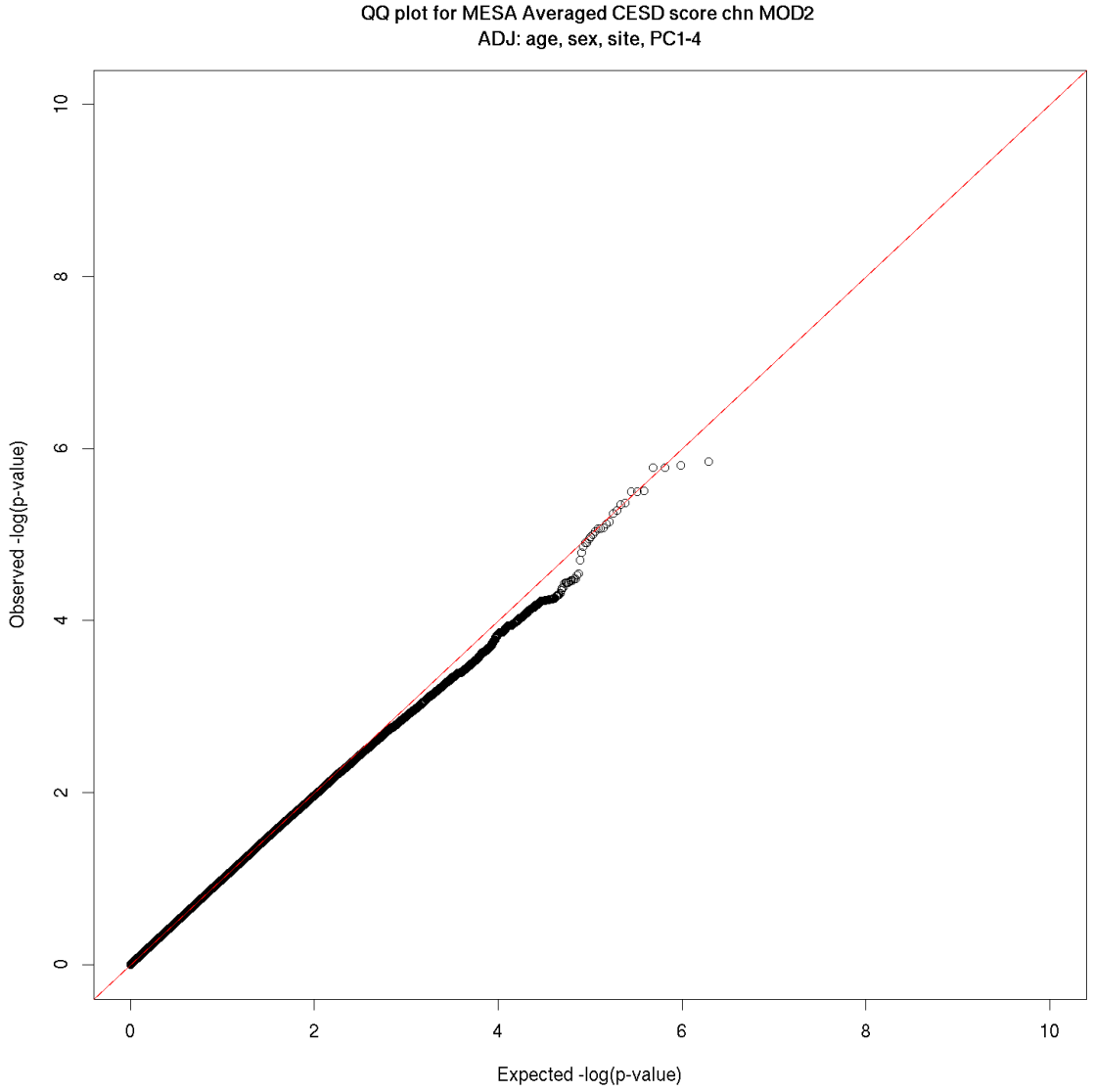
Appendix 7 QQ plot of p-values from SNP association with repeated measures depressive symptom score from European GWA analyses adjusted for age, sex, study site and top four principal components, minor allele frequency greater than 5%, Multi-Ethnic Study of Atherosclerosis



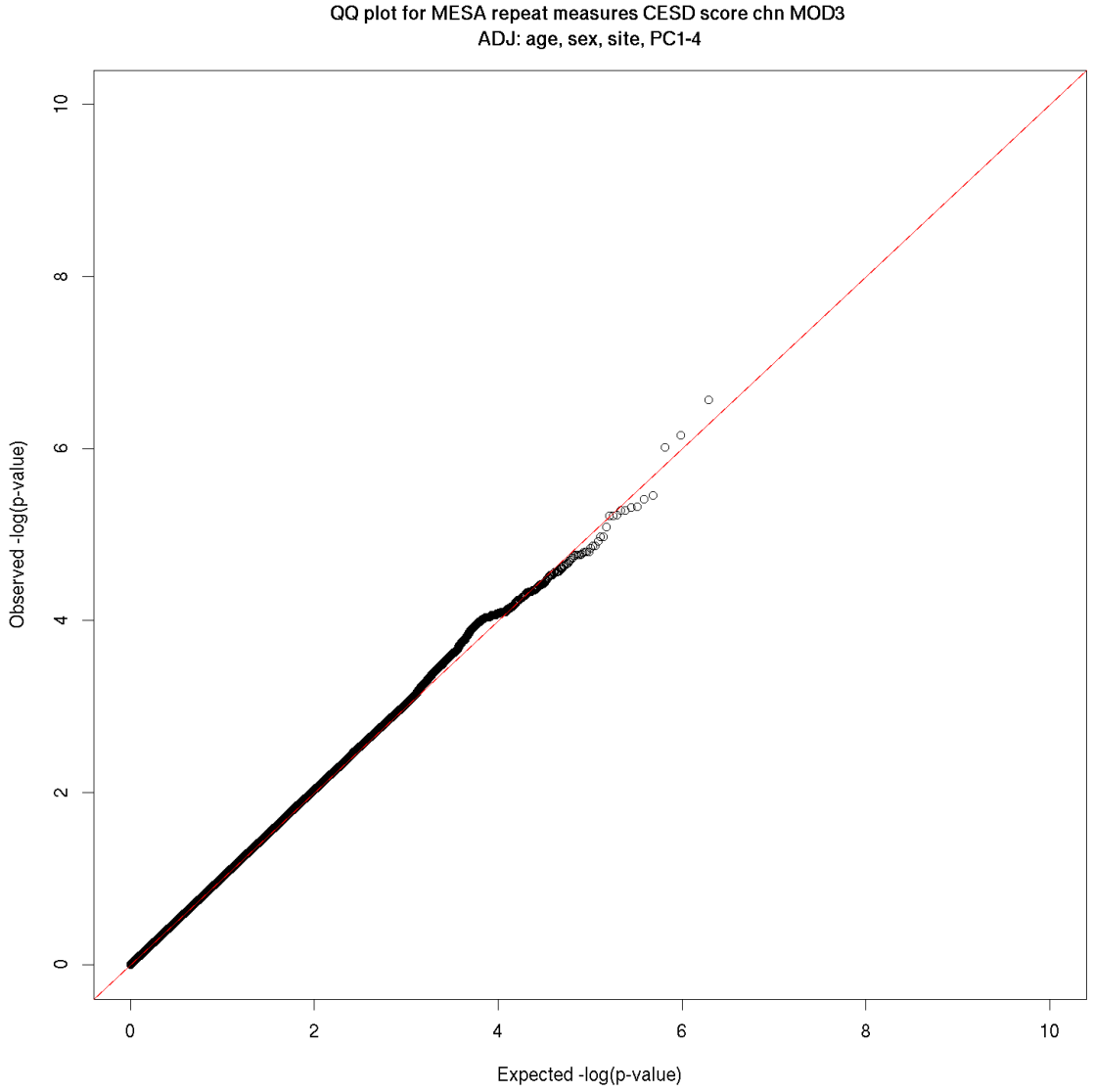
Appendix 8 QQ plot of p-values from SNP association with baseline depressive symptom score from Chinese GWA analyses adjusted for age, sex, study site and top four principal components, minor allele frequency greater than 5%, Multi-Ethnic Study of Atherosclerosis



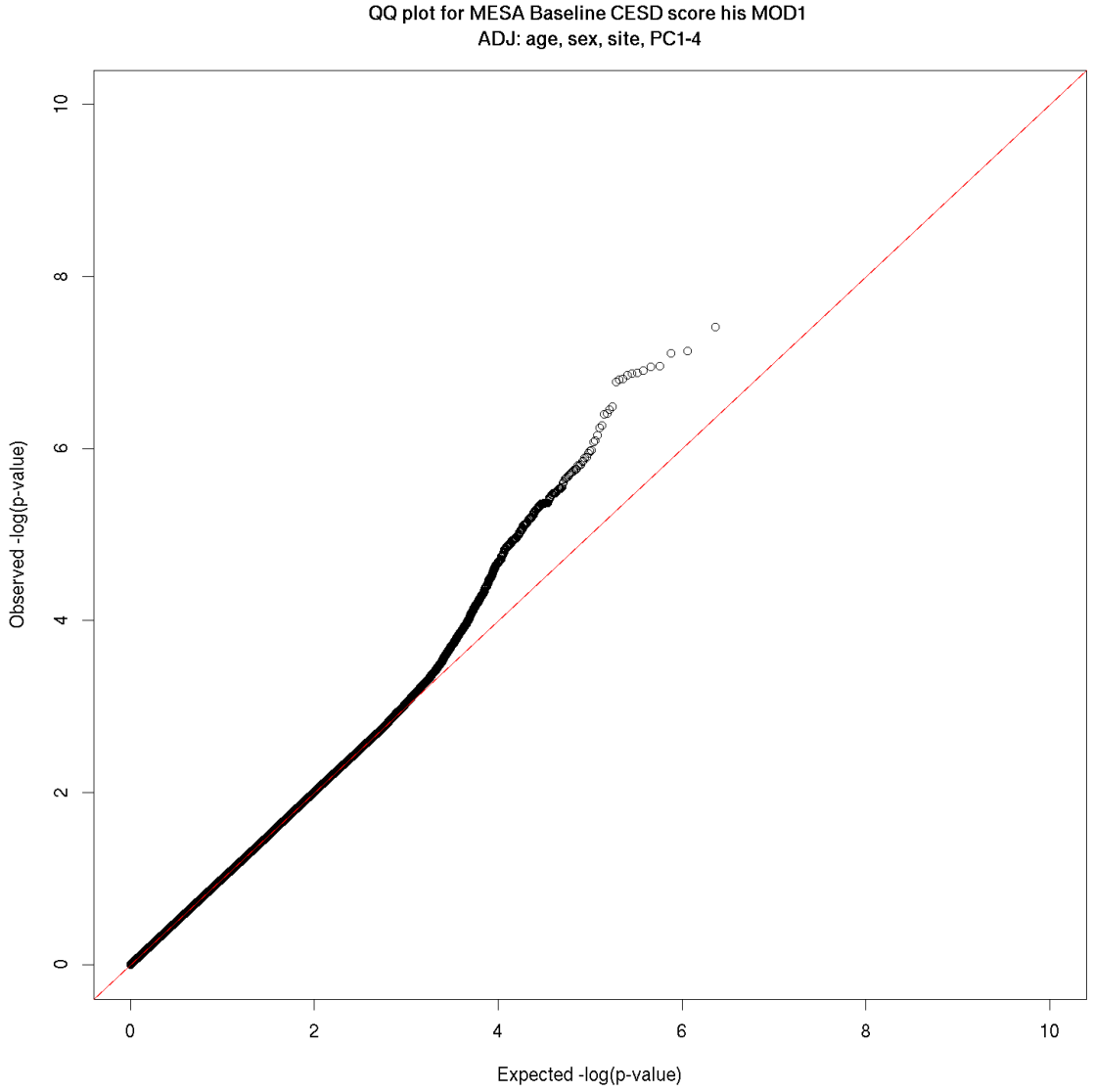
Appendix 9 QQ plot of p-values from SNP association with averaged depressive symptom score from Chinese GWA analyses adjusted for age, sex, study site and top four principal components, minor allele frequency greater than 5%, Multi-Ethnic Study of Atherosclerosis



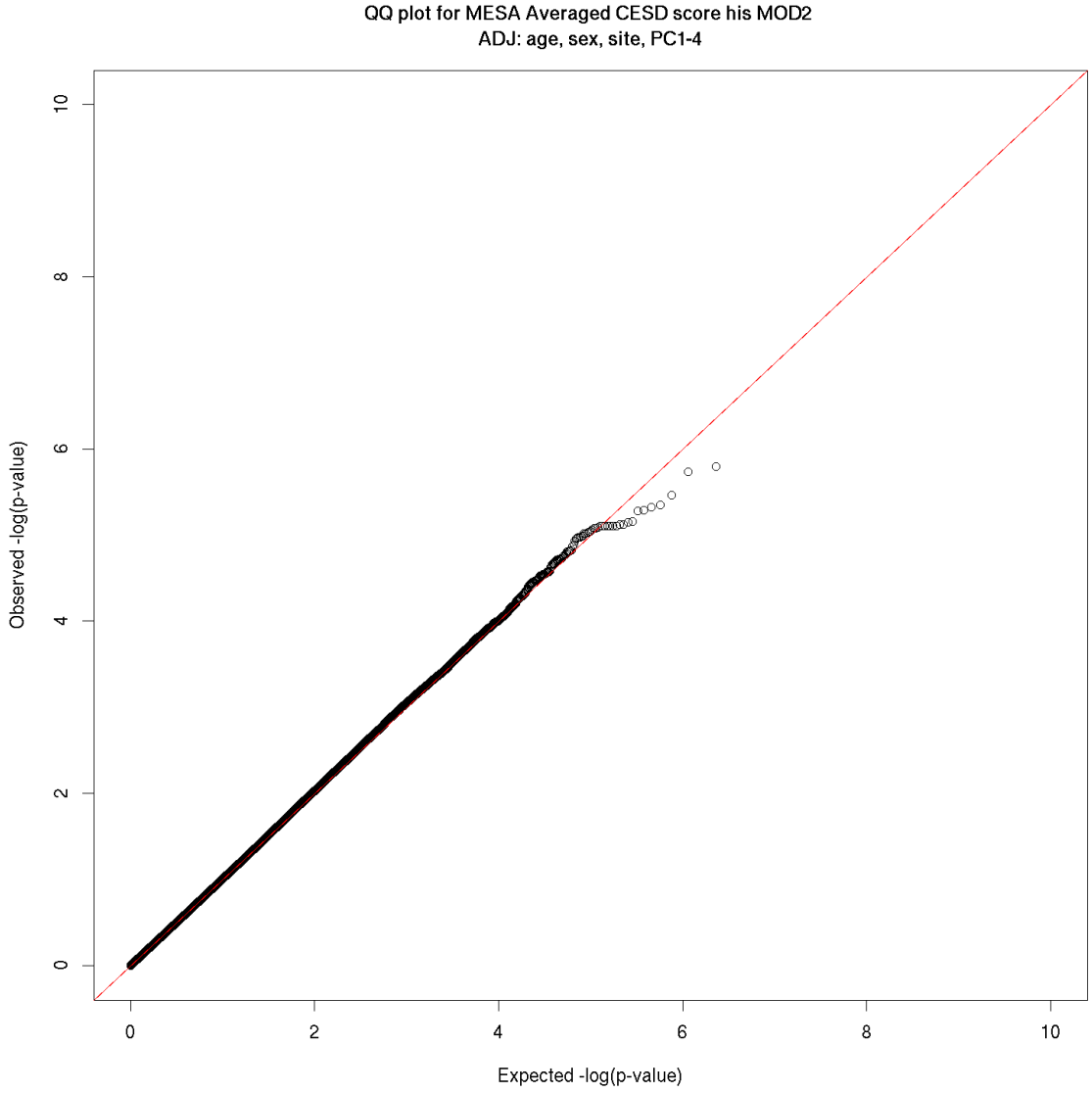
Appendix 10 QQ plot of p-values from SNP association with repeated measures depressive symptom score from Chinese GWA analyses adjusted for age, sex, study site and top four principal components, minor allele frequency greater than 5%, Multi-Ethnic Study of Atherosclerosis



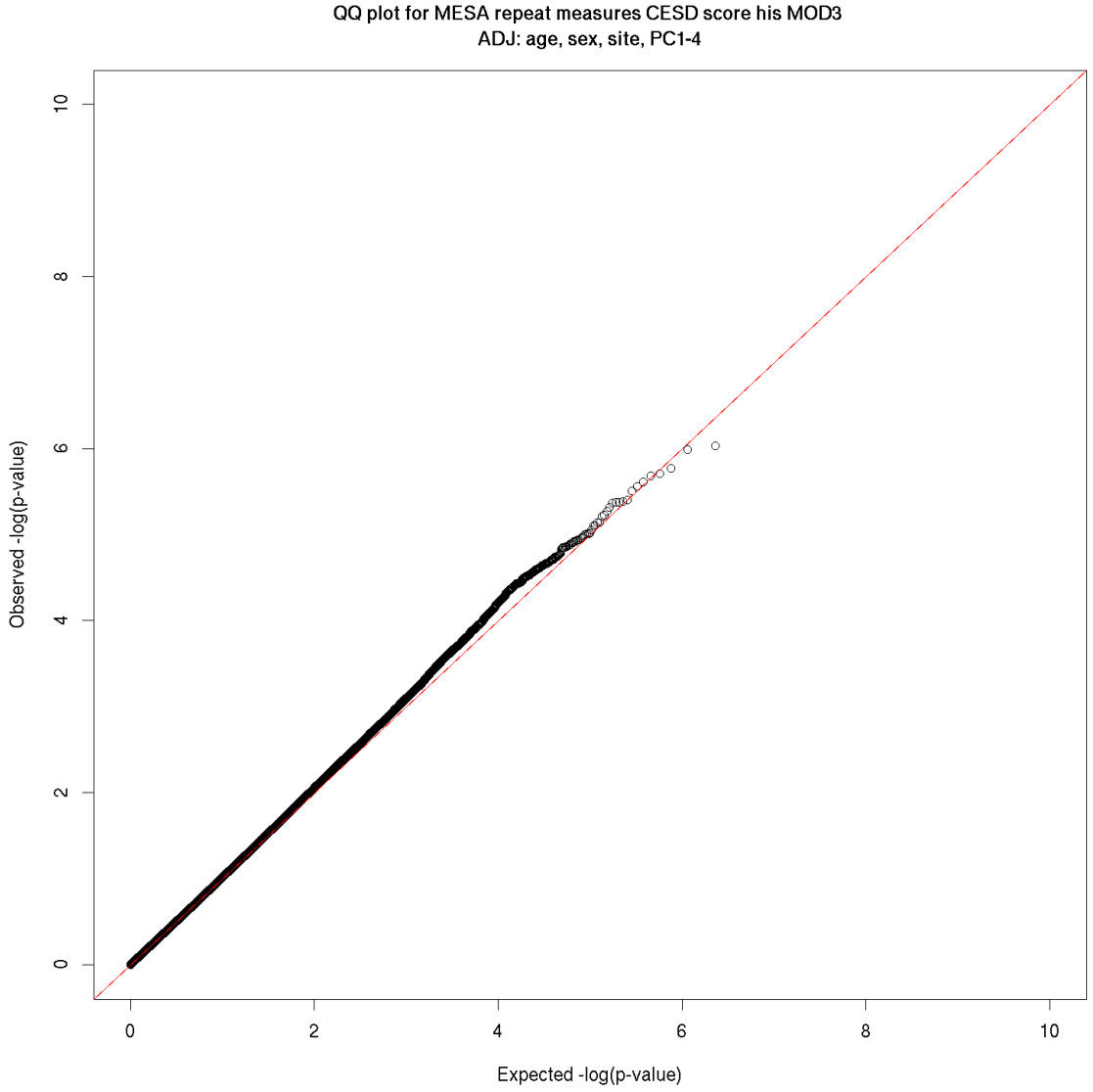
Appendix 11 QQ plot of p-values from SNP association with baseline depressive symptom score from Hispanic GWA analyses adjusted for age, sex, study site and top four principal components, minor allele frequency greater than 5%, Multi-Ethnic Study of Atherosclerosis



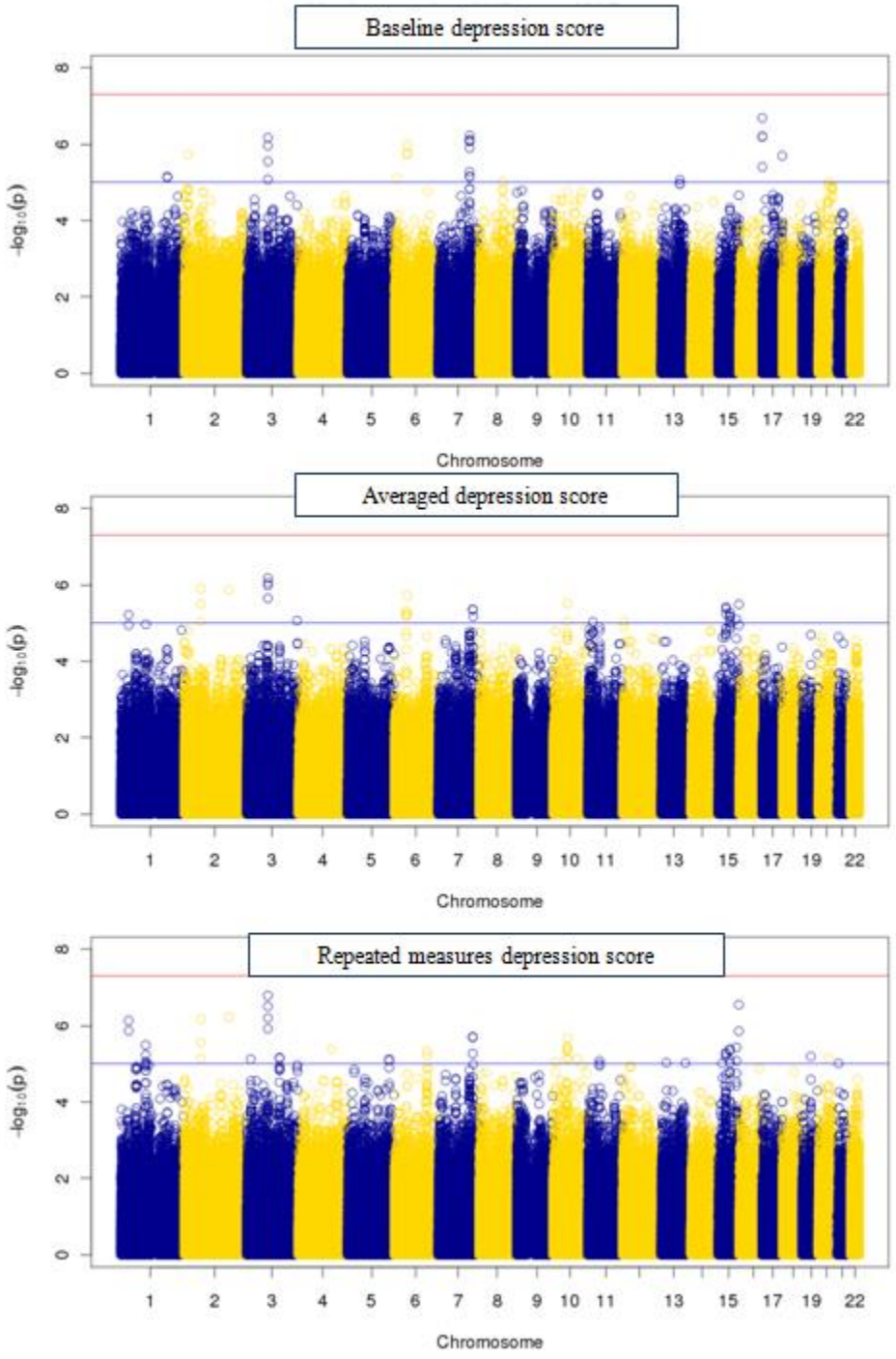
Appendix 12 QQ plot of p-values from SNP association with averaged depressive symptom score from Hispanic GWA analyses adjusted for age, sex, study site and top four principal components, minor allele frequency greater than 5%, Multi-Ethnic Study of Atherosclerosis



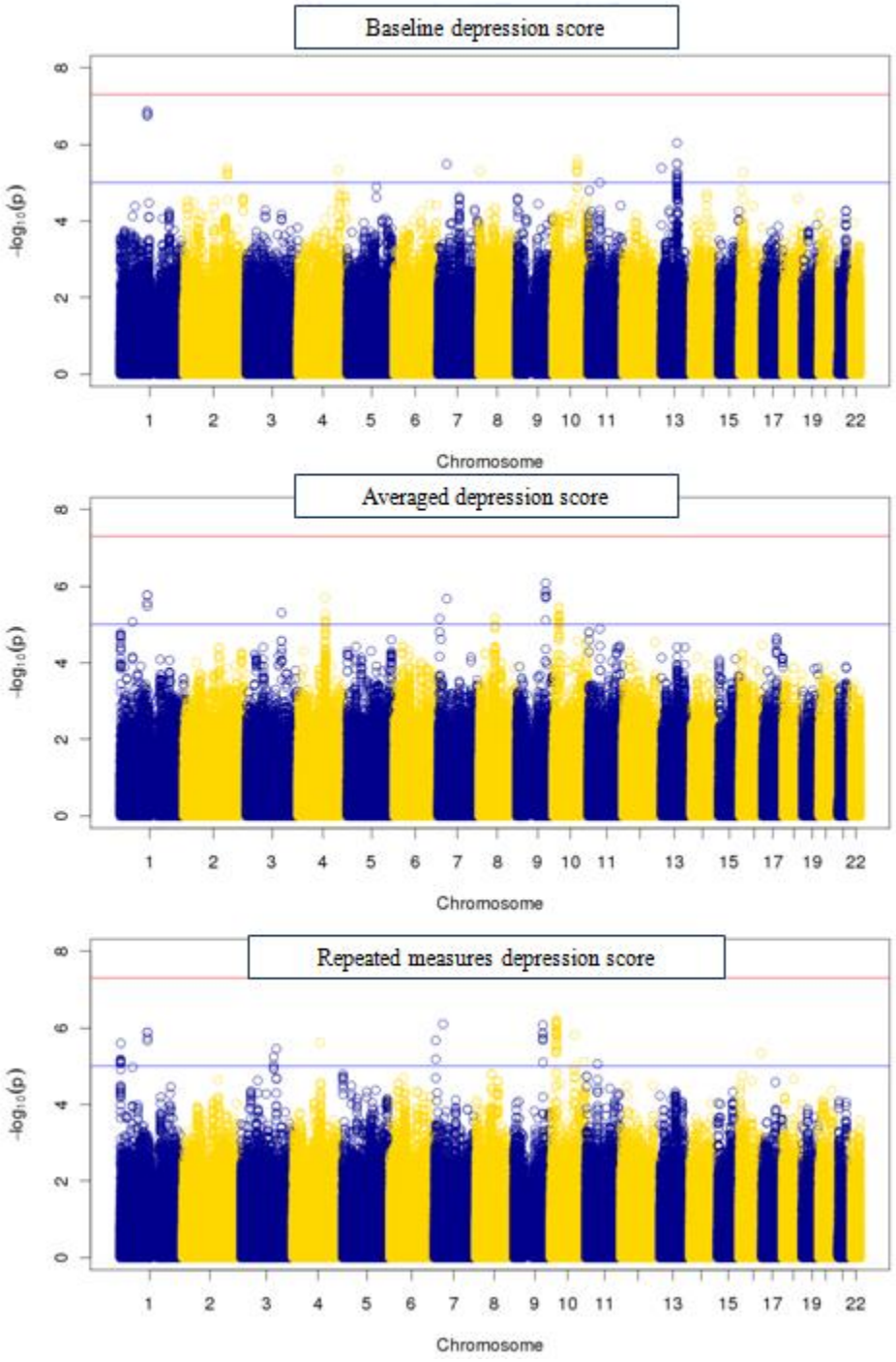
Appendix 13 QQ plot of p-values from SNP association with repeated measures depressive symptom score from Hispanic GWA analyses adjusted for age, sex, study site and top four principal components, minor allele frequency greater than 5%, Multi-Ethnic Study of Atherosclerosis



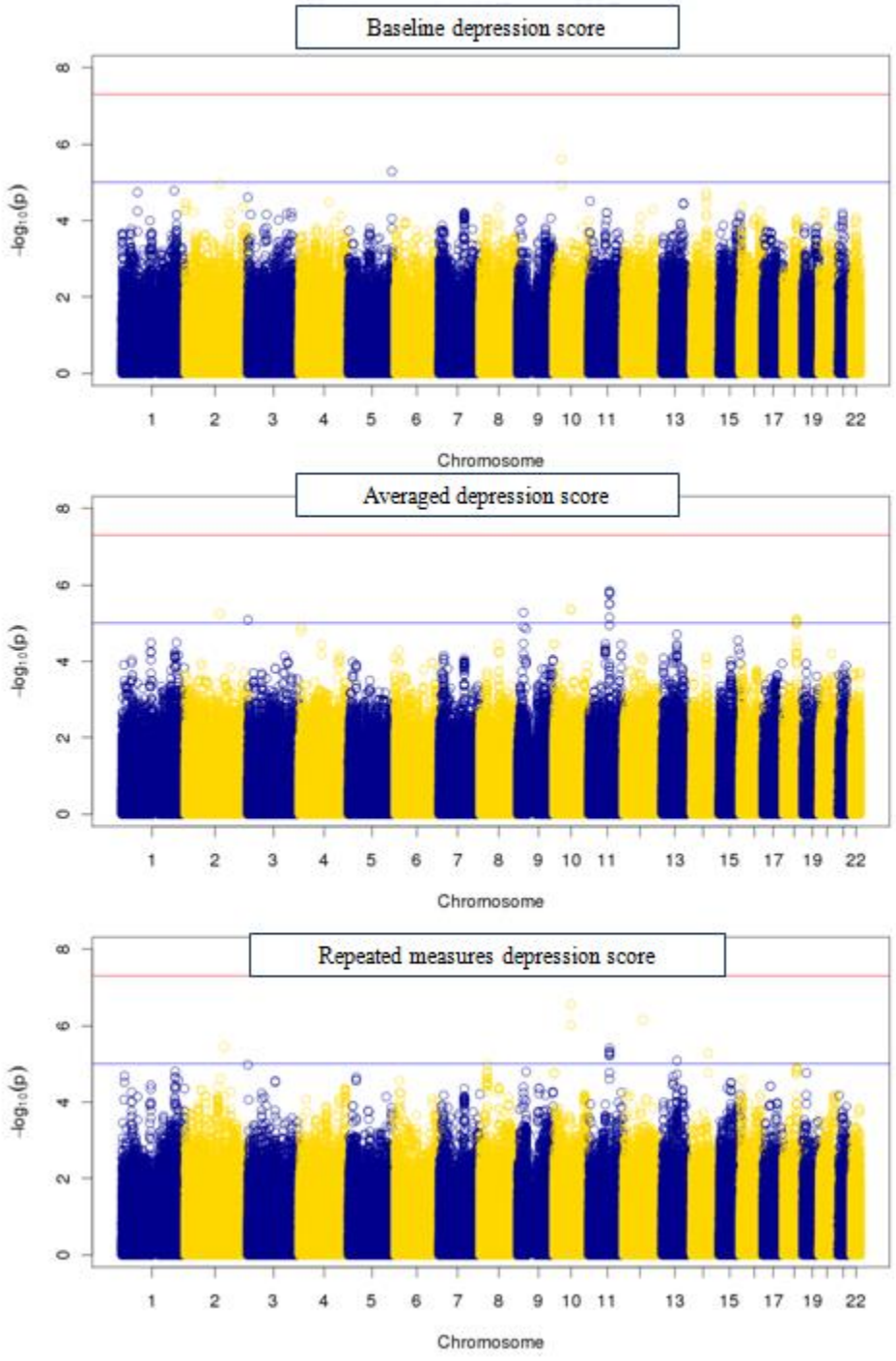
Appendix 14 Manhattan plots for baseline depressive symptoms score (top), averaged depressive symptom score (middle), and repeat measures depressive symptom score (bottom) from the African American GWAS, filtered at MAF>0.05, MESA



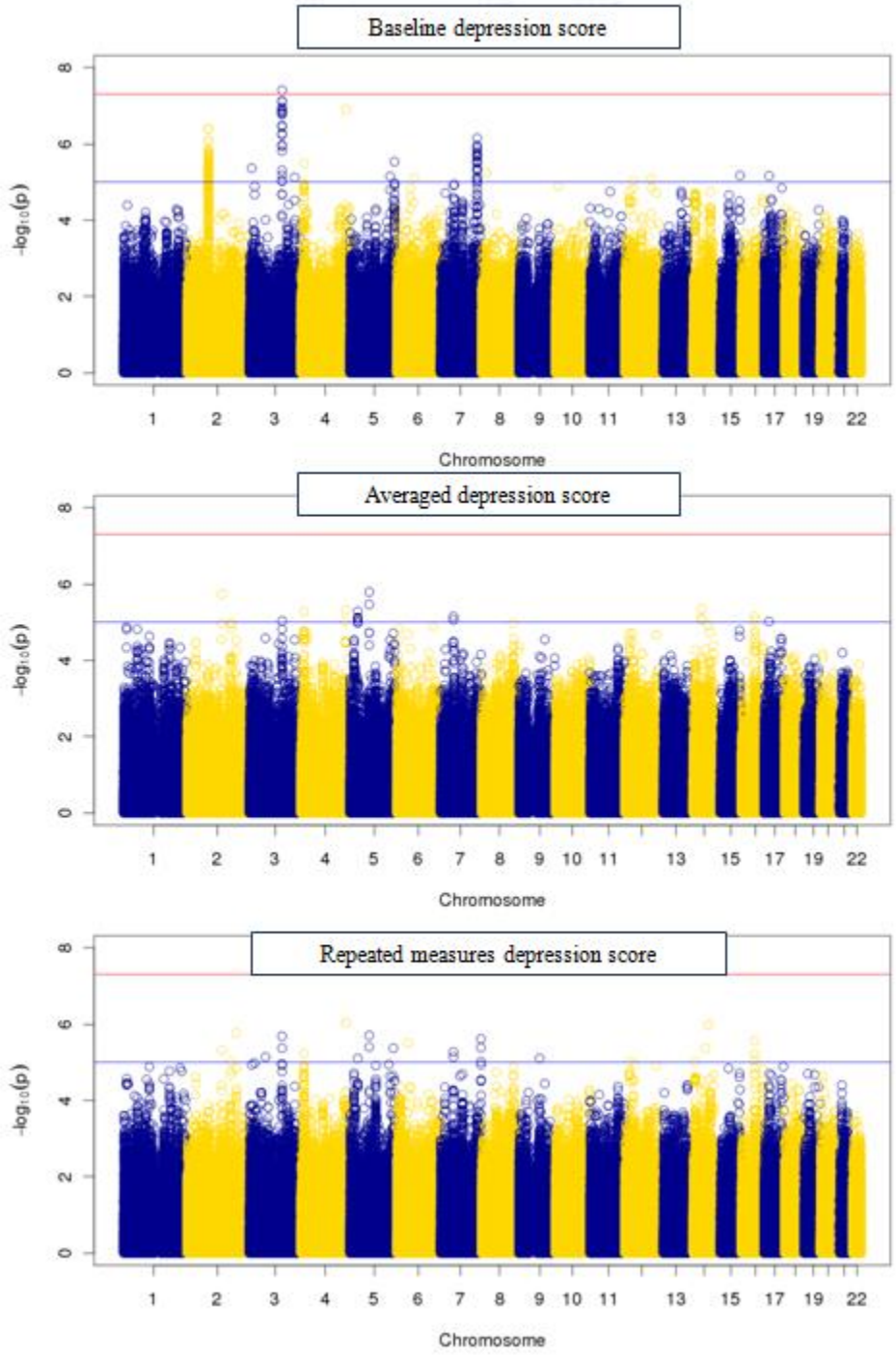
Appendix 15 Manhattan plots for baseline depressive symptoms score (top), averaged depressive symptom score (middle), and repeat measures depressive symptom score (bottom) from the European GWAS, filtered at MAF>0.05, MESA



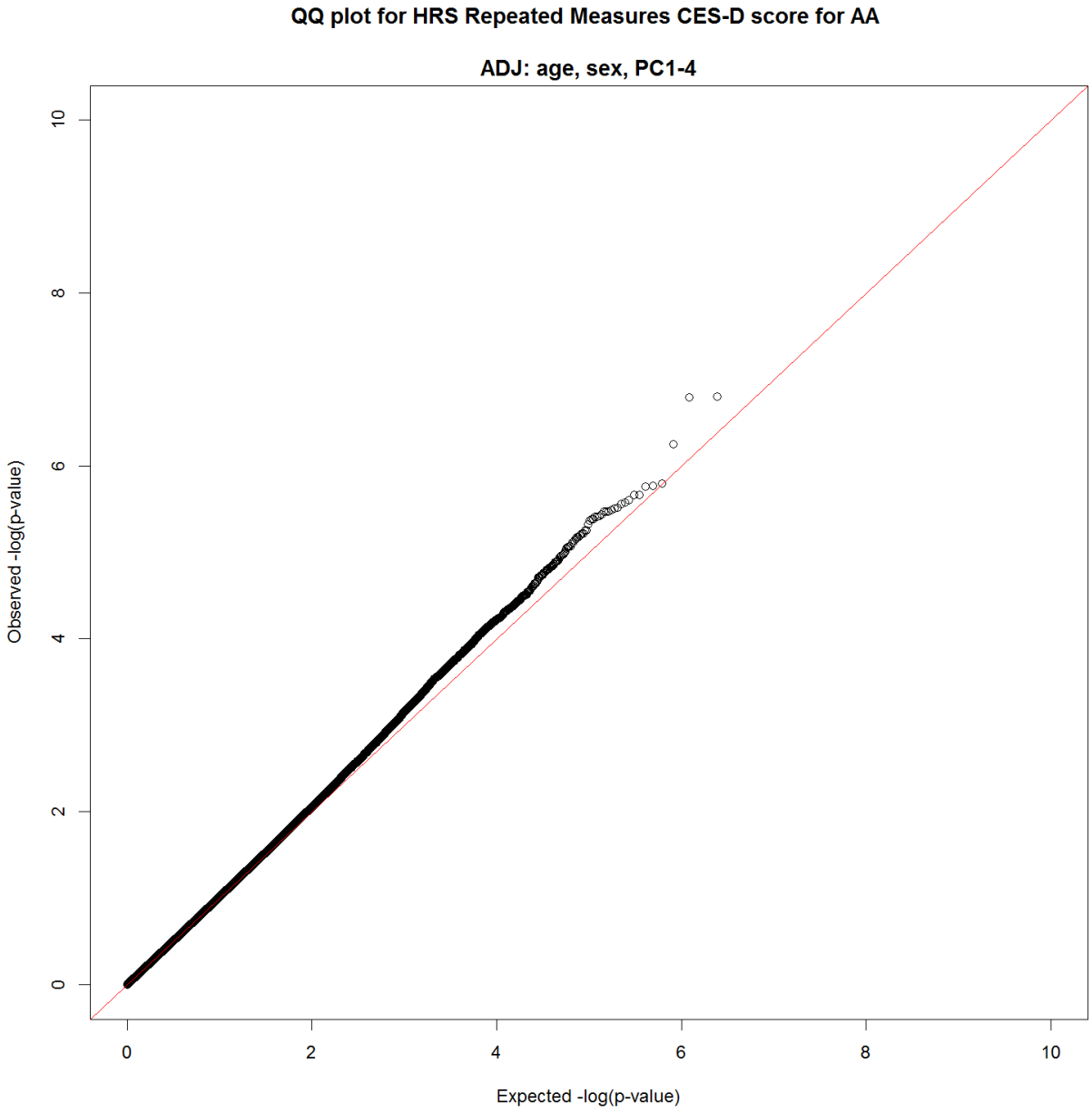
Appendix 16 Manhattan plots for baseline depressive symptoms score (top), averaged depressive symptom score (middle), and repeat measures depressive symptom score (bottom) from the Chinese GWAS, filtered at MAF>0.05, MESA



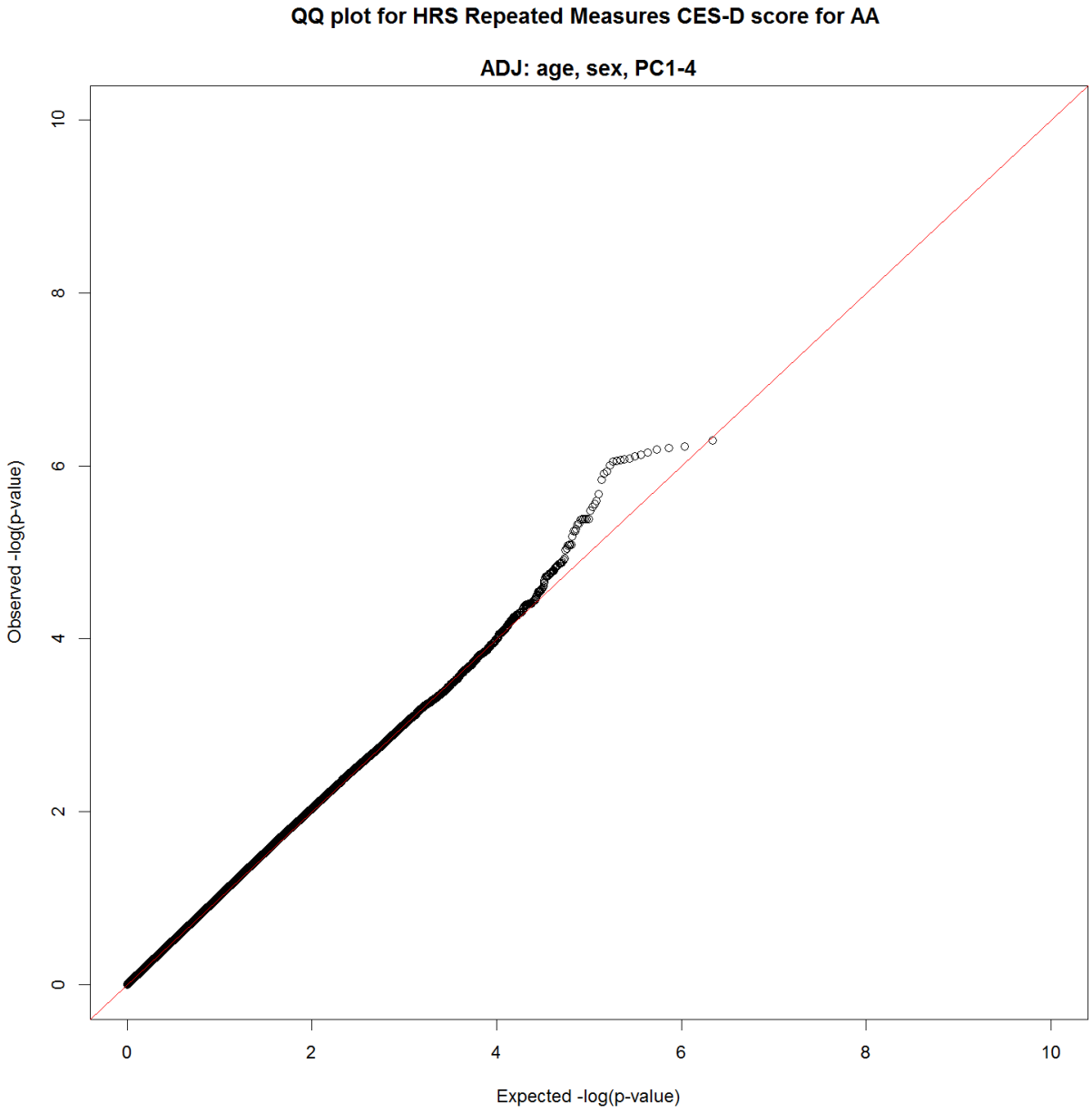
Appendix 17 Manhattan plots for baseline depressive symptoms score (top), averaged depressive symptom score (middle), and repeat measures depressive symptom score (bottom) from the Hispanic GWAS, filtered at MAF>0.05, MESA



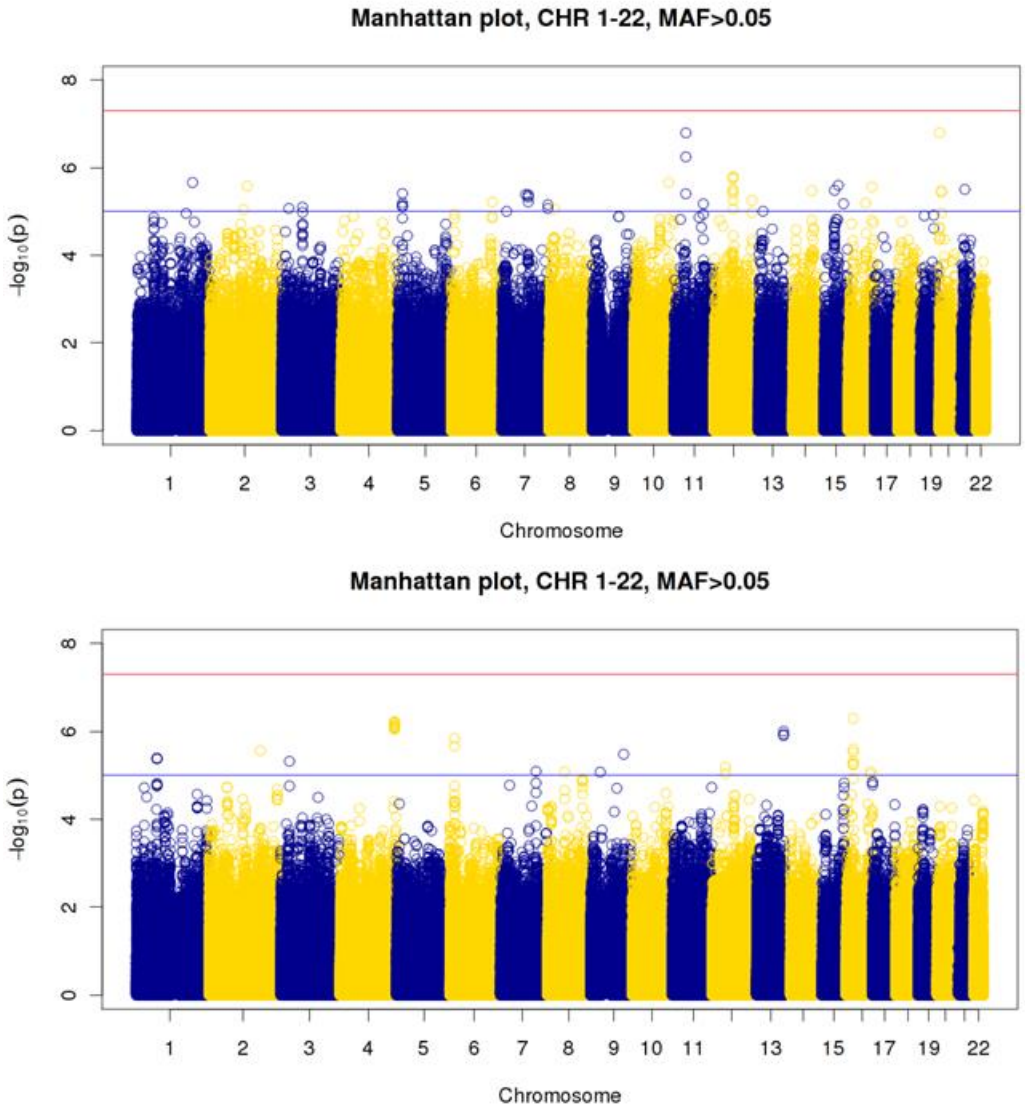
Appendix 18 QQ plot of p-values from SNP association with repeated measures depressive symptom score from African American GWA analyses adjusted for age, sex, and top four principal components, minor allele frequency greater than 5%, Health and Retirement Study



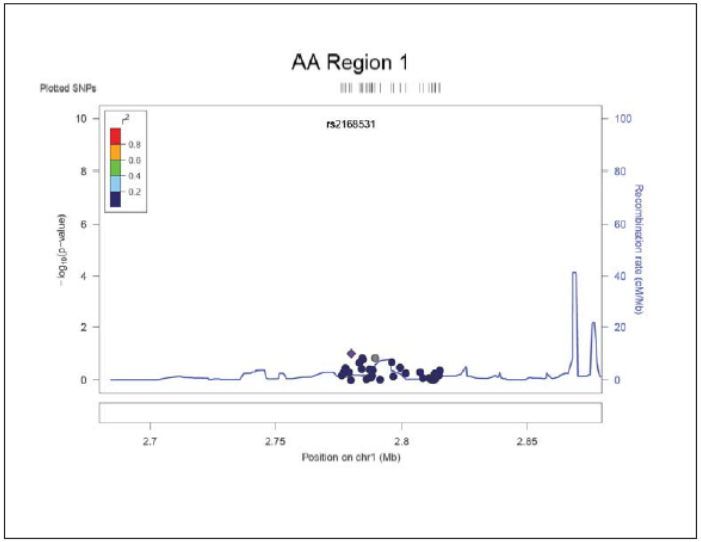
Appendix 19 QQ plot of p-values from SNP association with repeated measures depressive symptom score from European GWA analyses adjusted for age, sex, and top four principal components, minor allele frequency greater than 5%, Health and Retirement Study

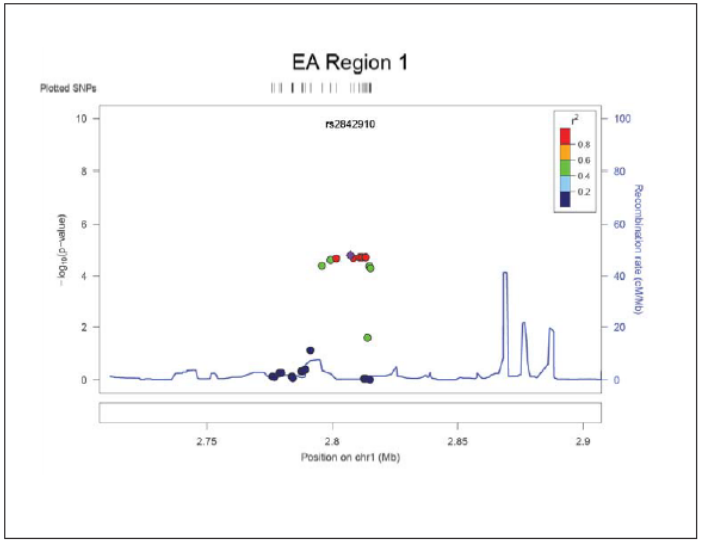


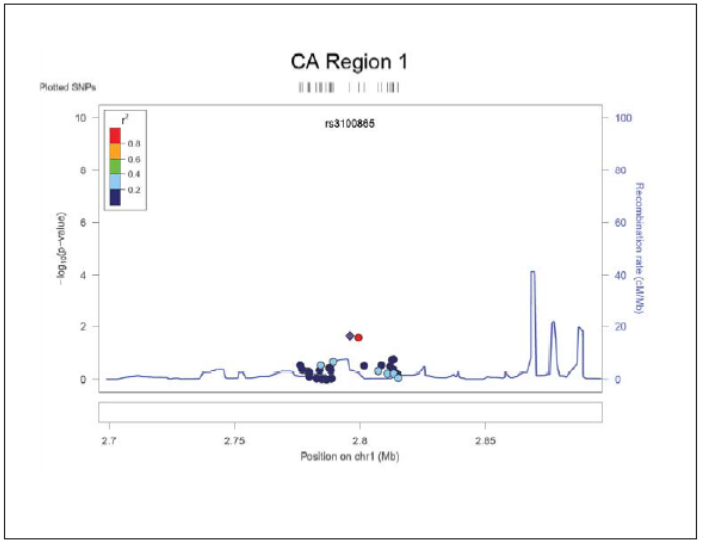
Appendix 20 Manhattan plots for repeated measures depressive symptom score from the African American (top) and European (bottom) GWAS, filtered at MAF>0.05, Health and Retirement Study

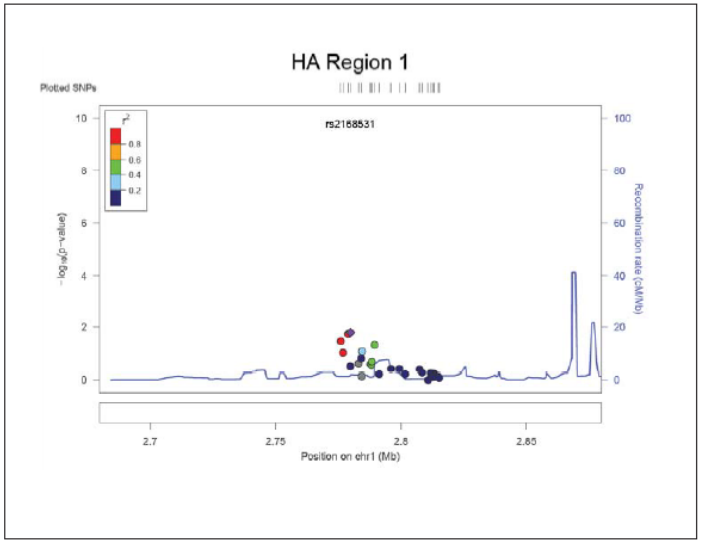


Appendix 21 LocusZoom plots for region 1, all ethnicities

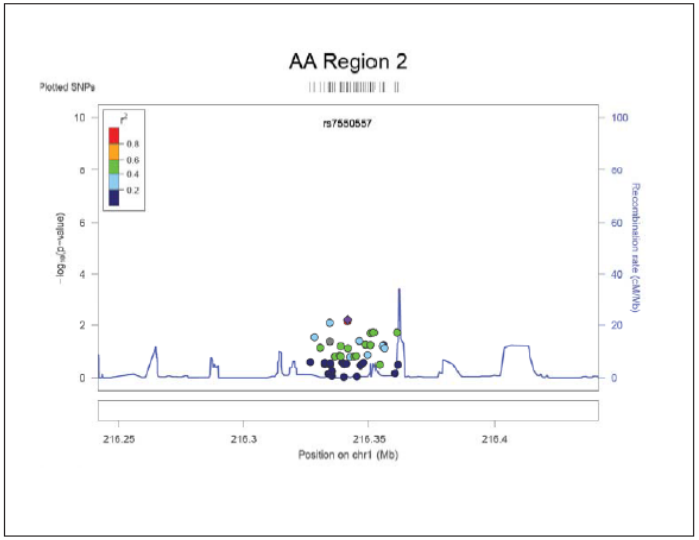


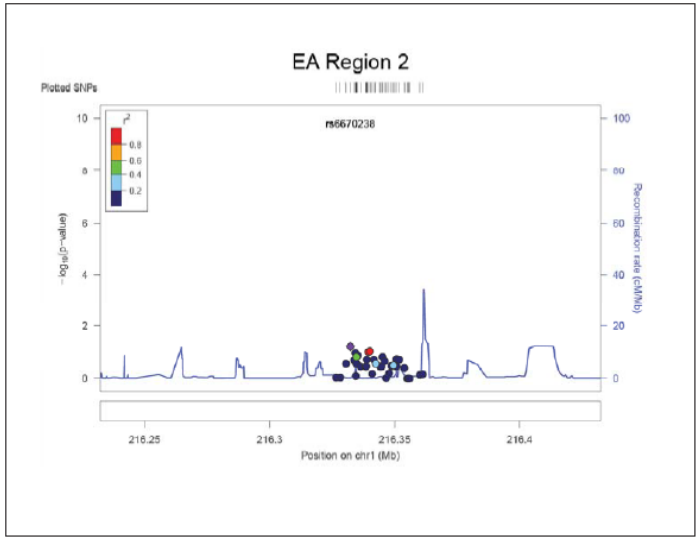


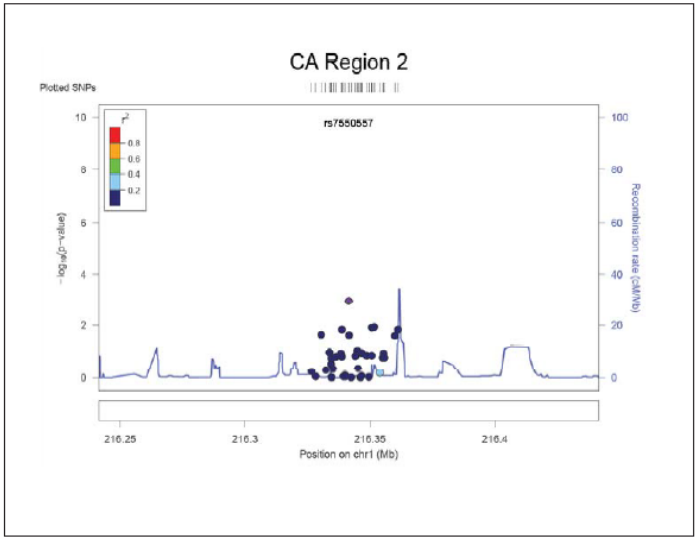


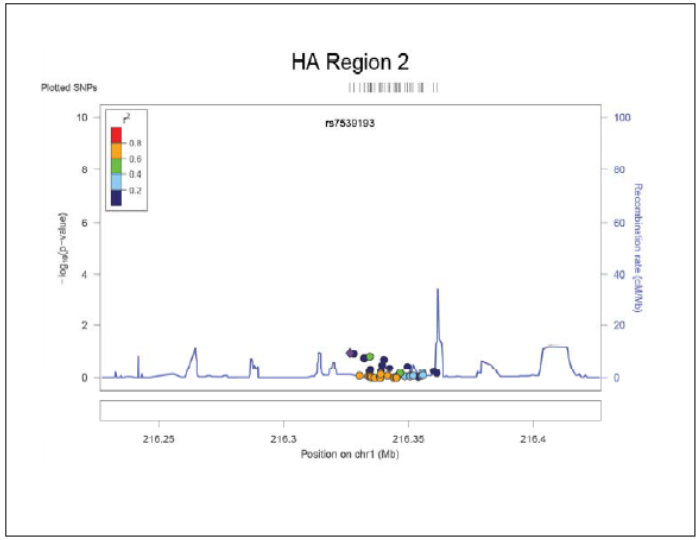


Appendix 22 LocusZoom plots for region 2, all ethnicities

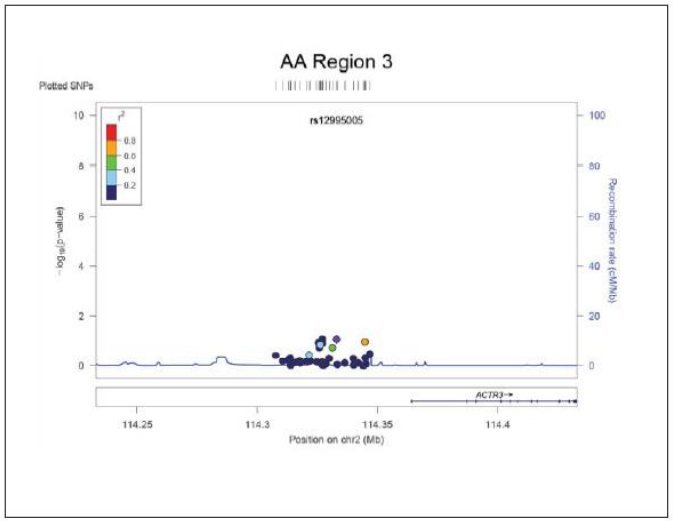


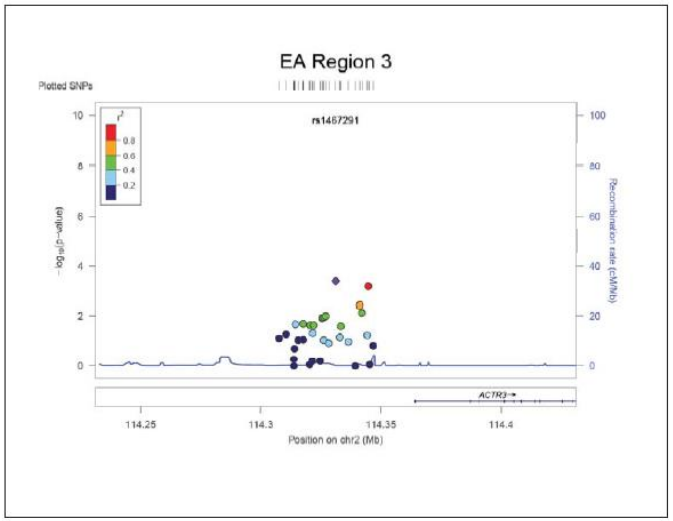


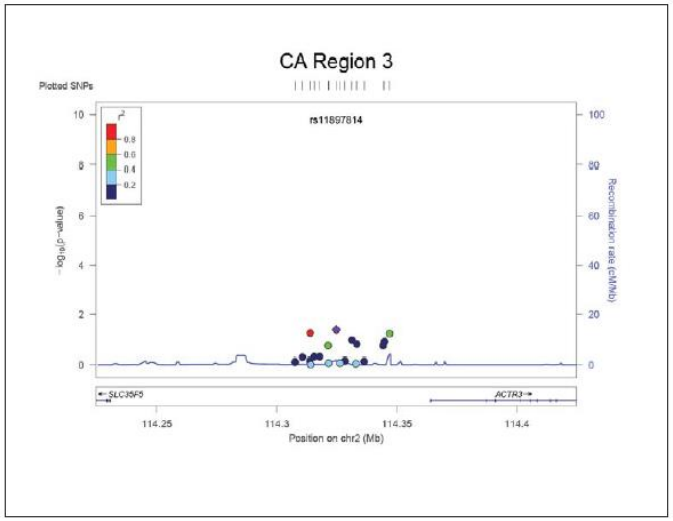


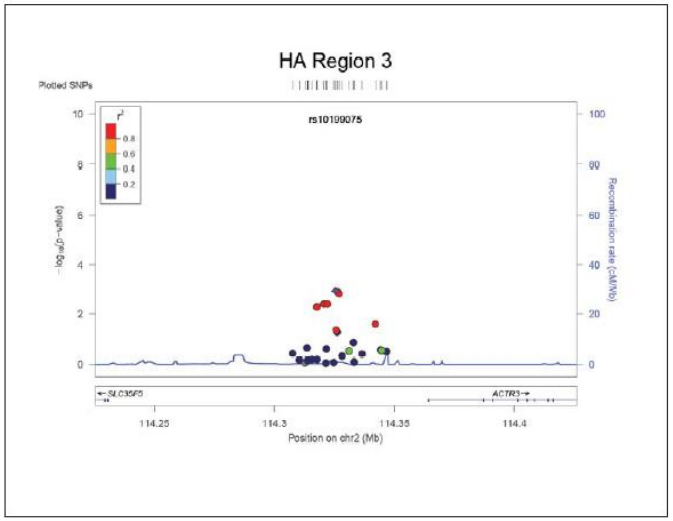


Appendix 23 LocusZoom plots for region 3, all ethnicities

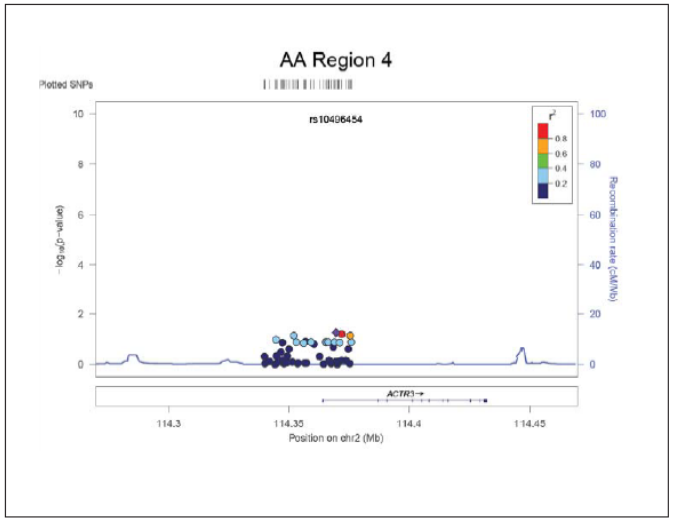


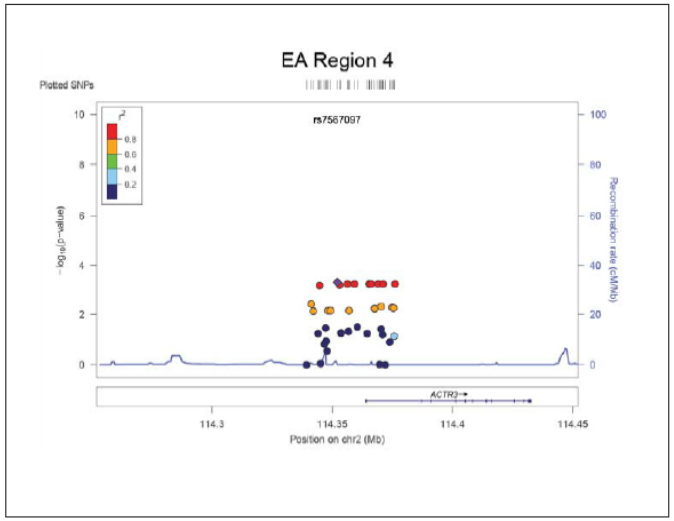


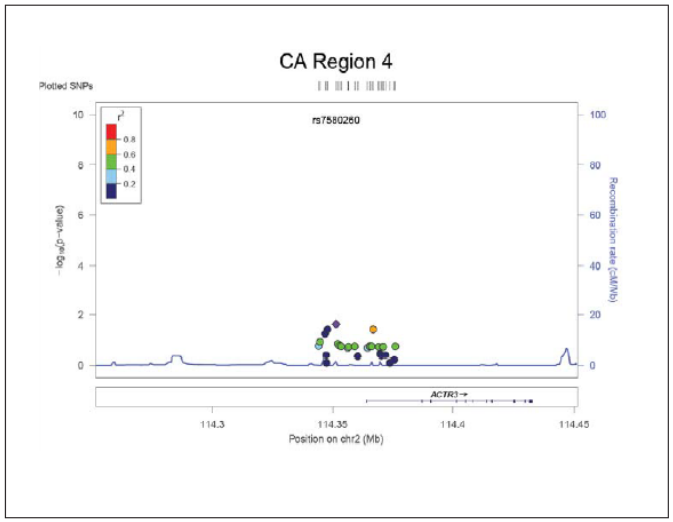


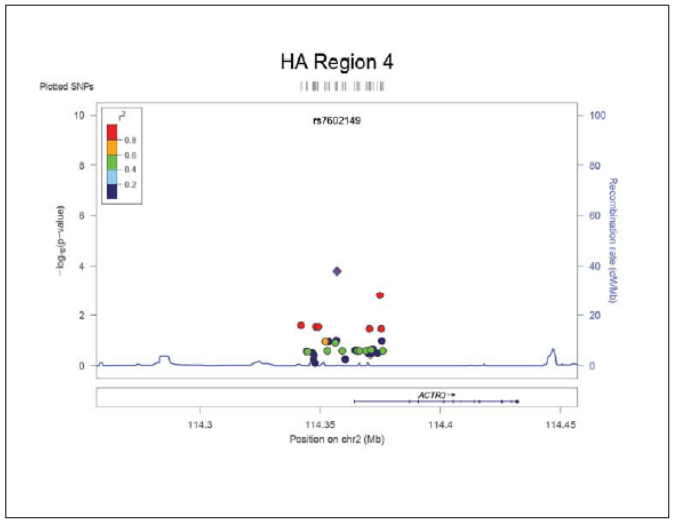


Appendix 24 LocusZoom plots for region 4, all ethnicities

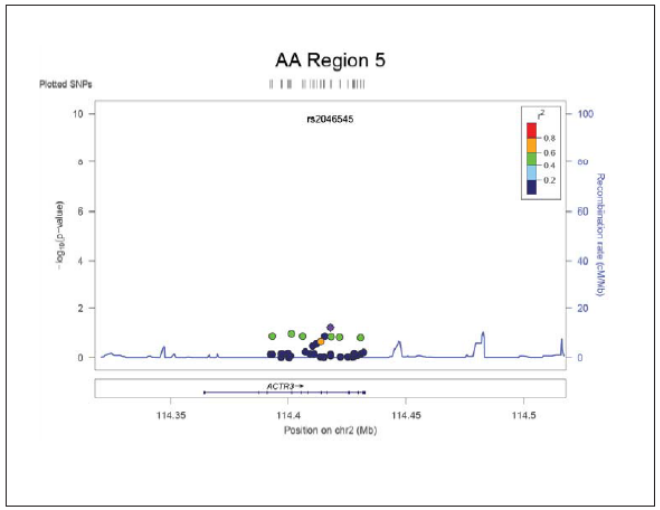


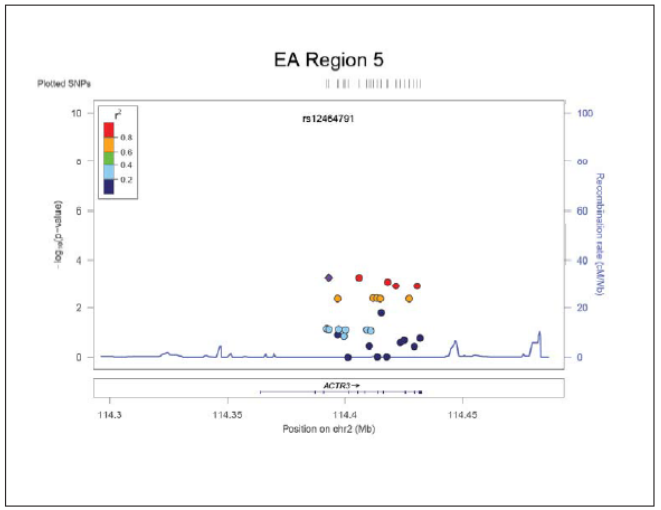


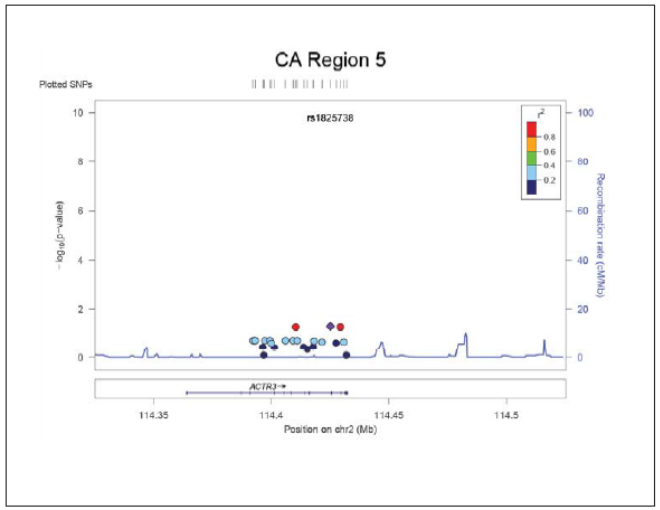


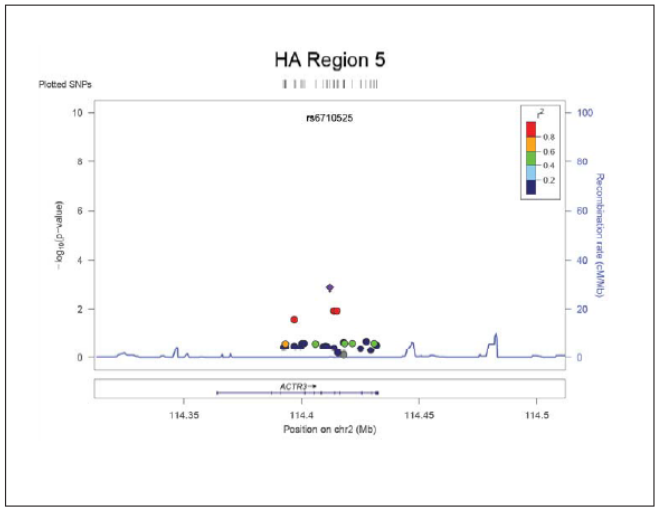


Appendix 25 LocusZoom plots for region 5, all ethnicities

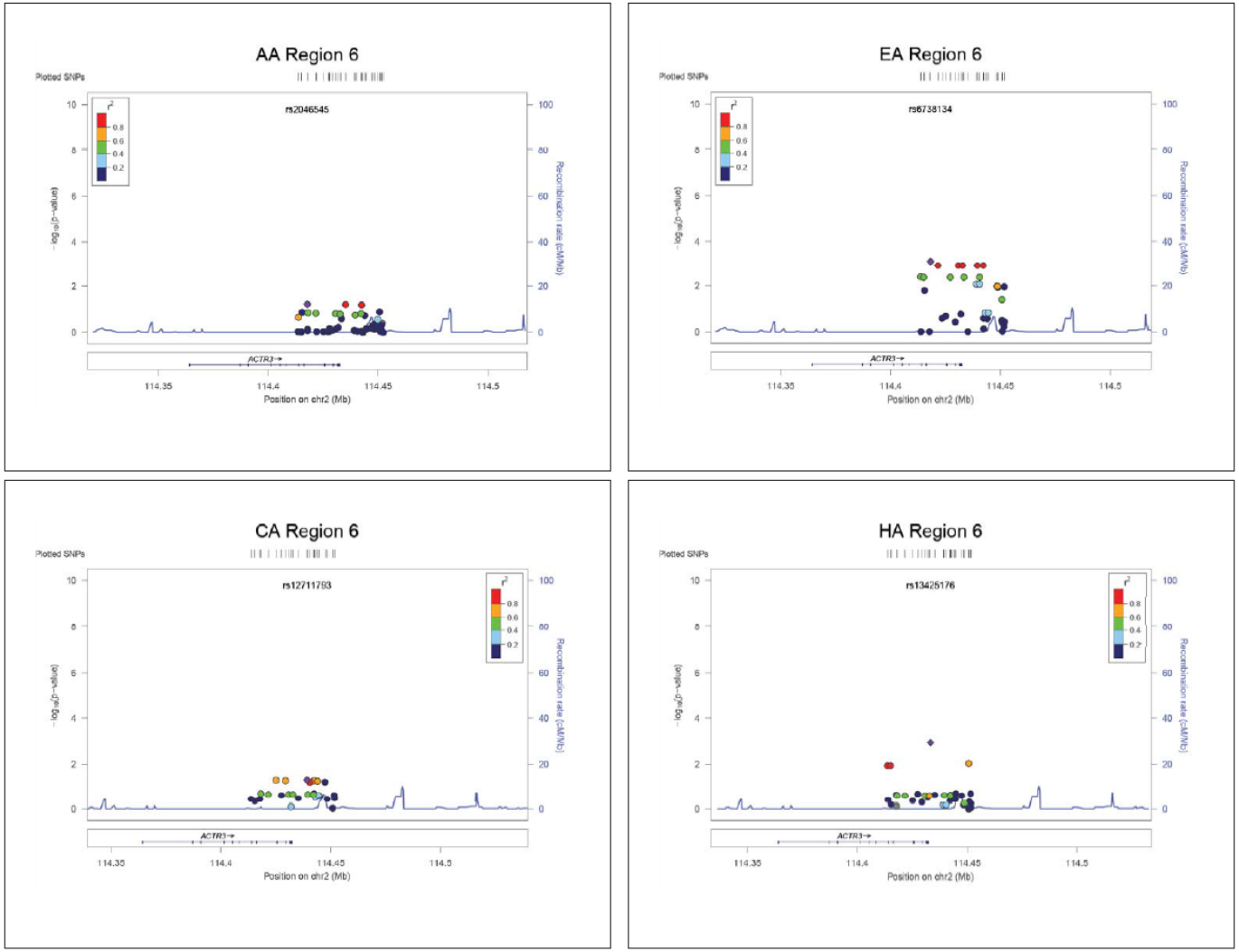




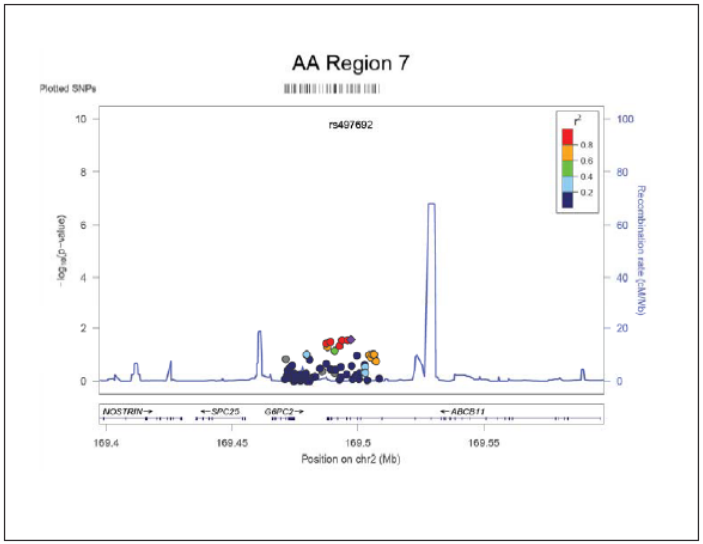


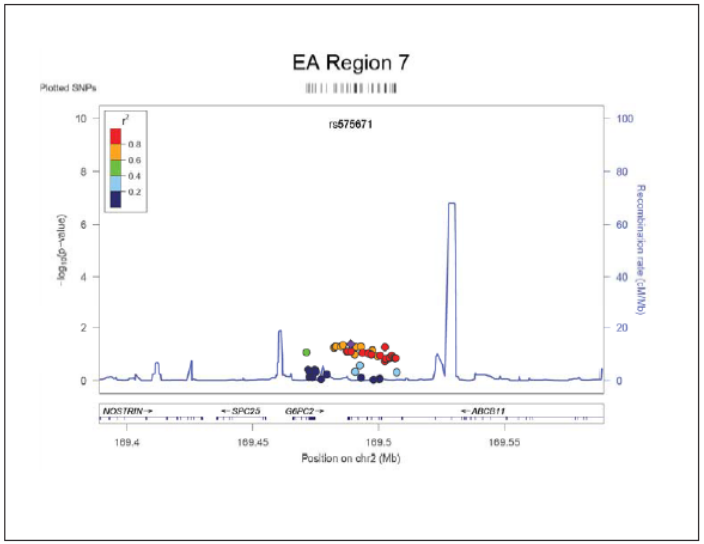


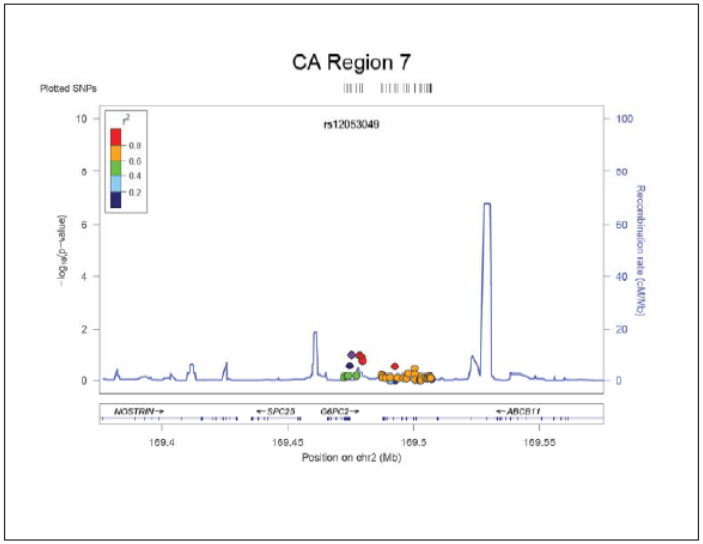
Appendix 26 LocusZoom plots for region 6, all ethnicities

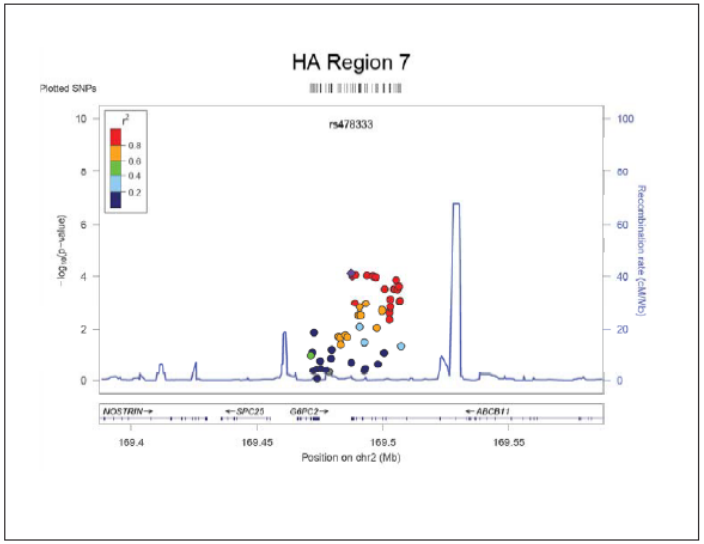


Appendix 27 LocusZoom plots for region 7, all ethnicities

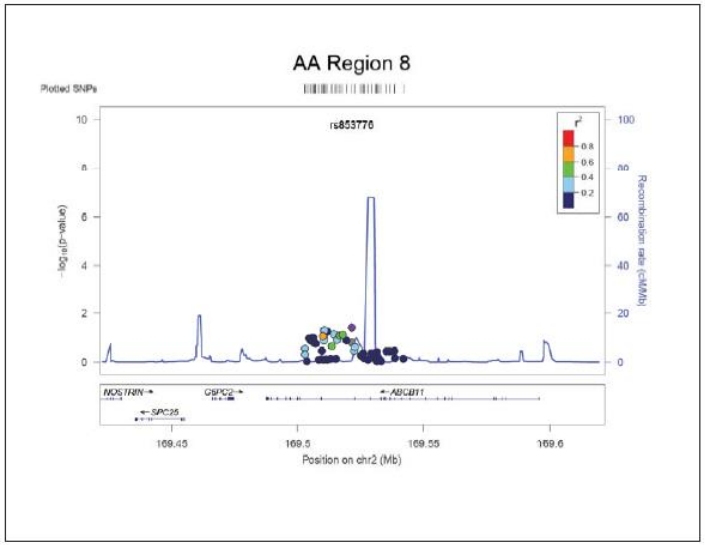


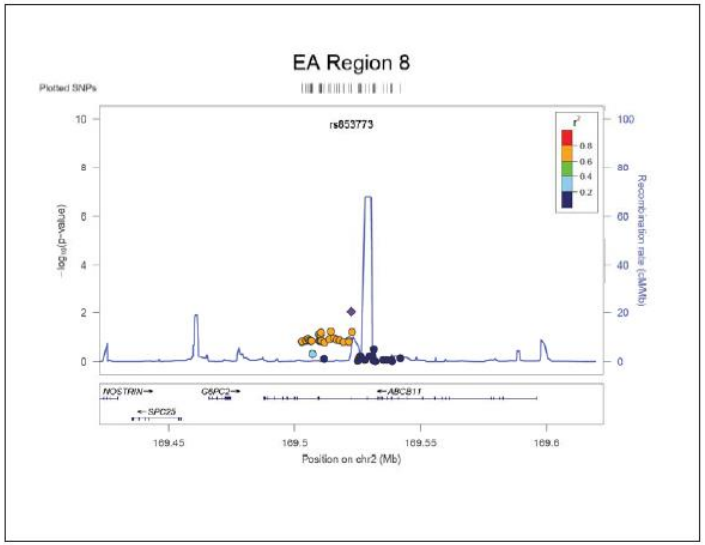


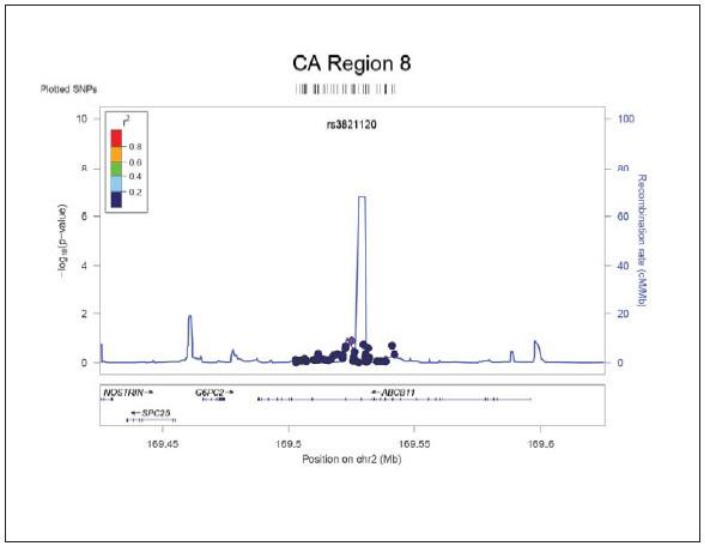


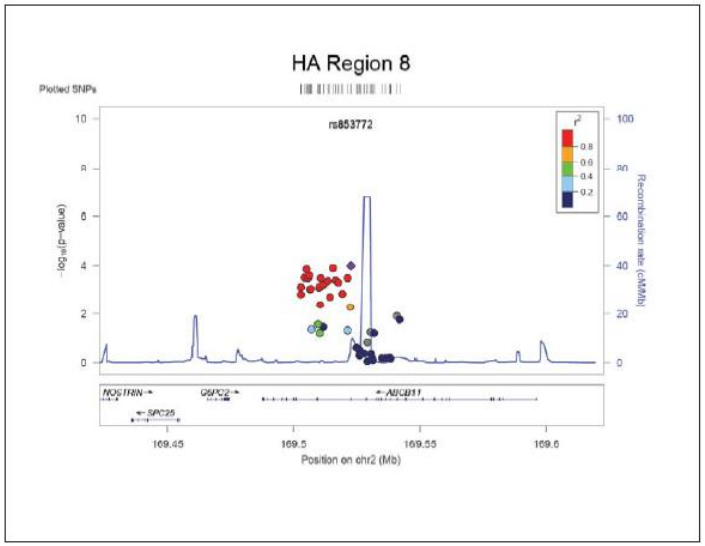


Appendix 28 LocusZoom plots for region 8, all ethnicities

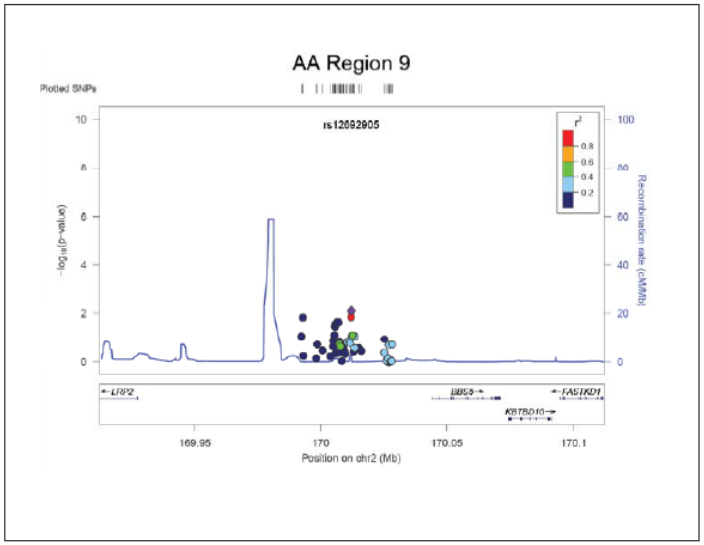


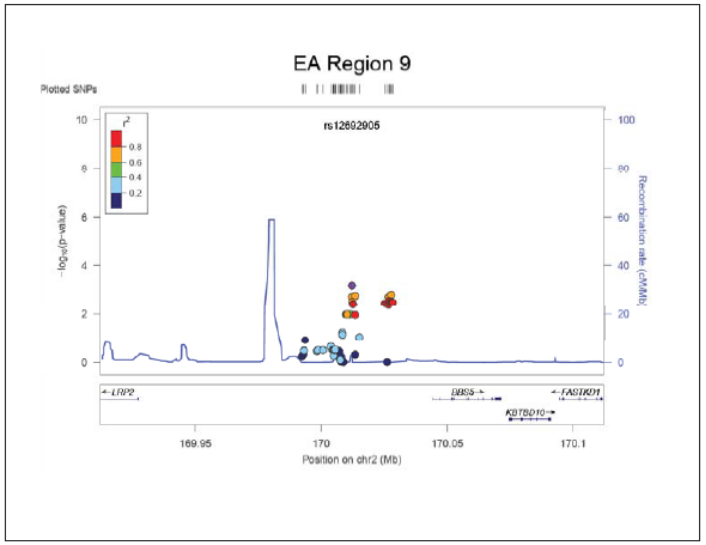


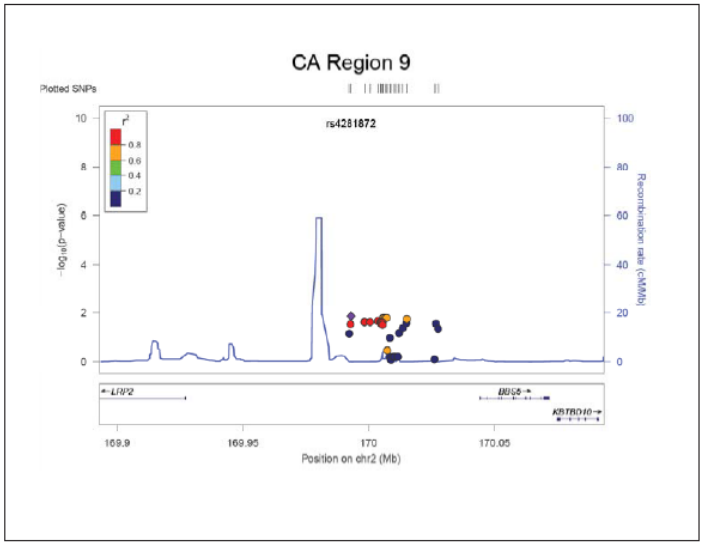


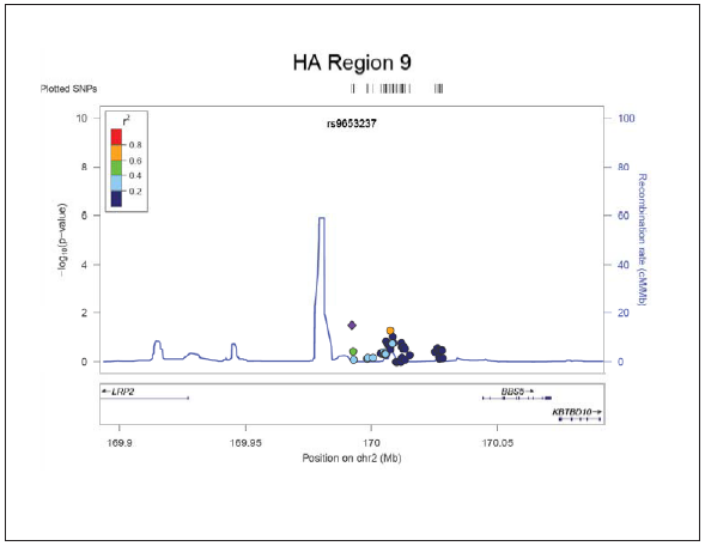


Appendix 29 LocusZoom plots for region 9, all ethnicities

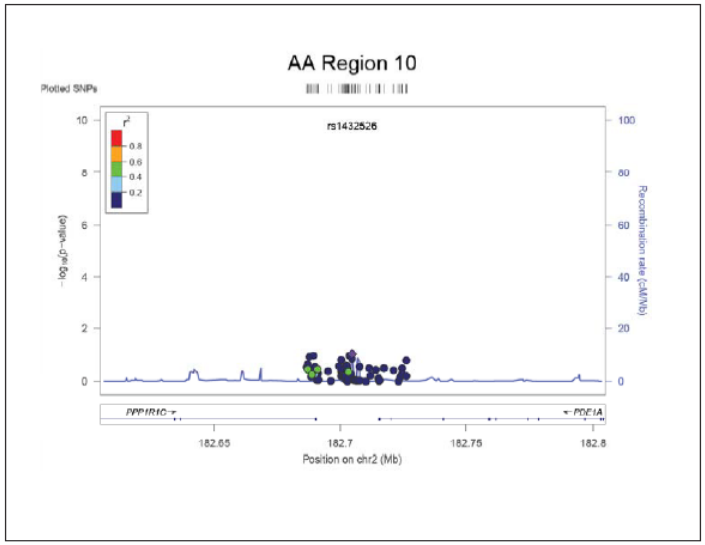


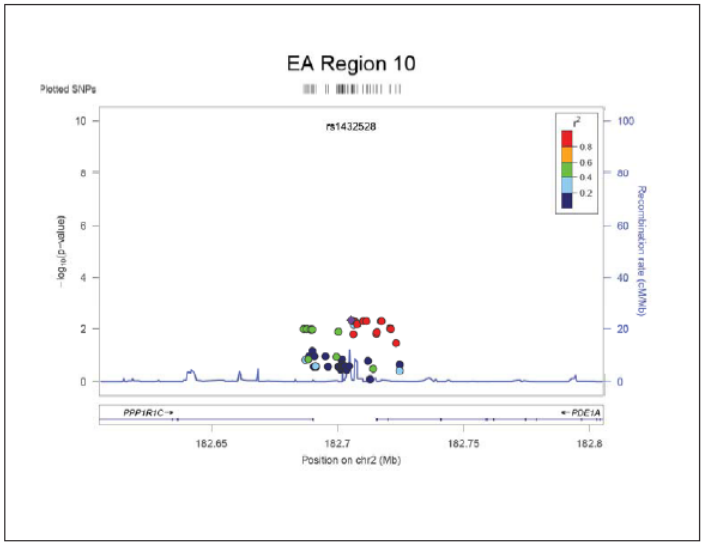


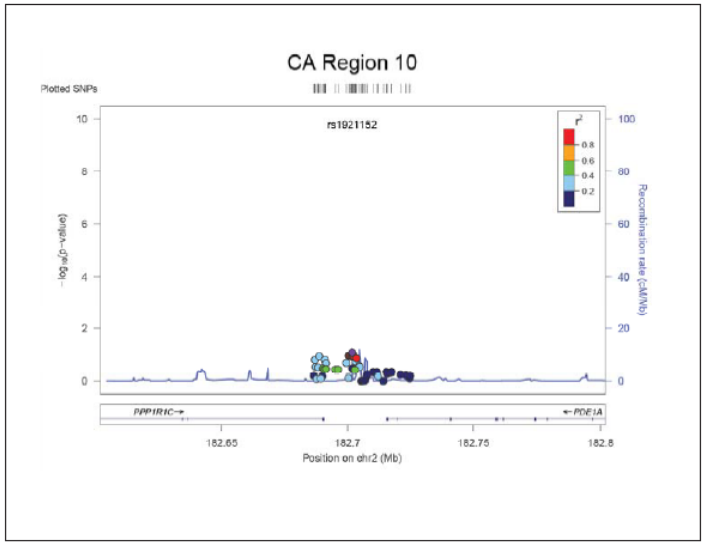


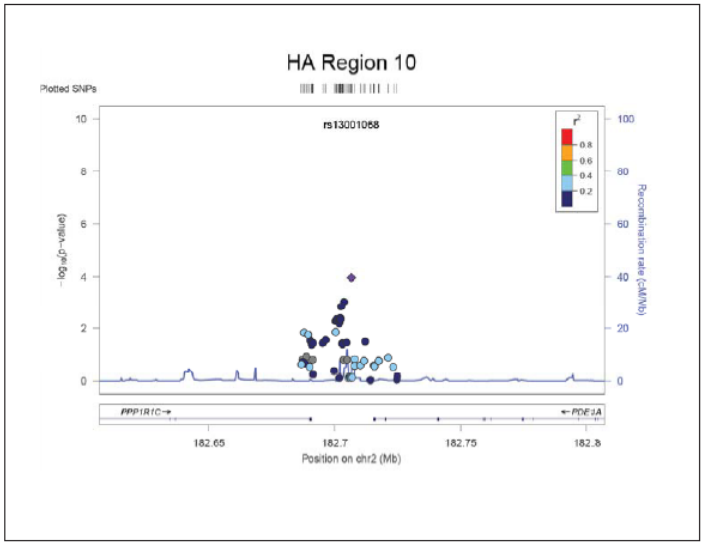


Appendix 30 LocusZoom plots for region 10, all ethnicities

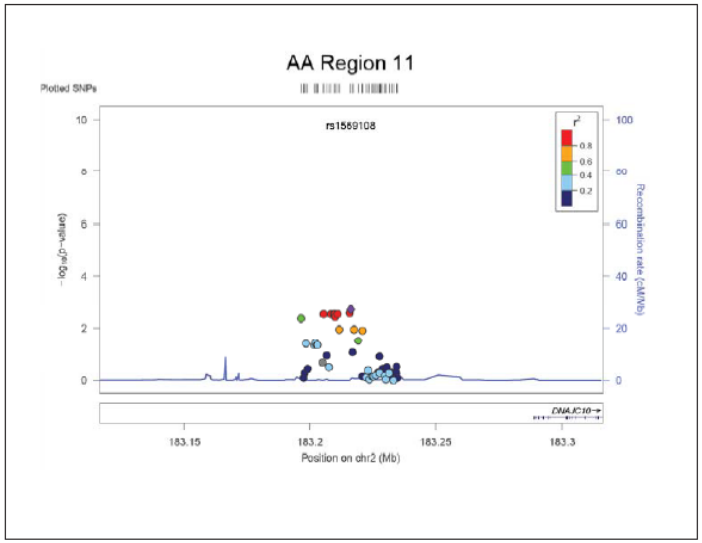


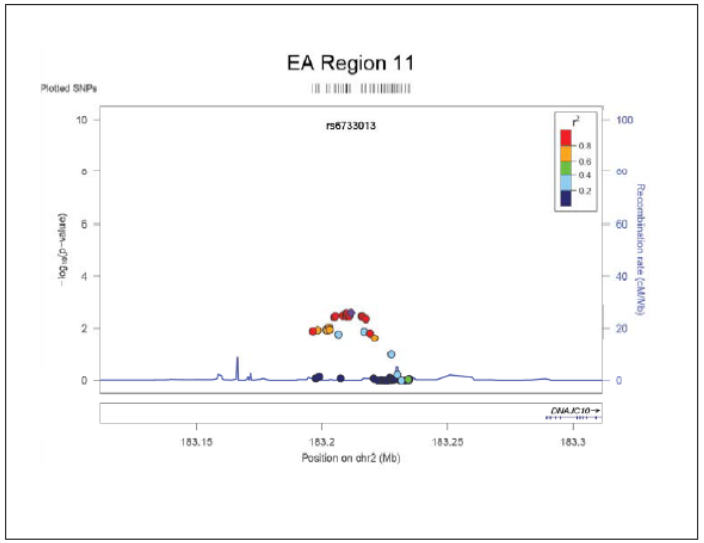


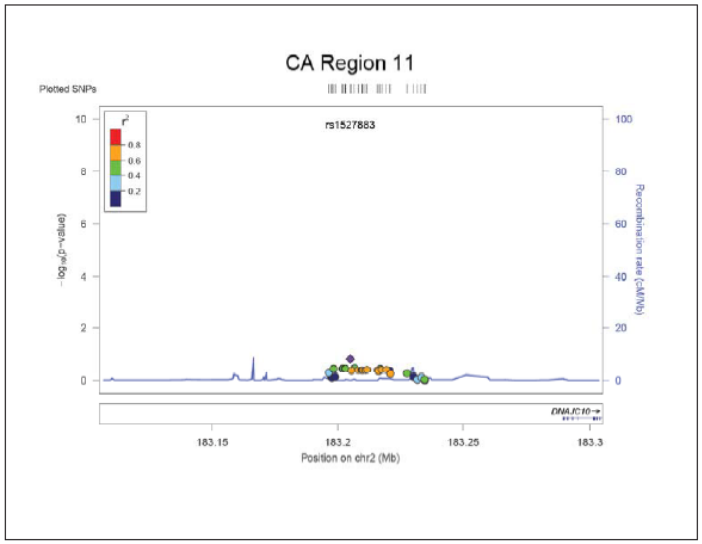


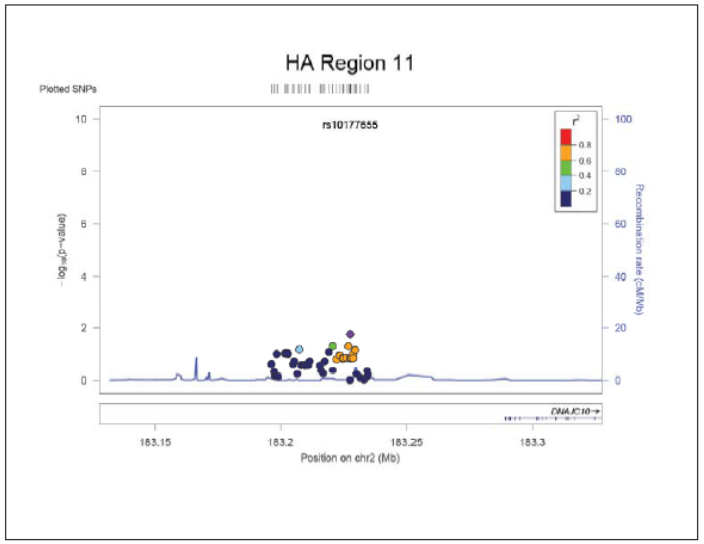


Appendix 31 LocusZoom plots for region 11, all ethnicities

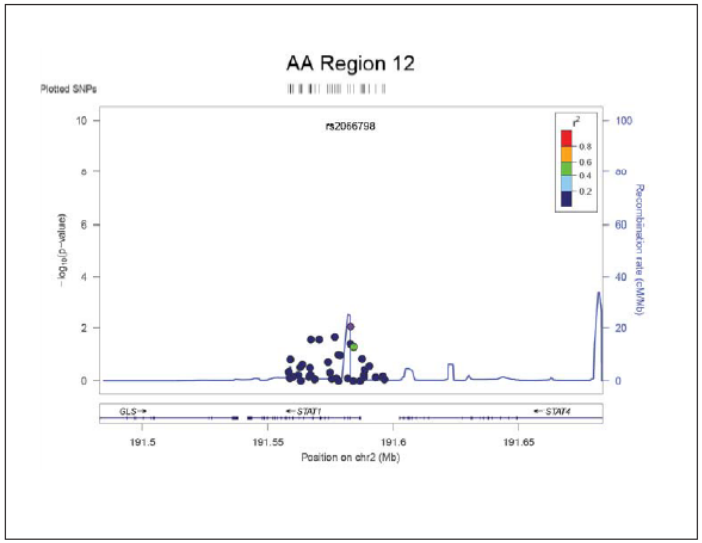


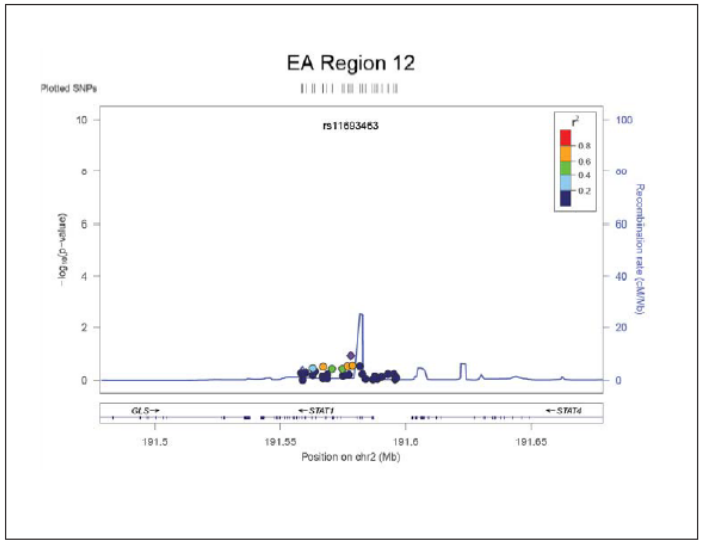


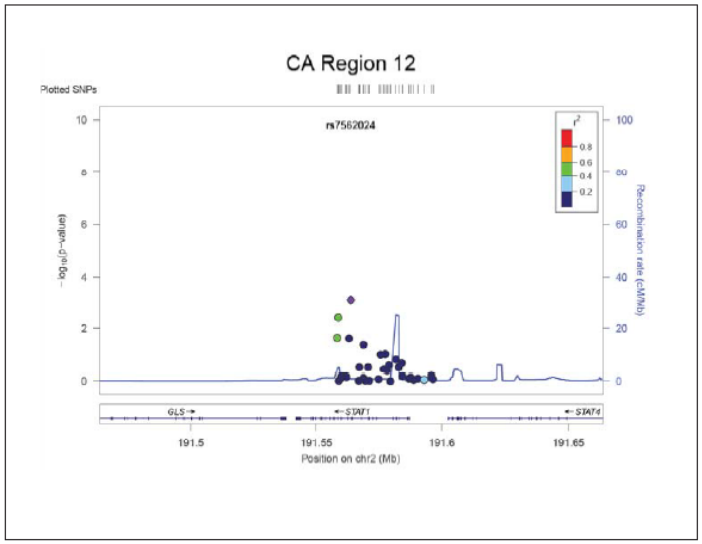


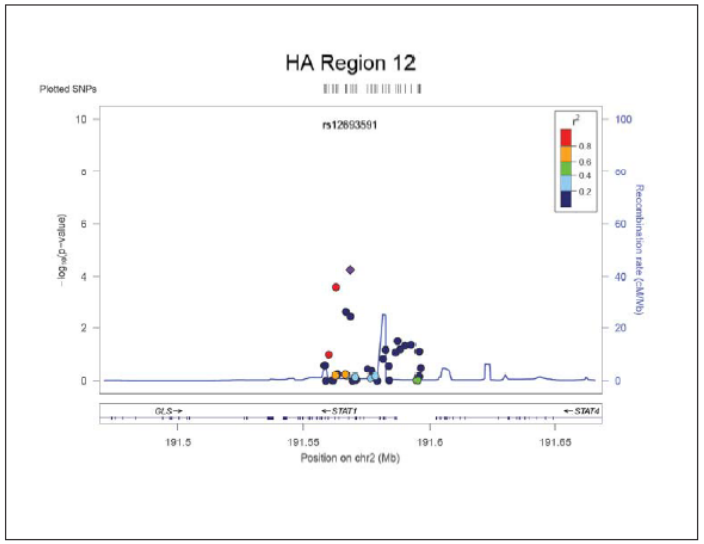


Appendix 32 LocusZoom plots for region 12, all ethnicities

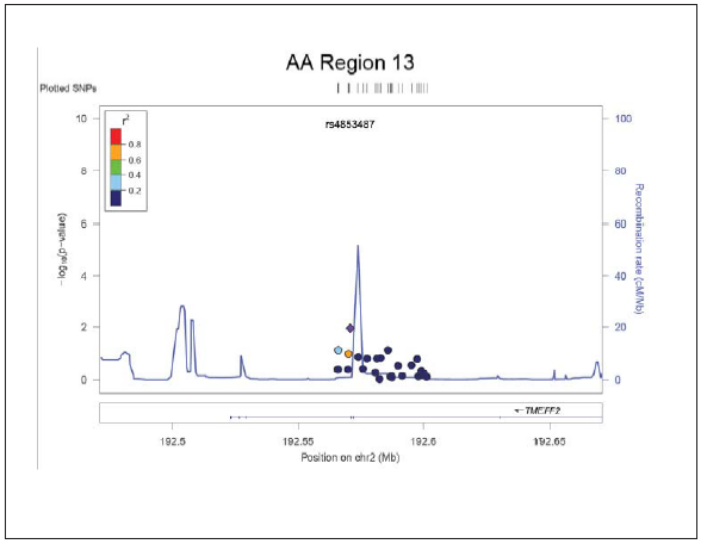


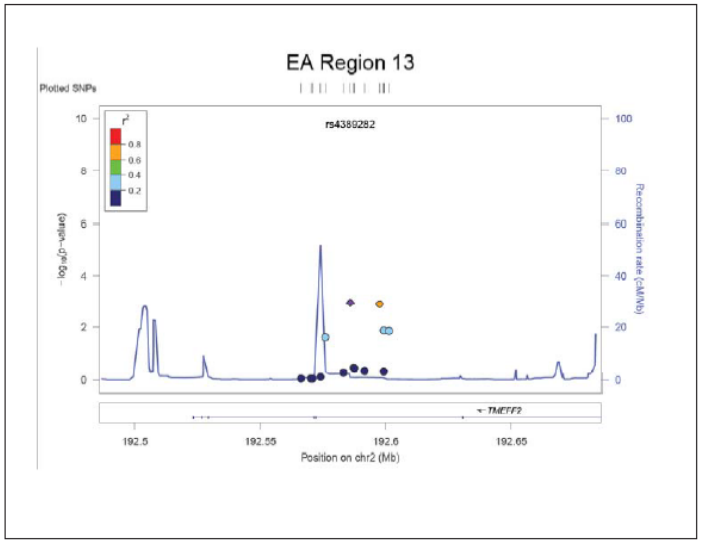


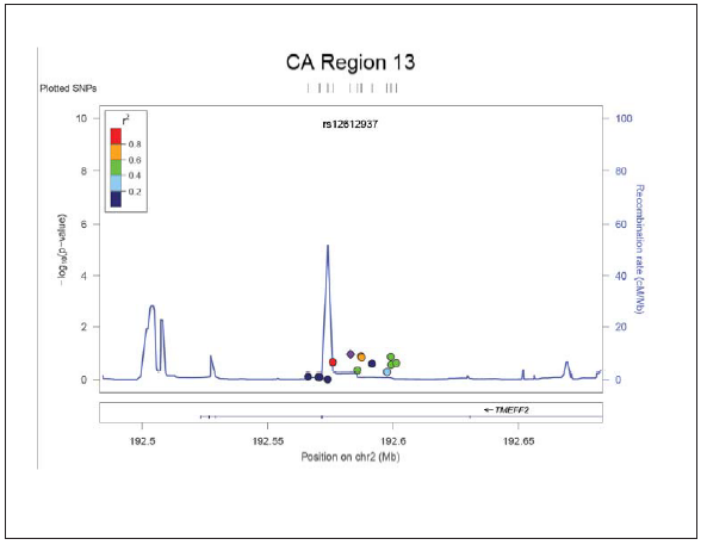


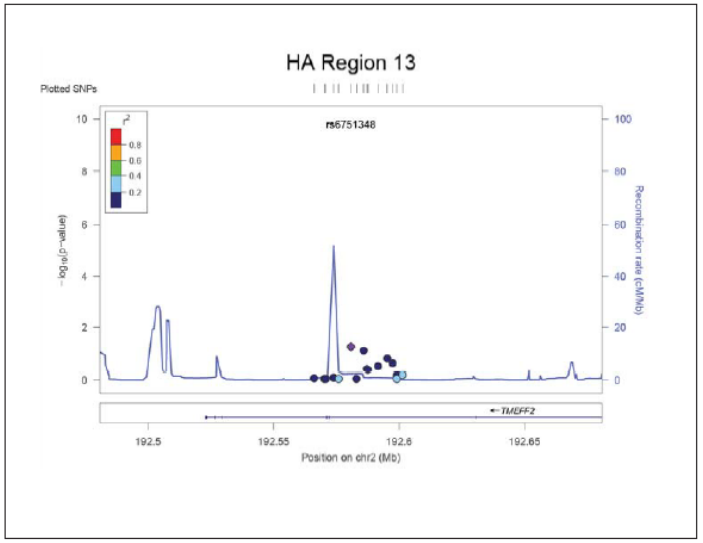


Appendix 33 LocusZoom plots for region 13, all ethnicities

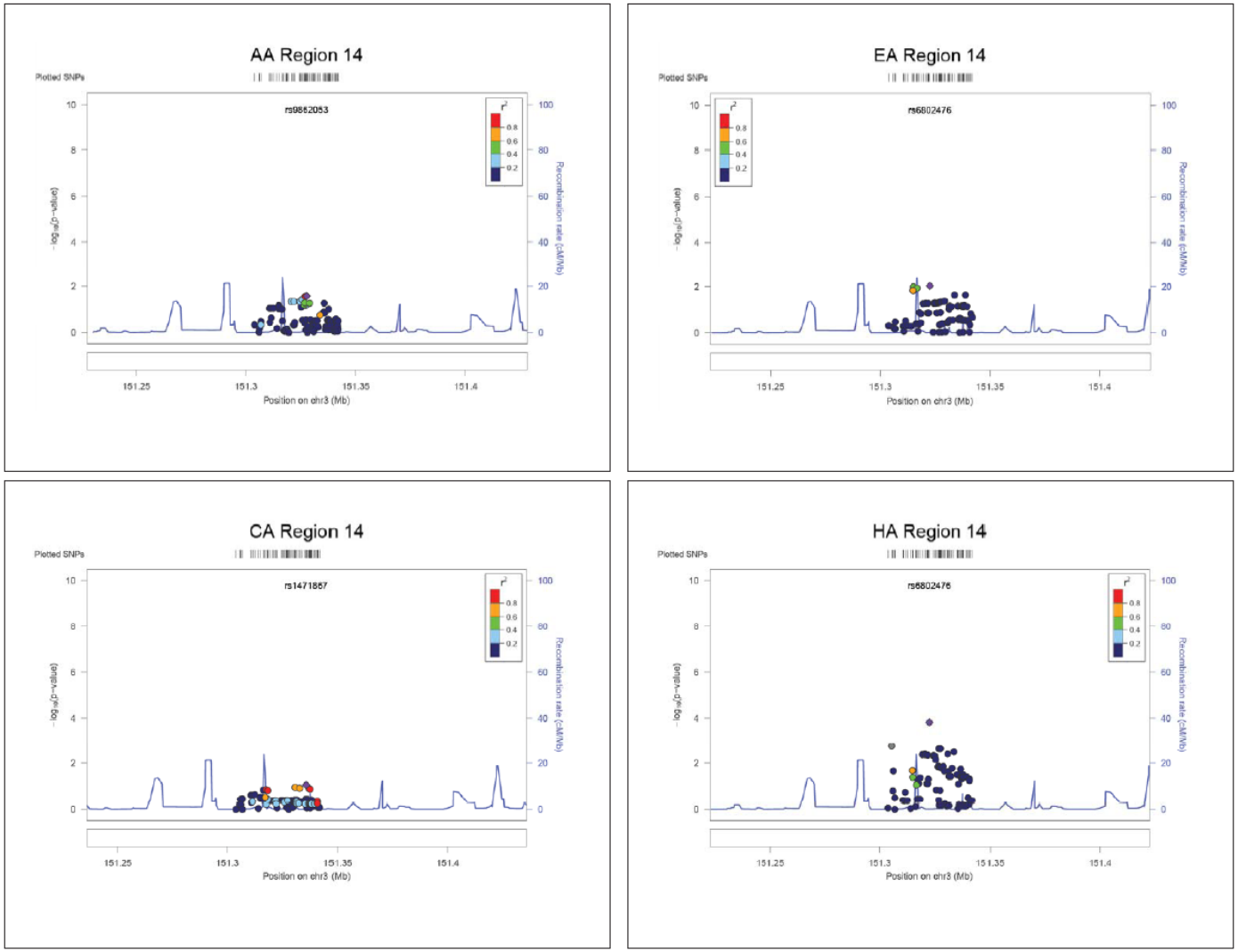




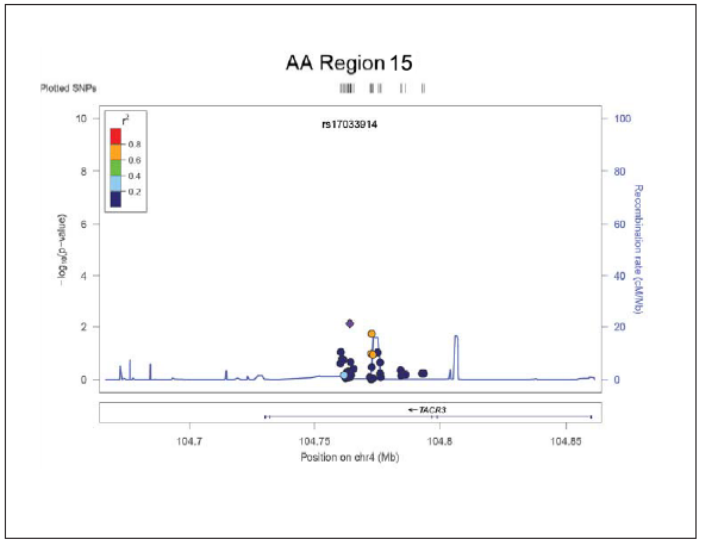


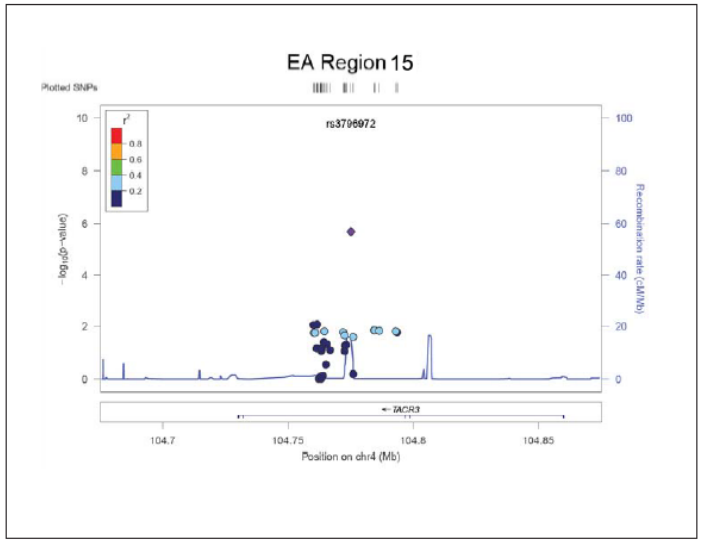


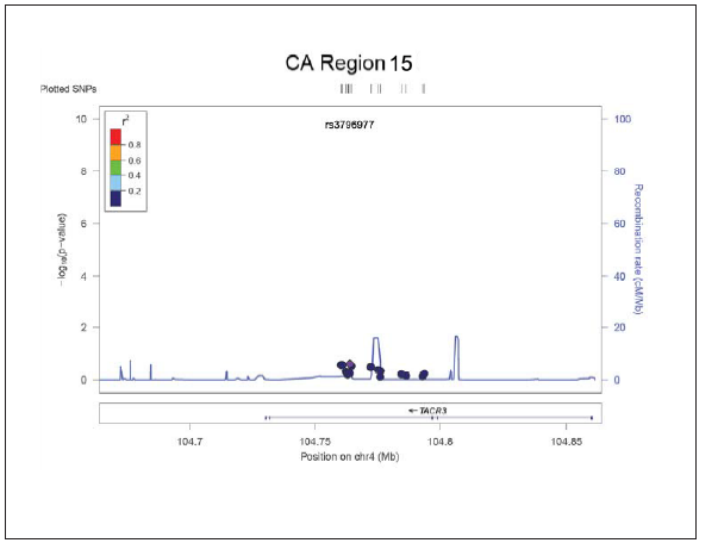
Appendix 34 LocusZoom plots for region 14, all ethnicities

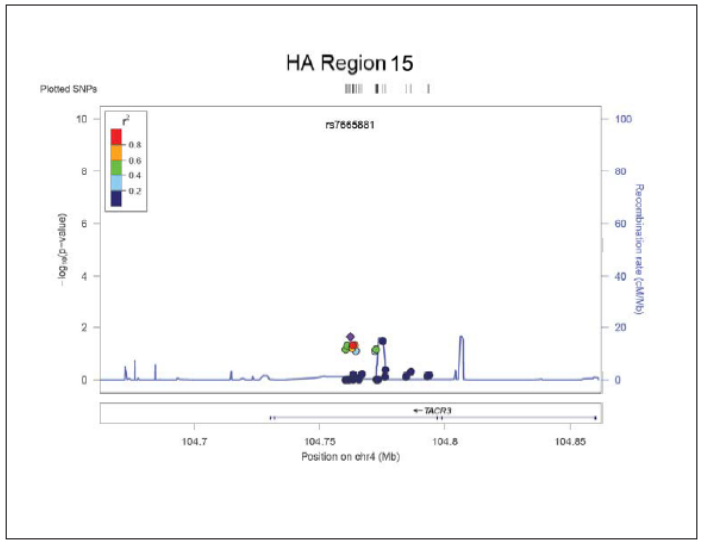


Appendix 35 LocusZoom plots for region 15, all ethnicities

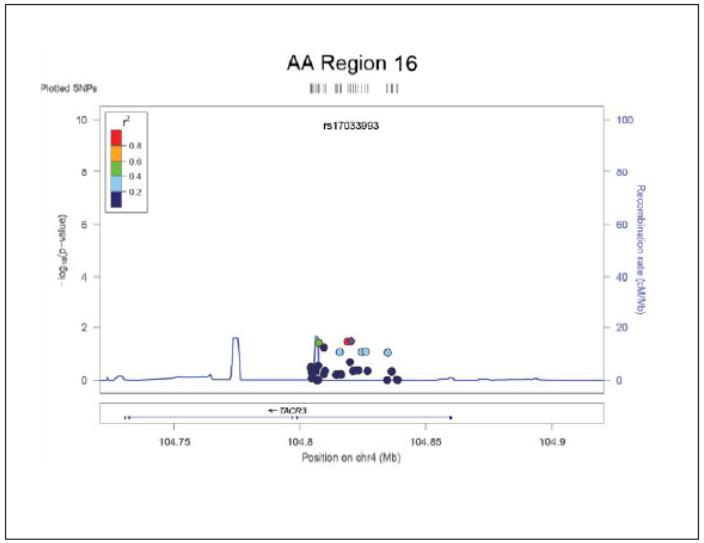


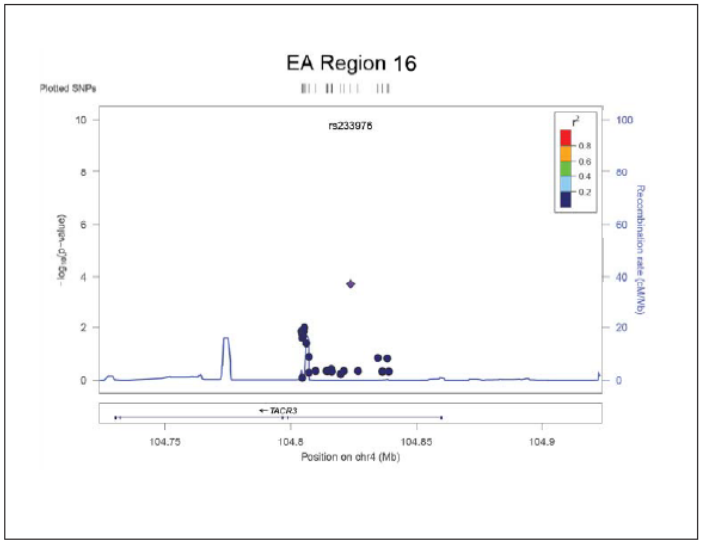


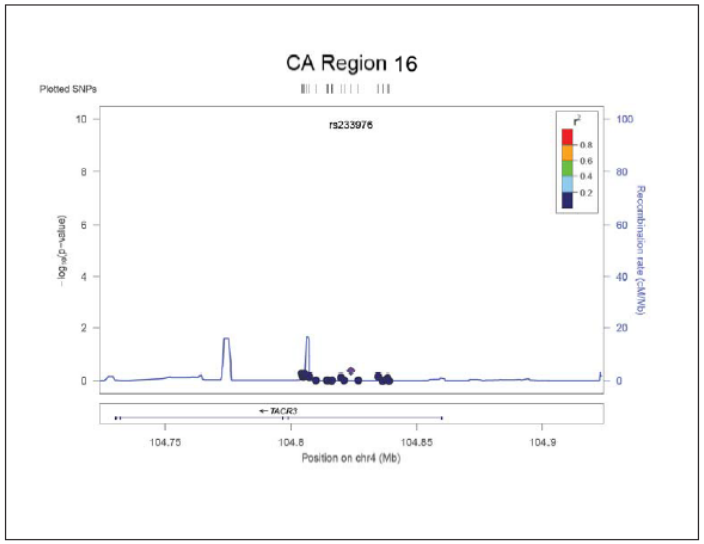


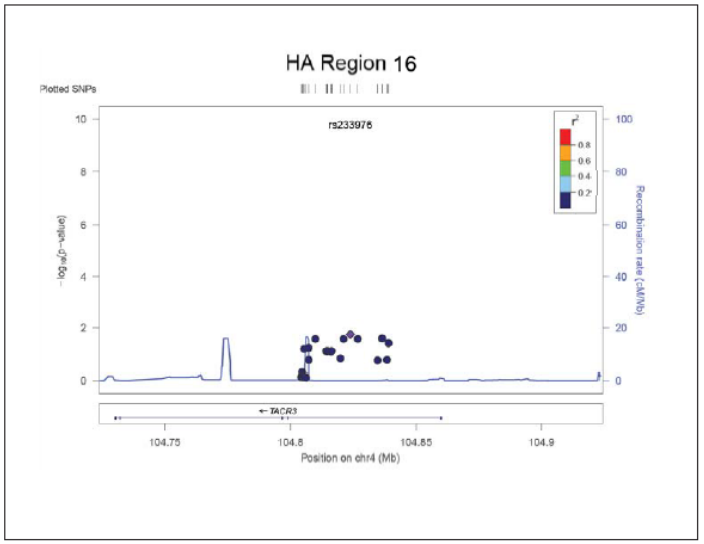


Appendix 36 LocusZoom plots for region 16, all ethnicities

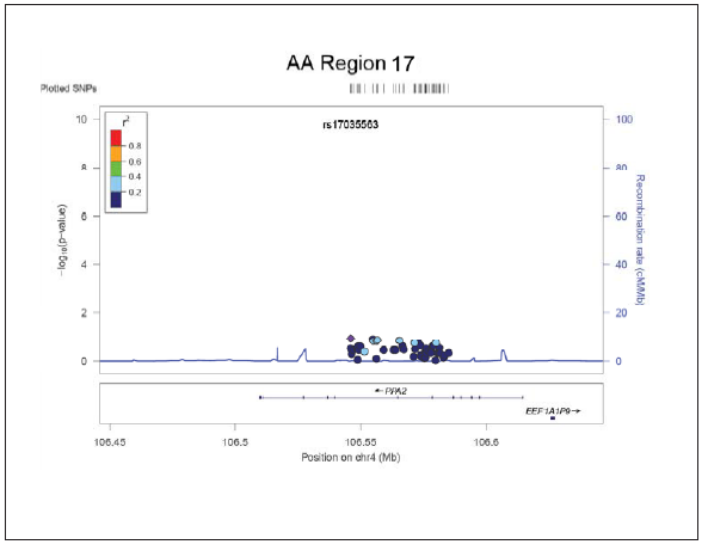


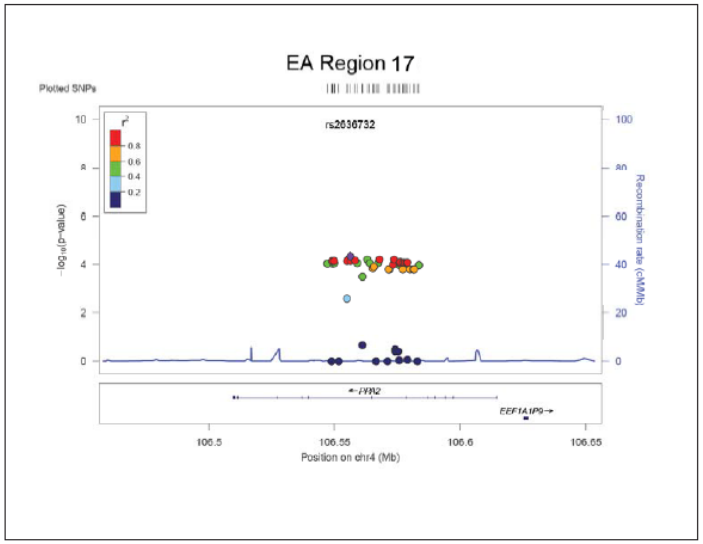


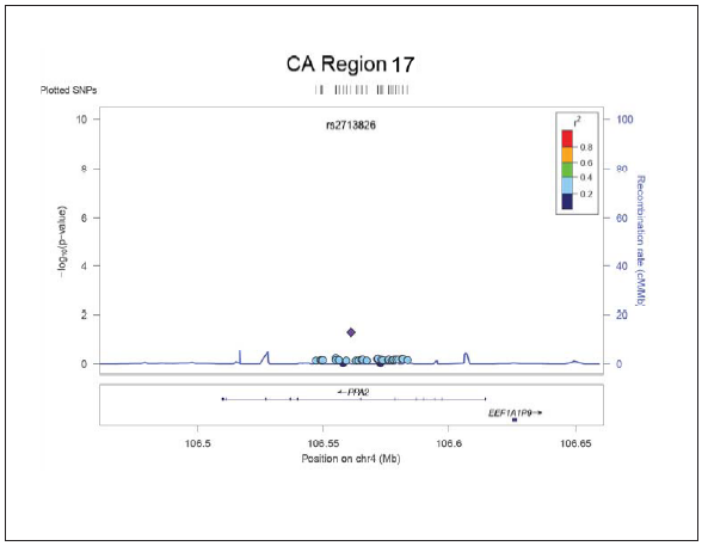


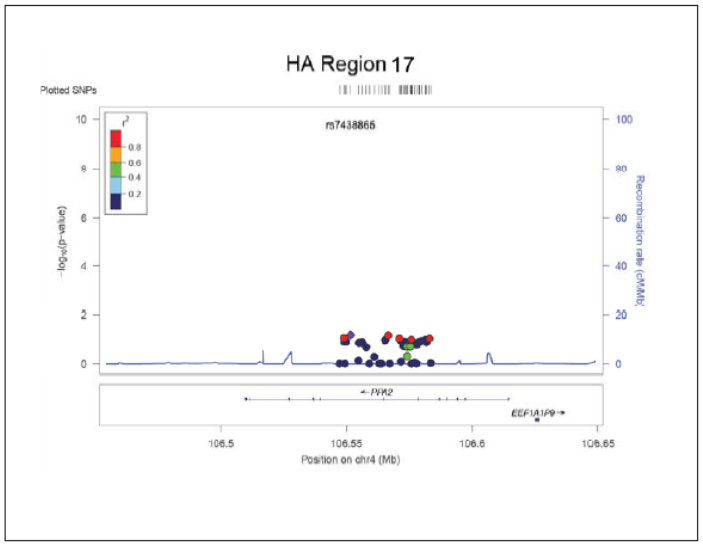


Appendix 37 LocusZoom plots for region 17, all ethnicities

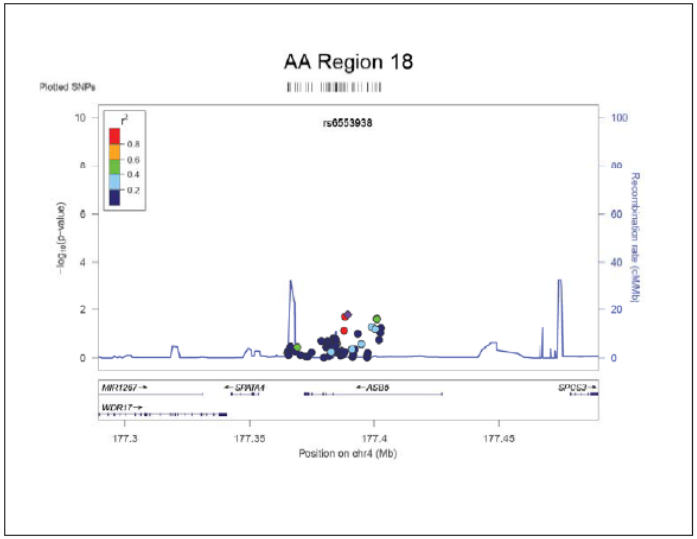


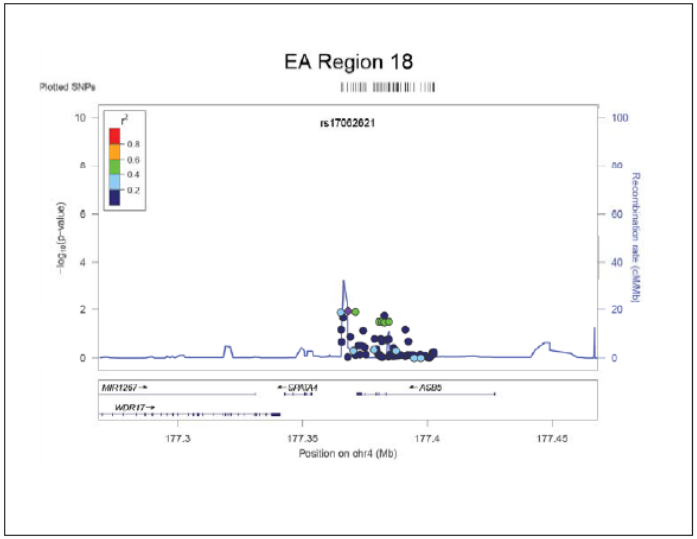


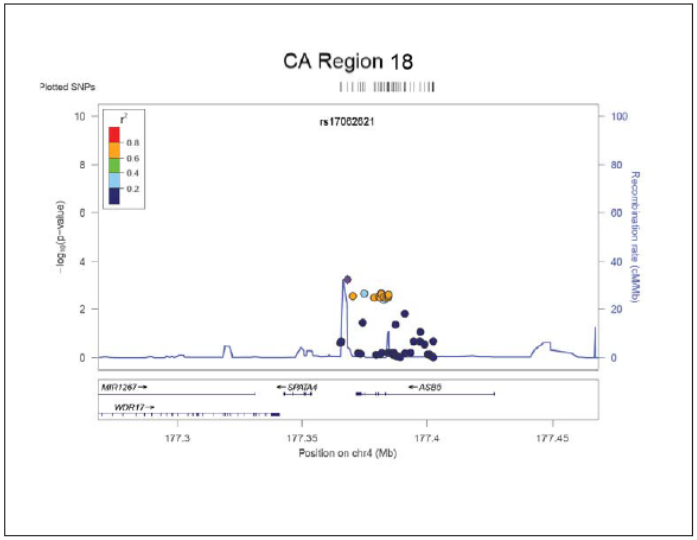


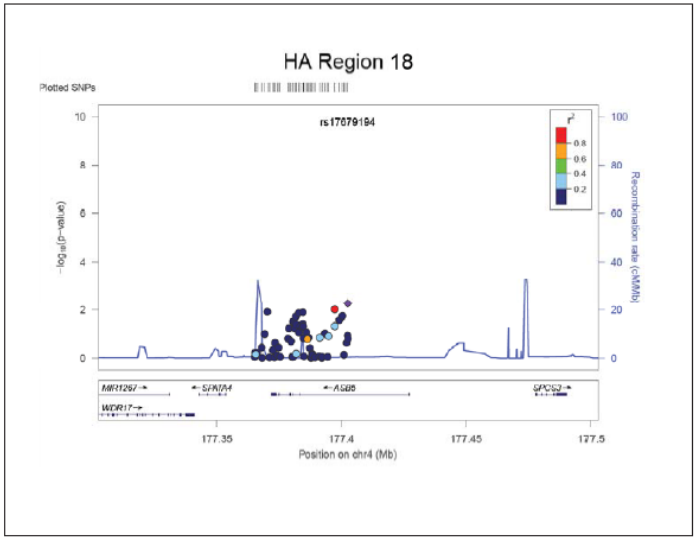


Appendix 38 LocusZoom plots for region 18, all ethnicities

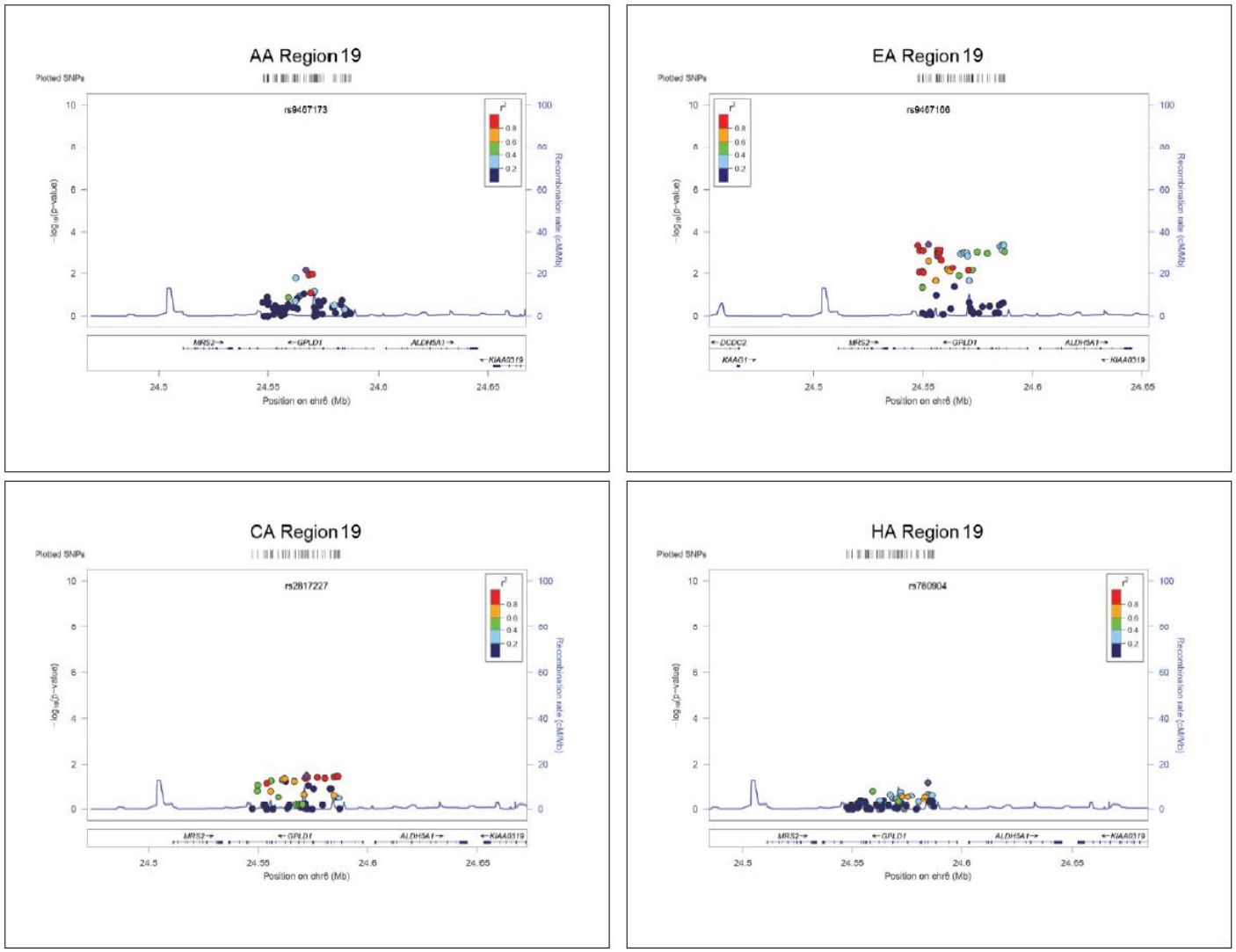




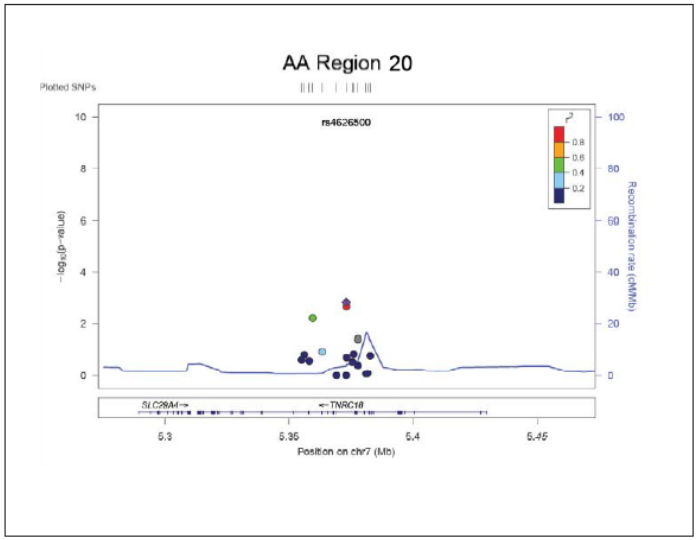


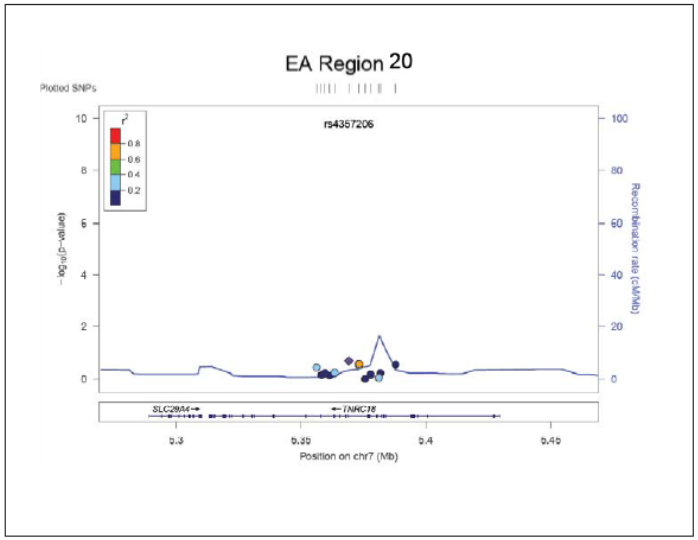


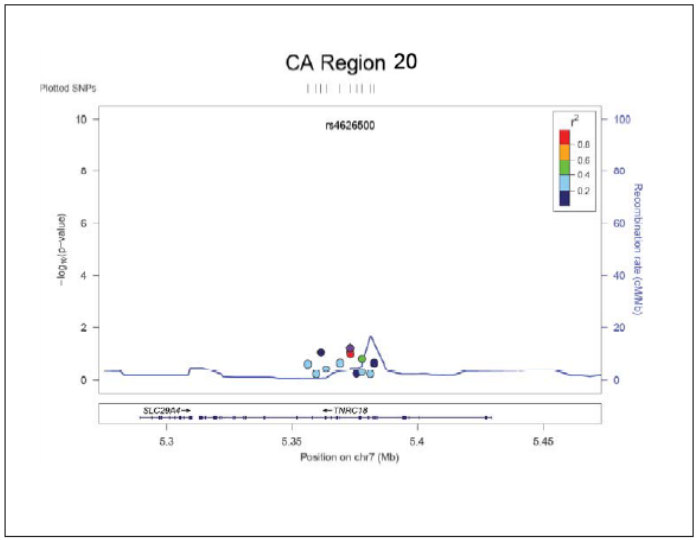
Appendix 39 LocusZoom plots for region 19, all ethnicities

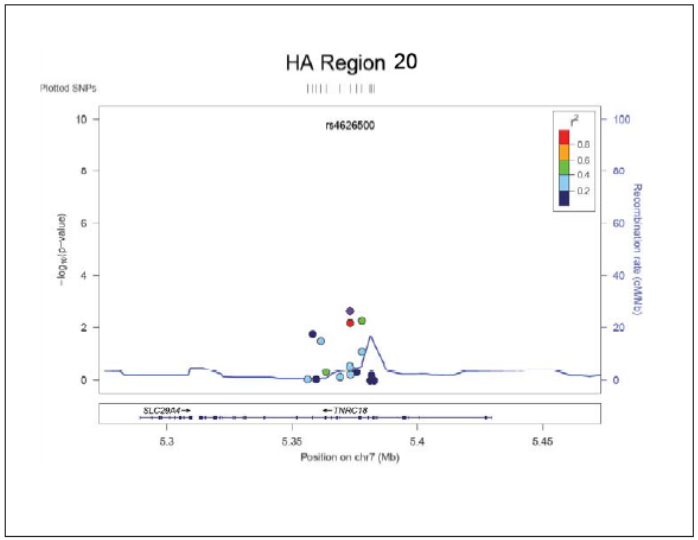


Appendix 40 LocusZoom plots for region 20, all ethnicities

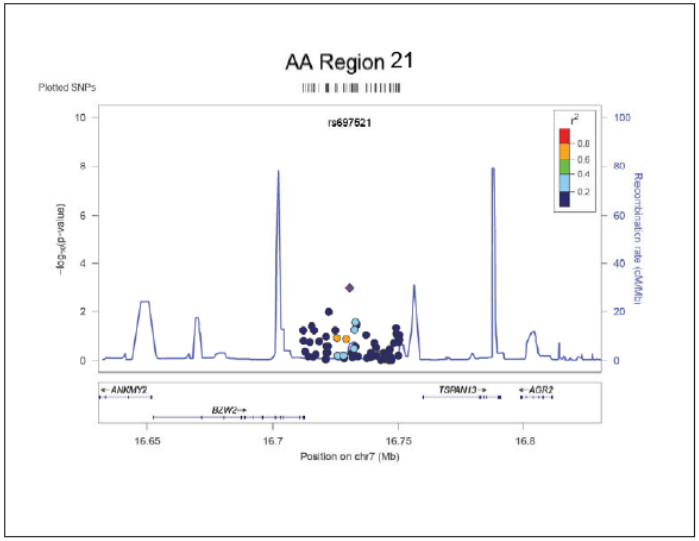


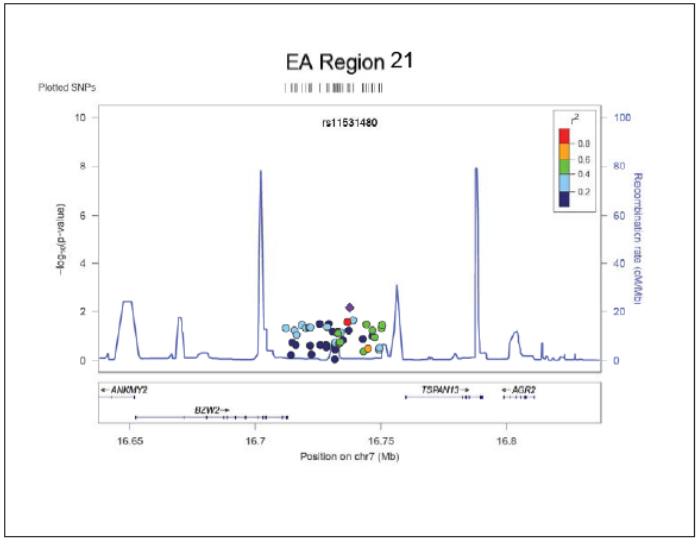


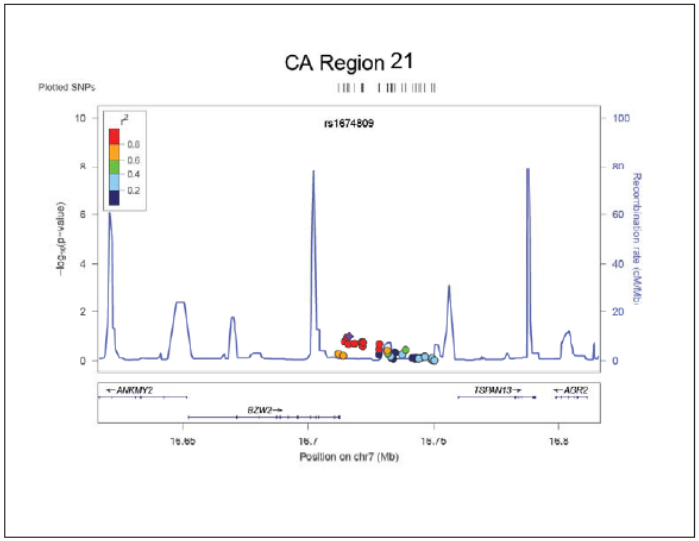


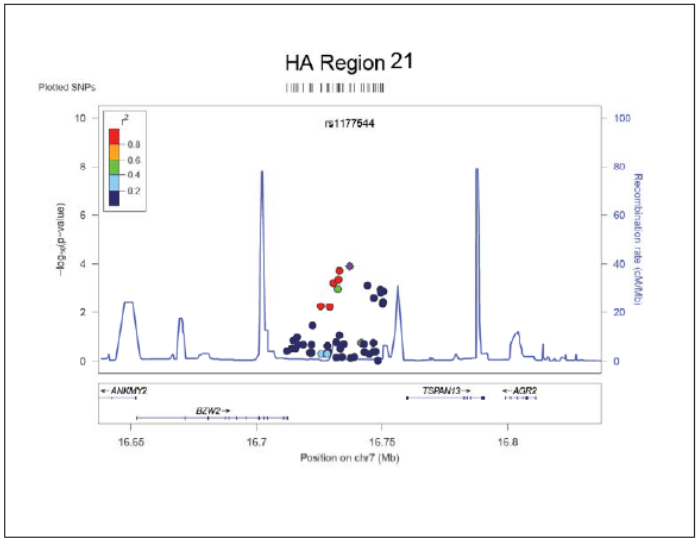


Appendix 41 LocusZoom plots for region 21, all ethnicities

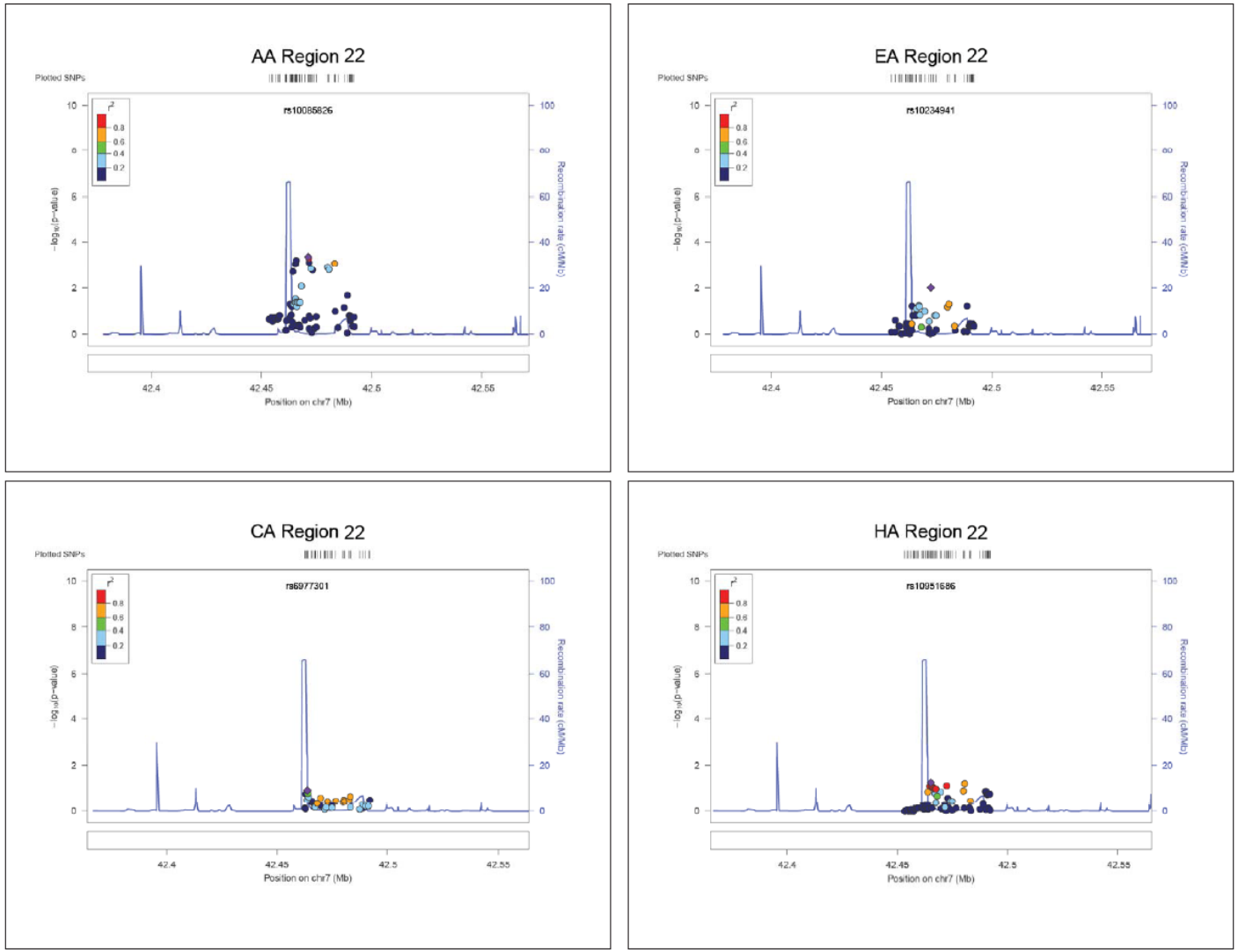




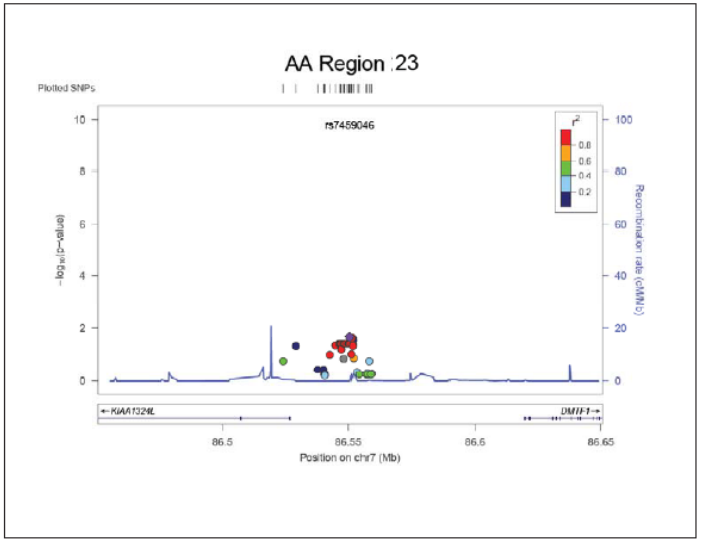


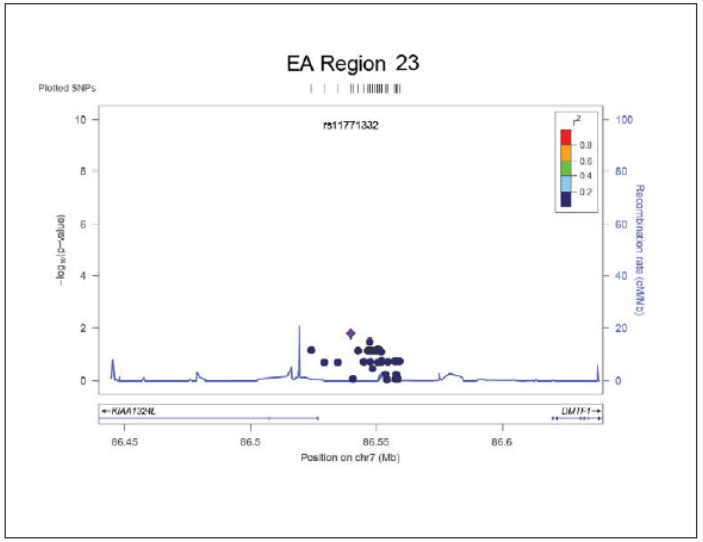


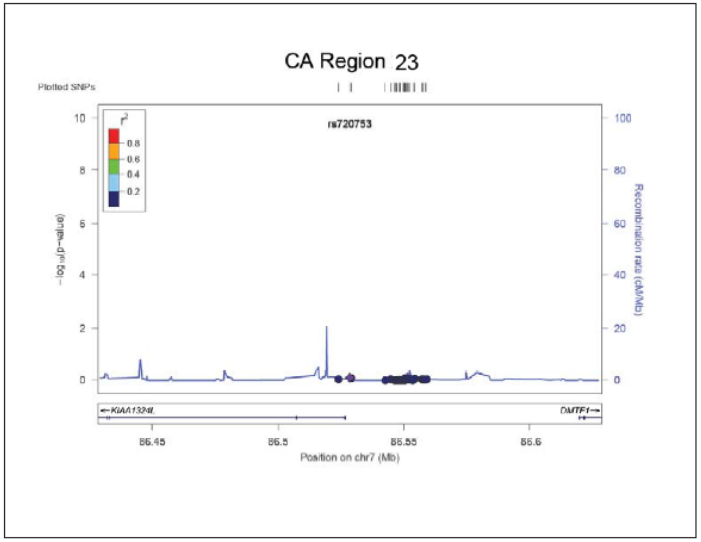
Appendix 42 LocusZoom plots for region 22, all ethnicities

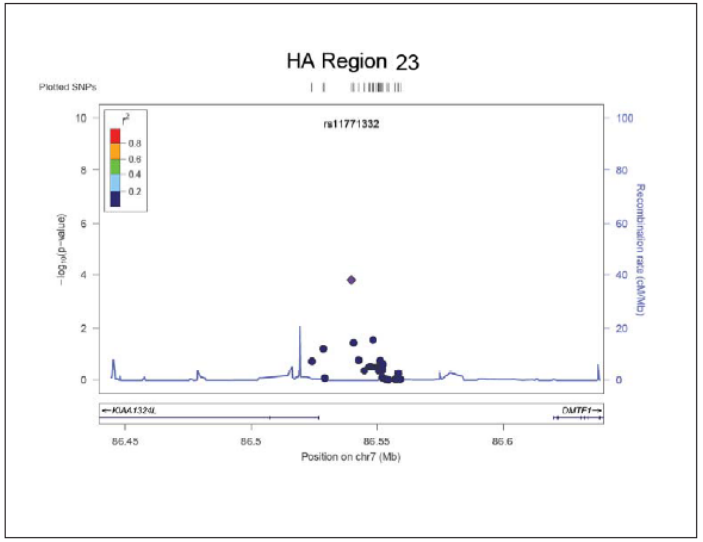


Appendix 43 LocusZoom plots for region 23, all ethnicities

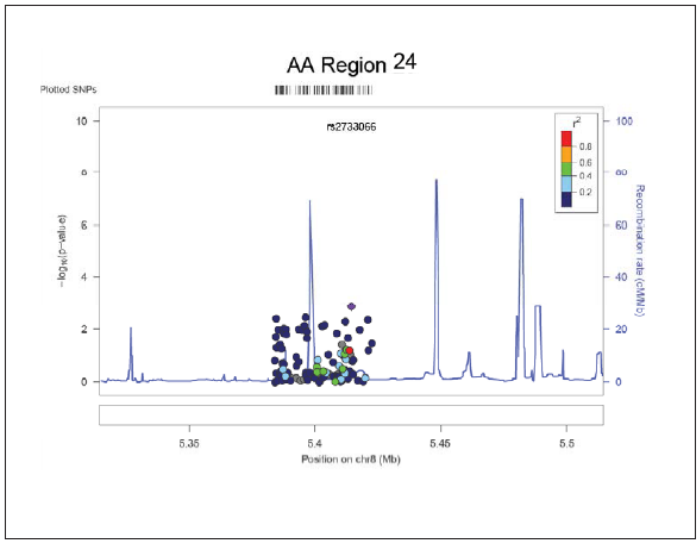


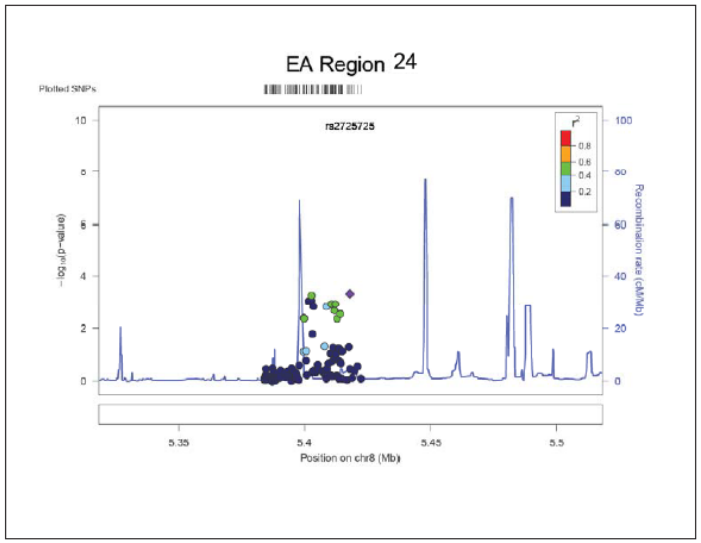


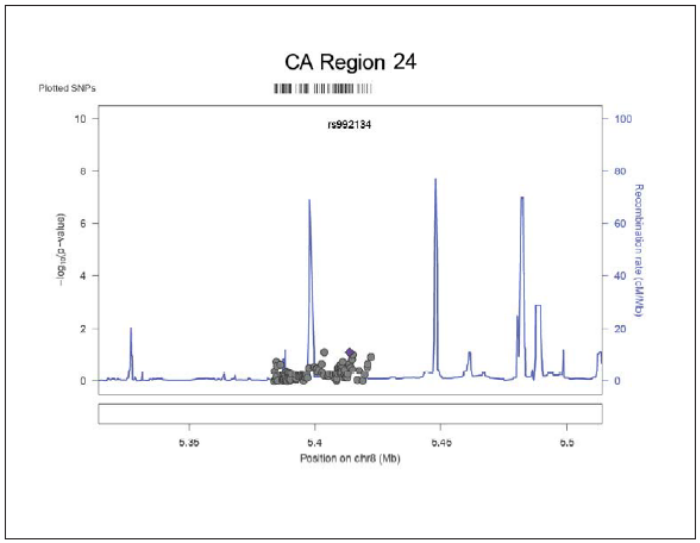


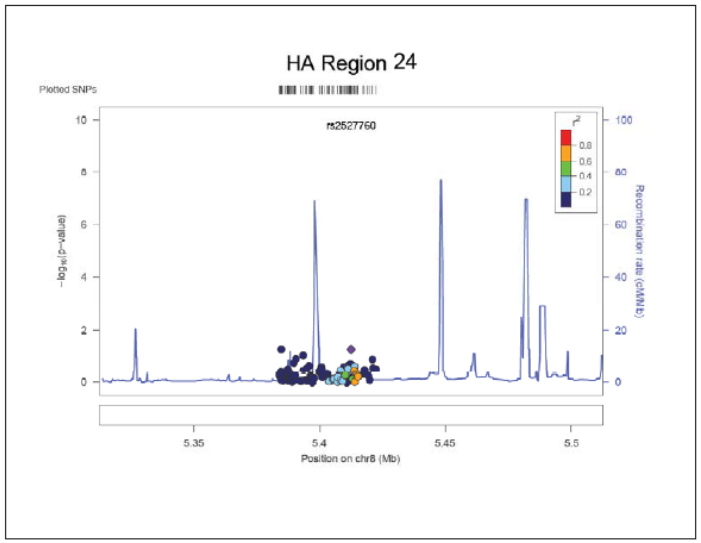


Appendix 44 LocusZoom plots for region 24, all ethnicities

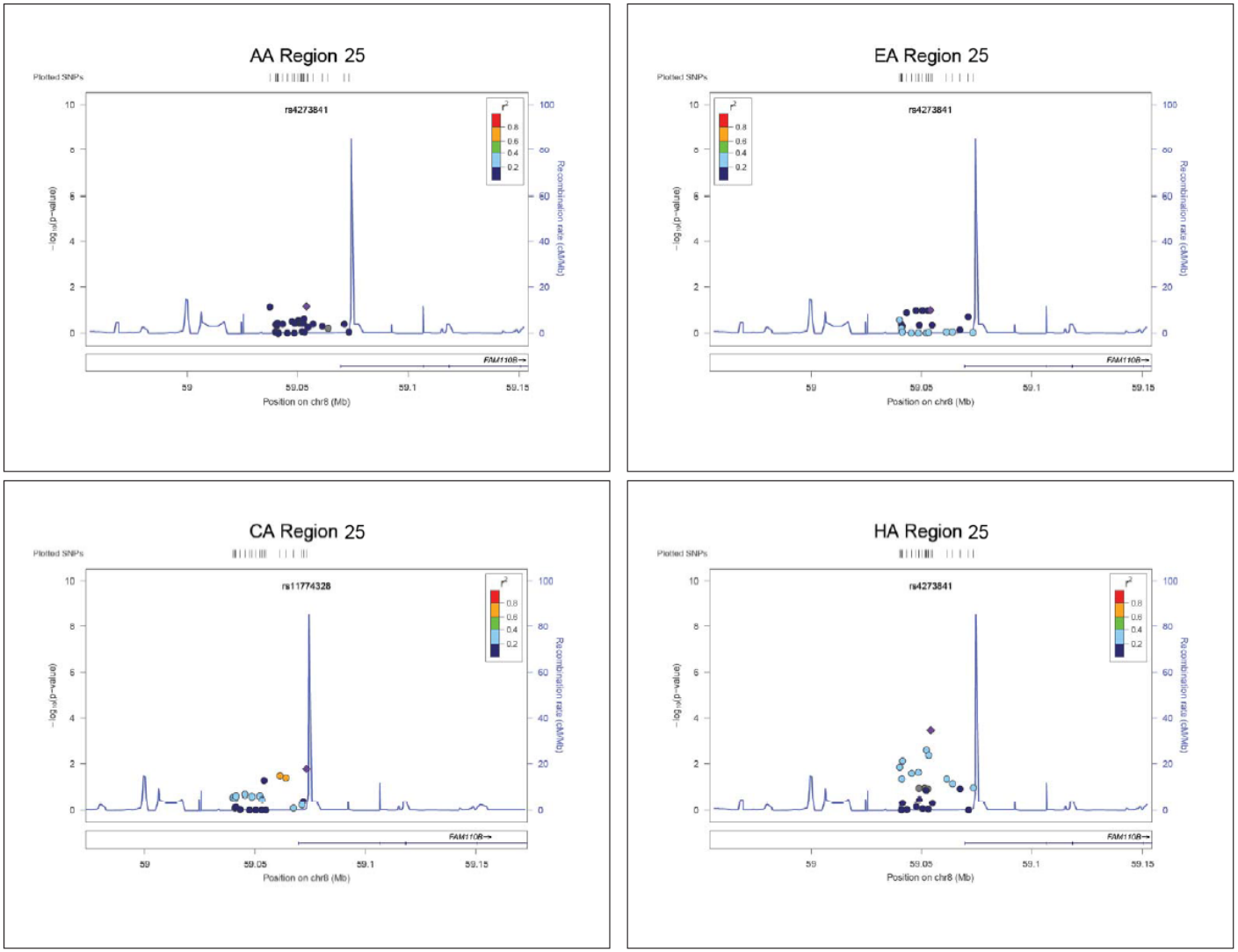




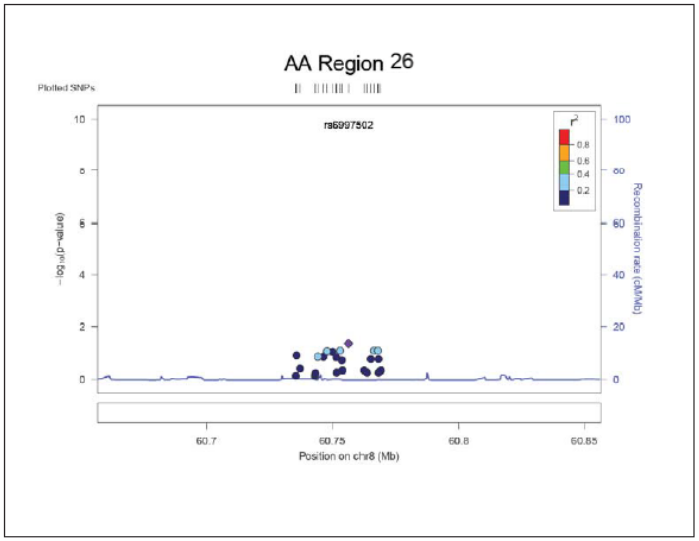


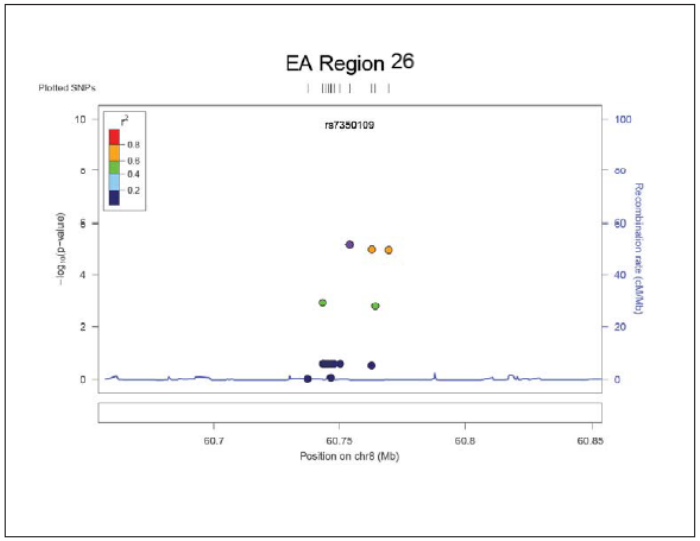


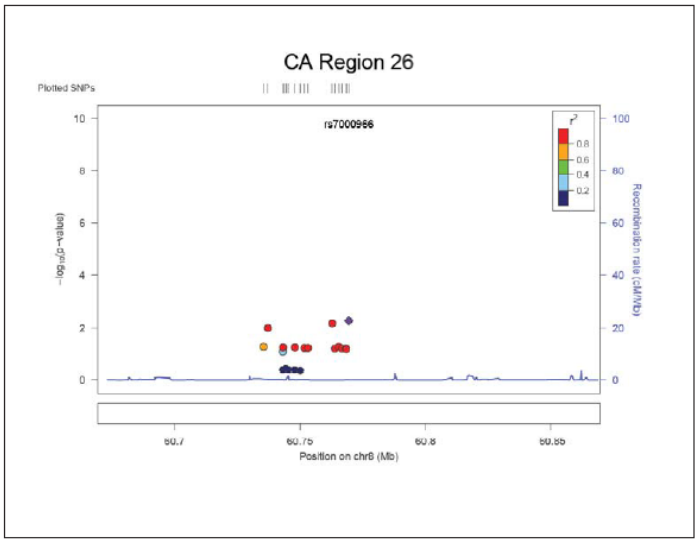
Appendix 45 LocusZoom plots for region 24, all ethnicities

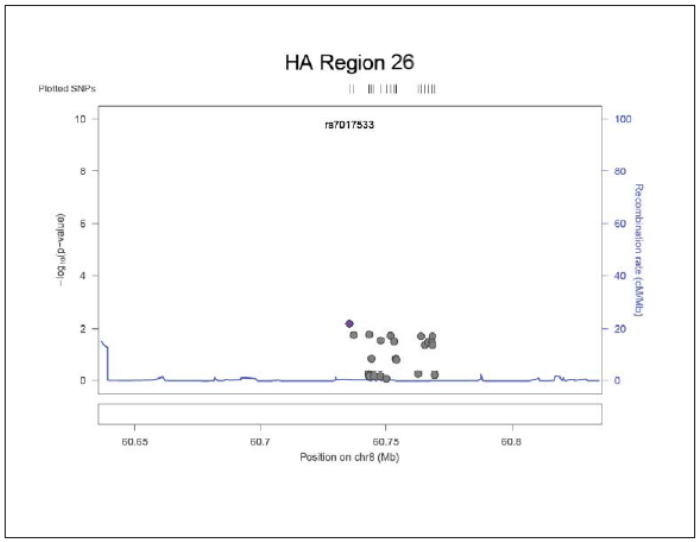


Appendix 46 LocusZoom plots for region 26, all ethnicities

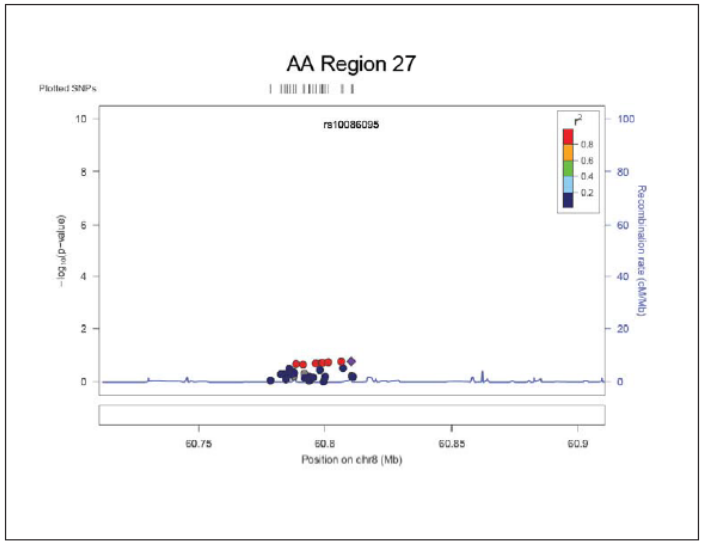


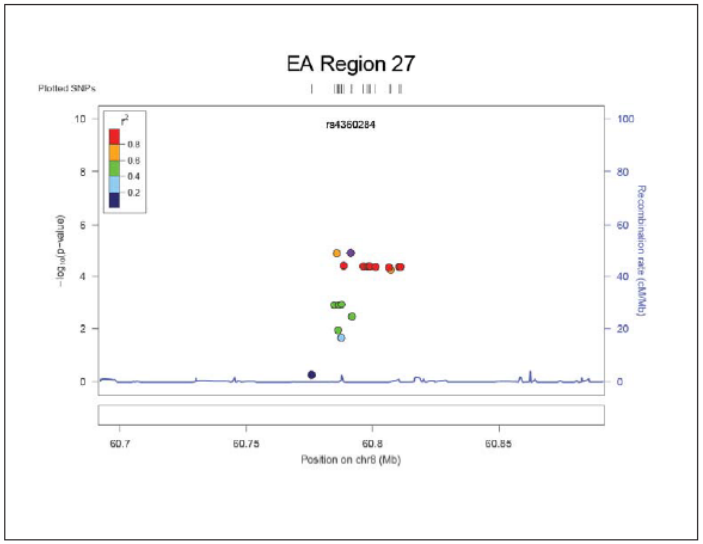


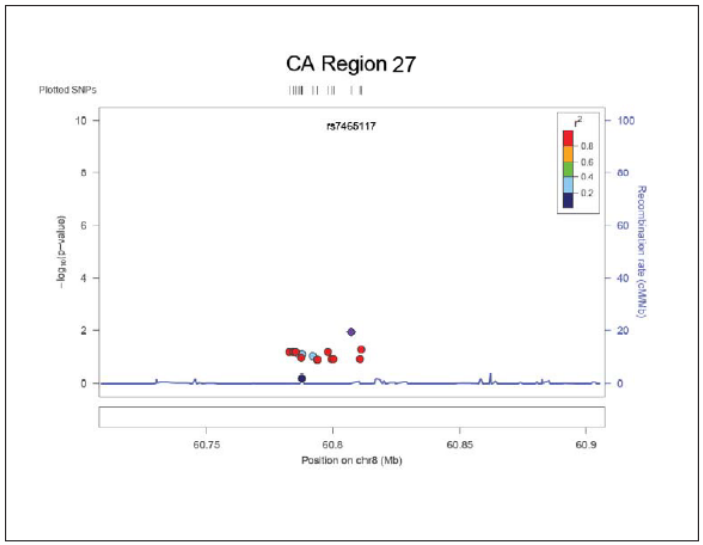


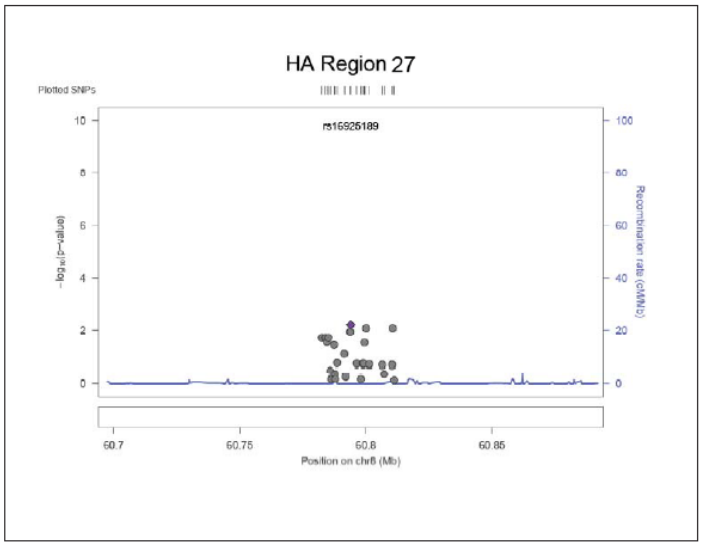


Appendix 47 LocusZoom plots for region 27, all ethnicities

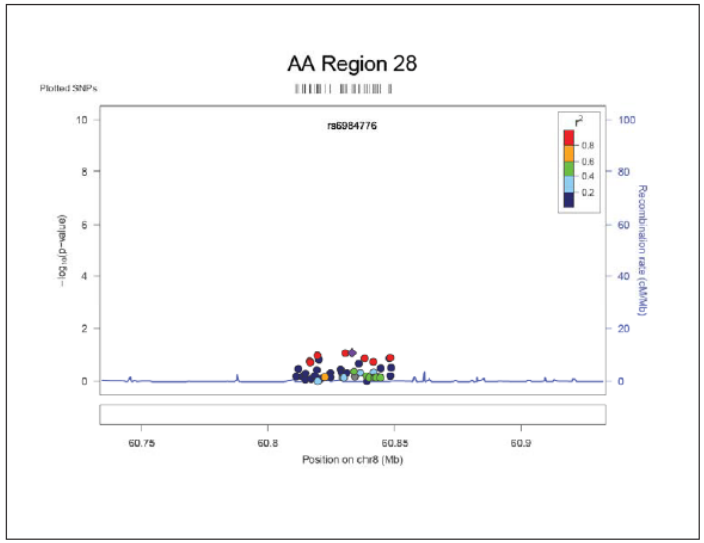


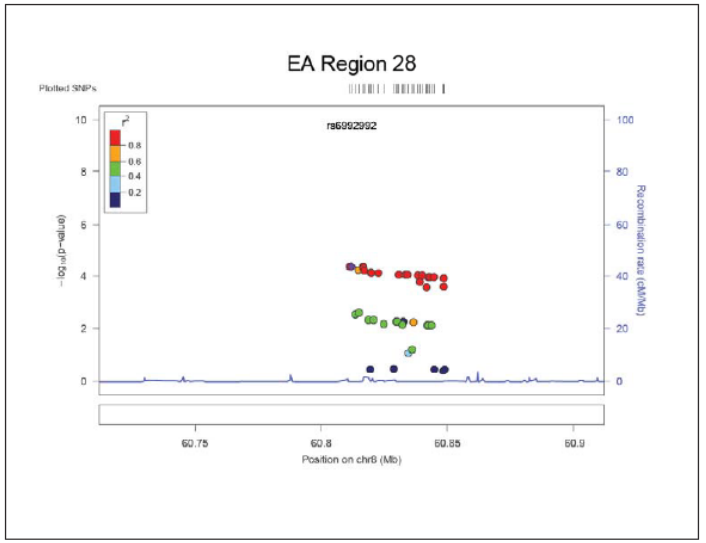


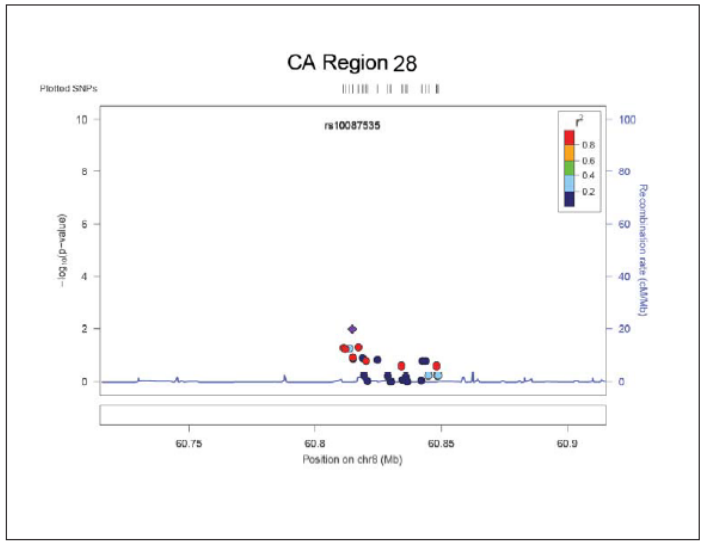


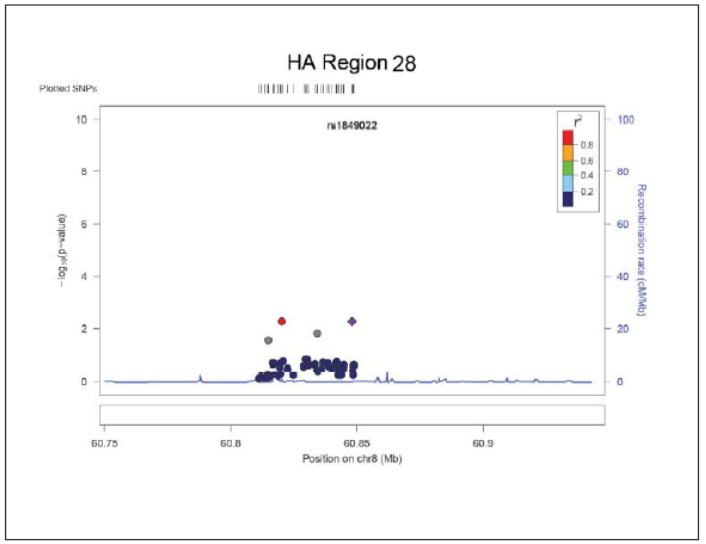


Appendix 48 LocusZoom plots for region 28, all ethnicities

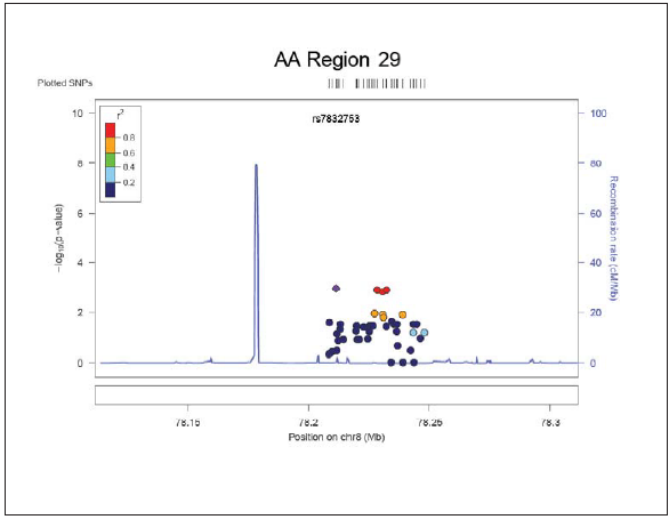


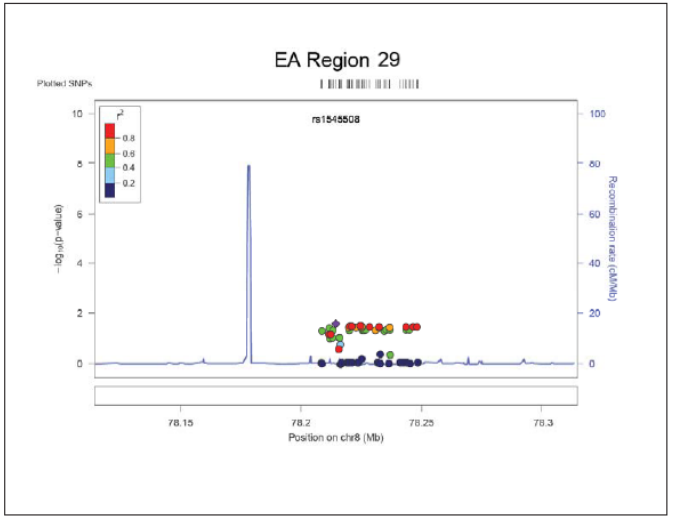


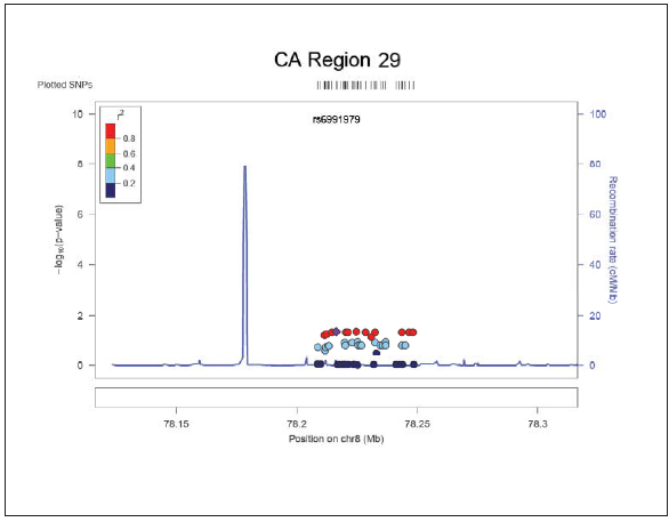


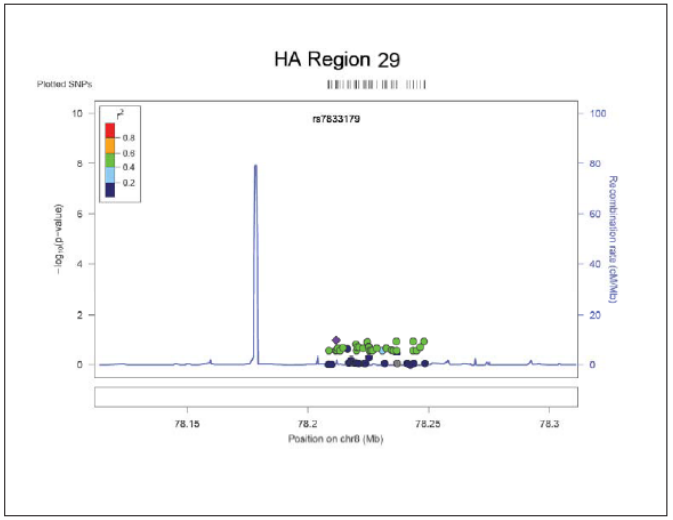


Appendix 49 LocusZoom plots for region 29, all ethnicities

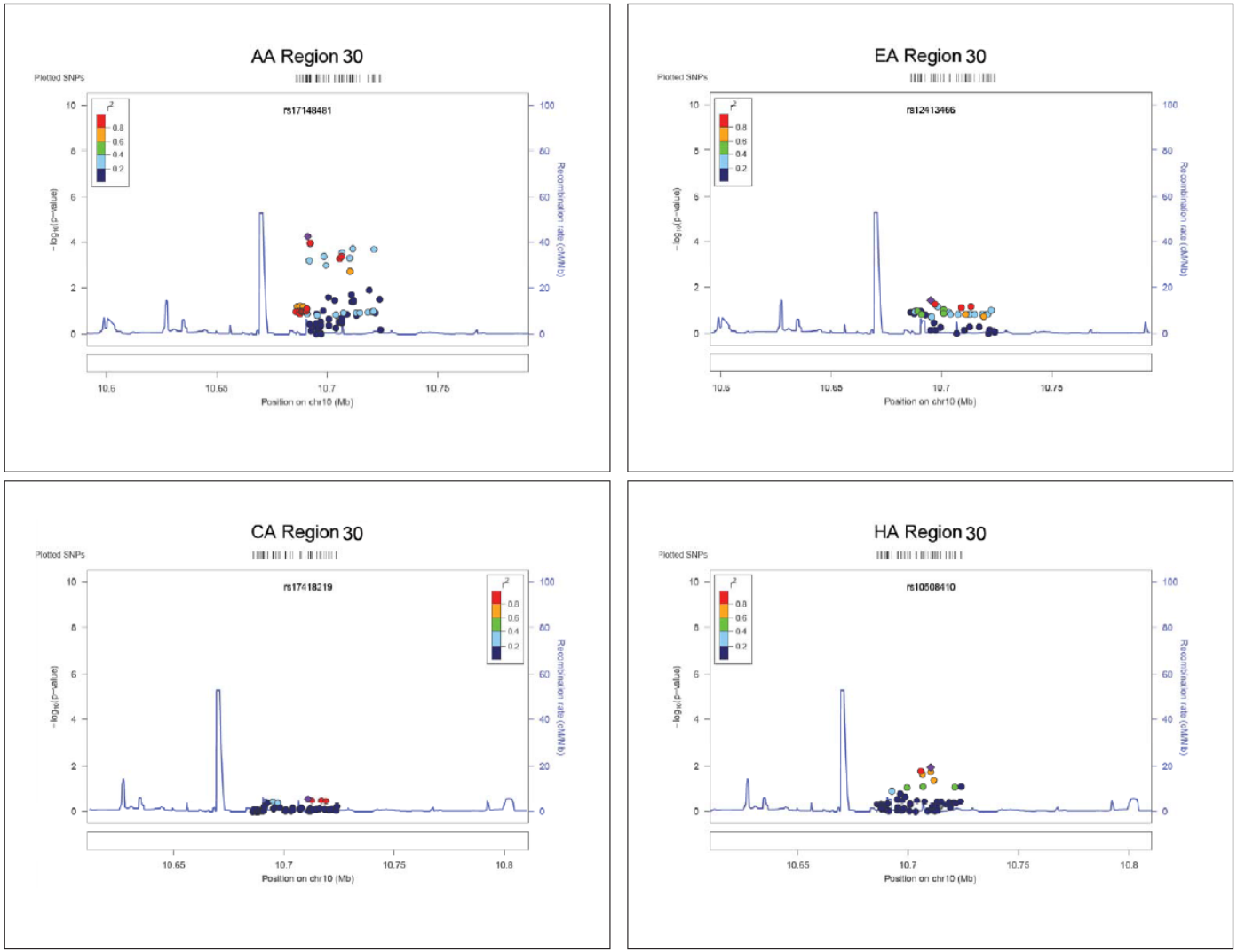




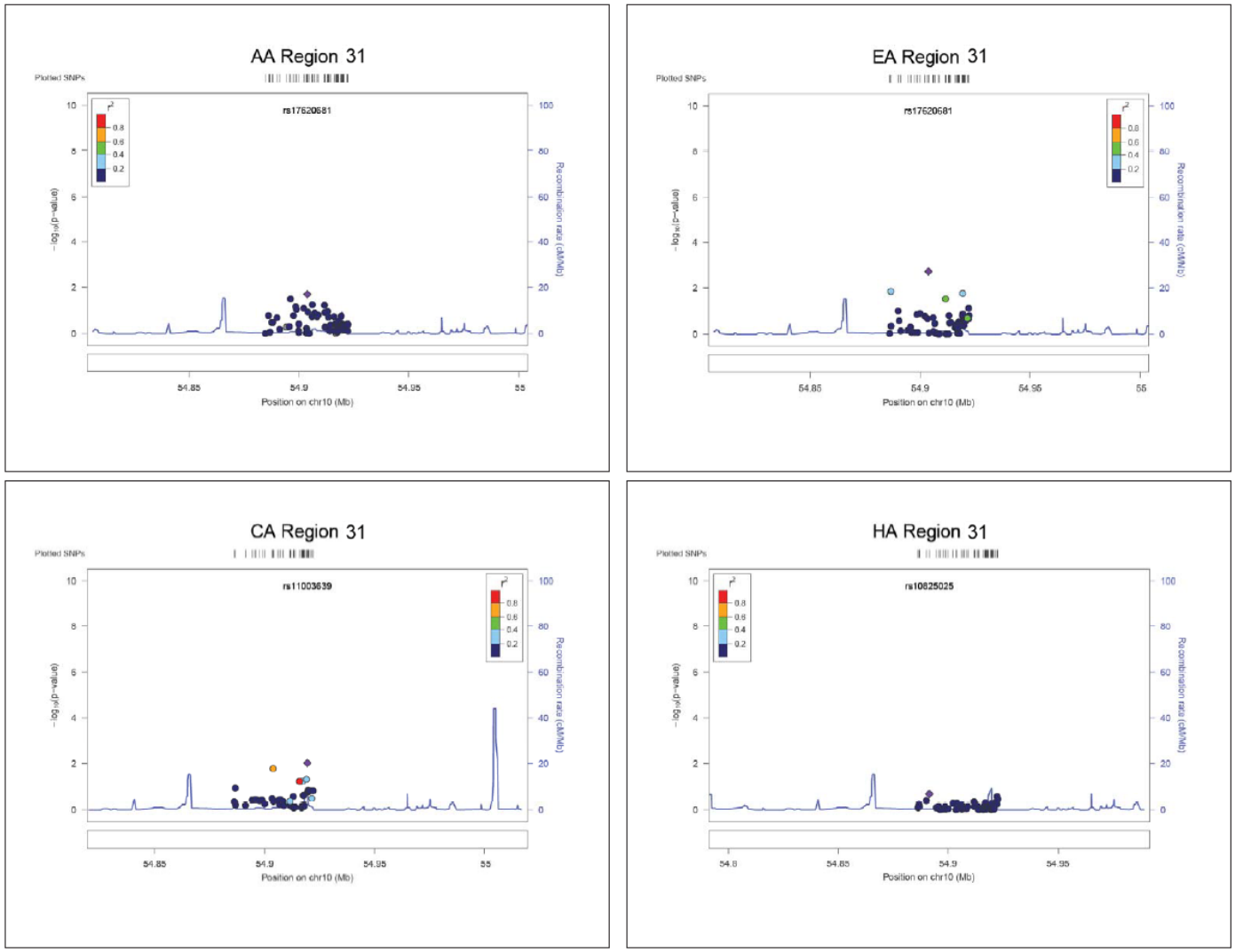




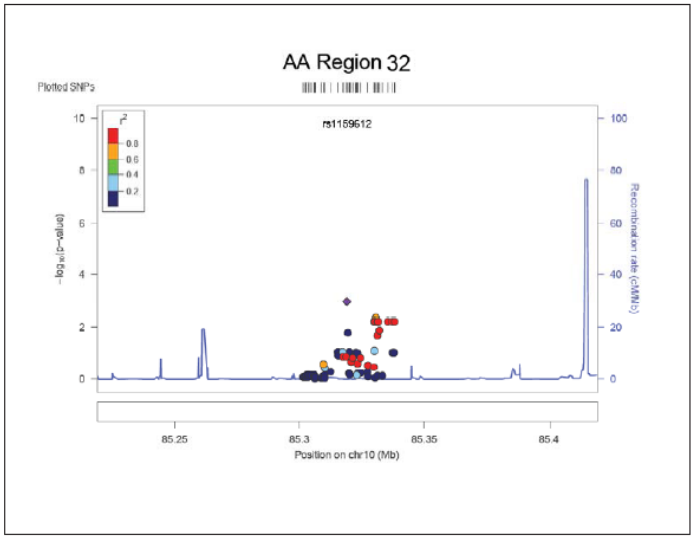
Appendix 50 LocusZoom plots for region 30, all ethnicities

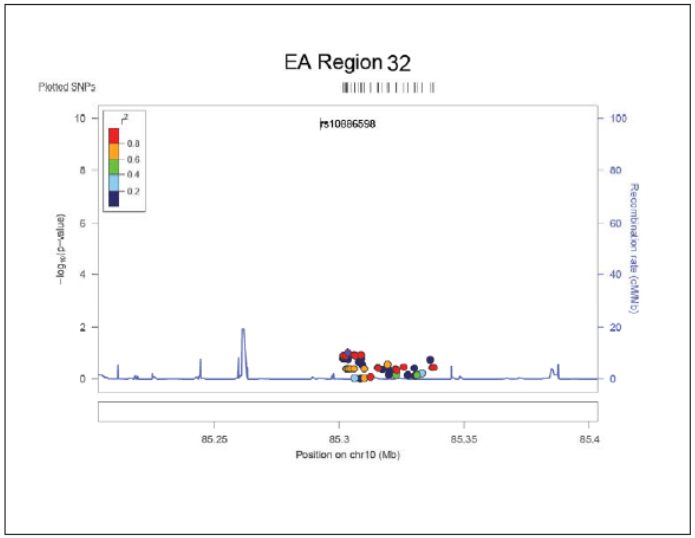


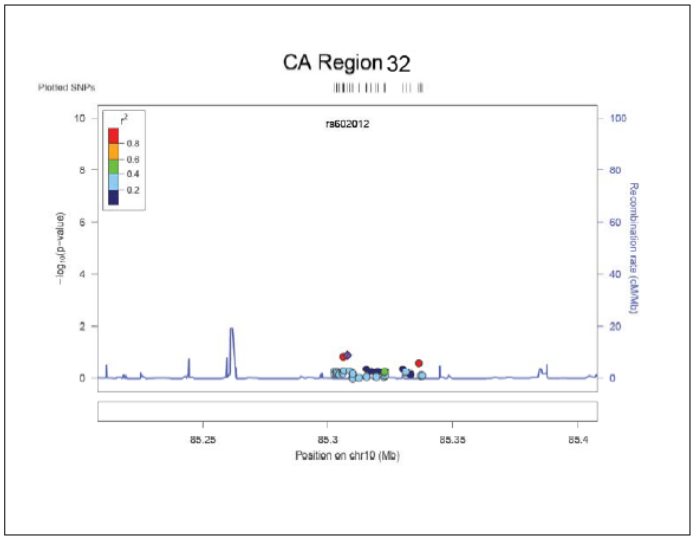
Appendix 51 LocusZoom plots for region 31, all ethnicities

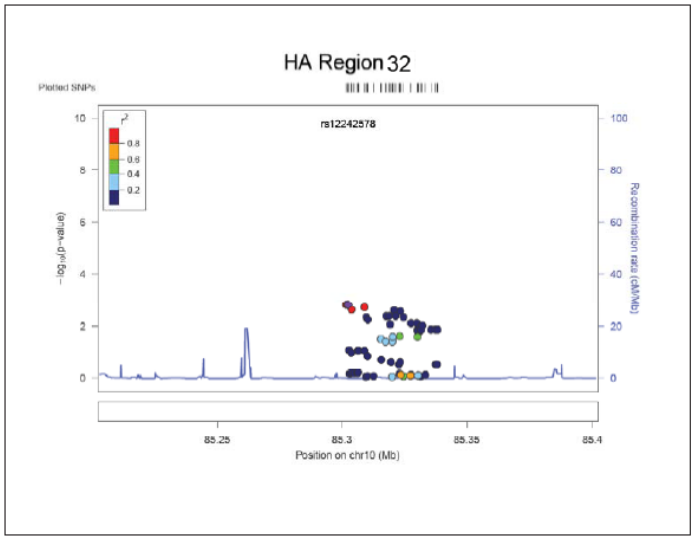


Appendix 52 LocusZoom plots for region 32, all ethnicities

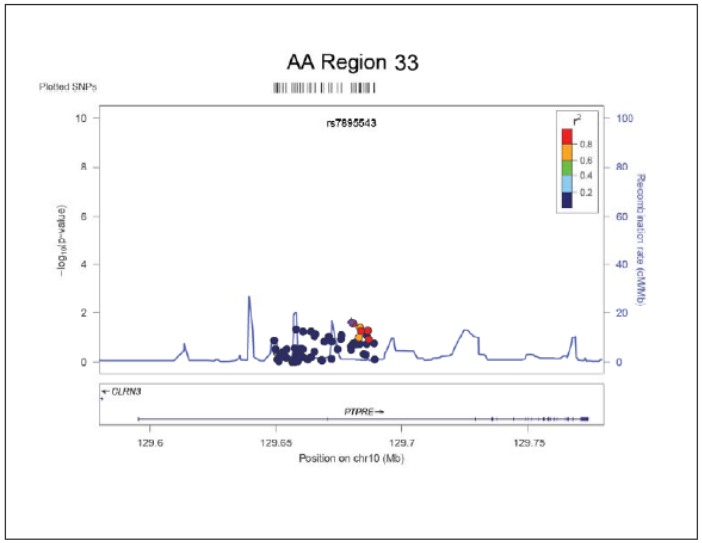


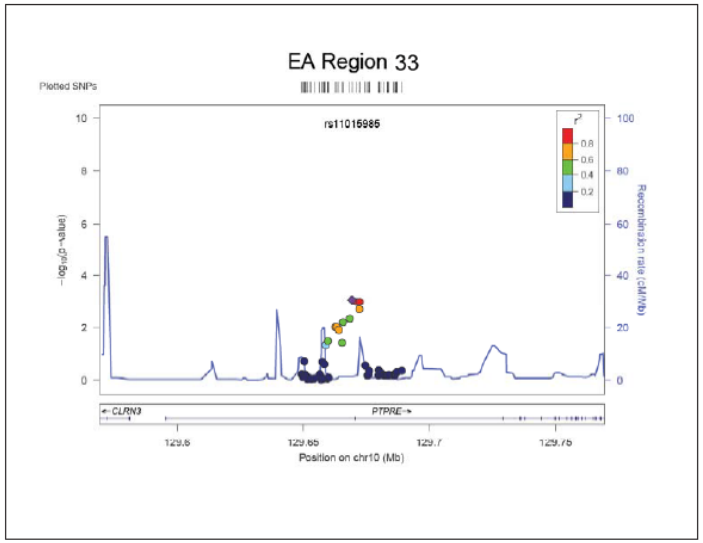


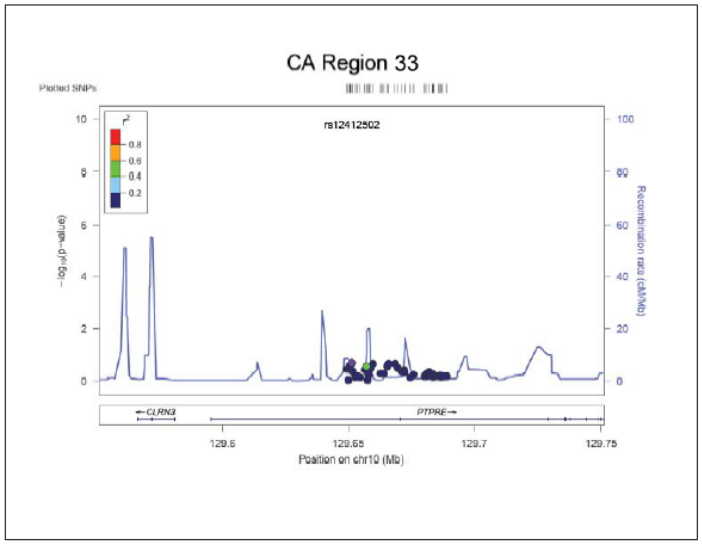


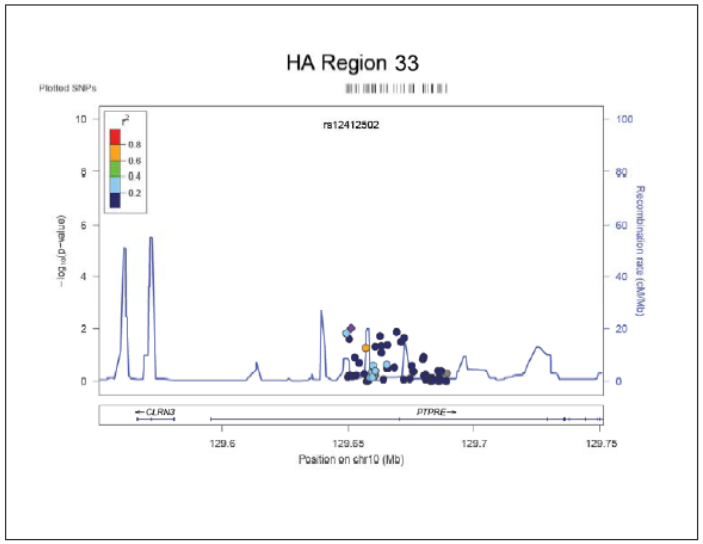


Appendix 53 LocusZoom plots for region 33, all ethnicities

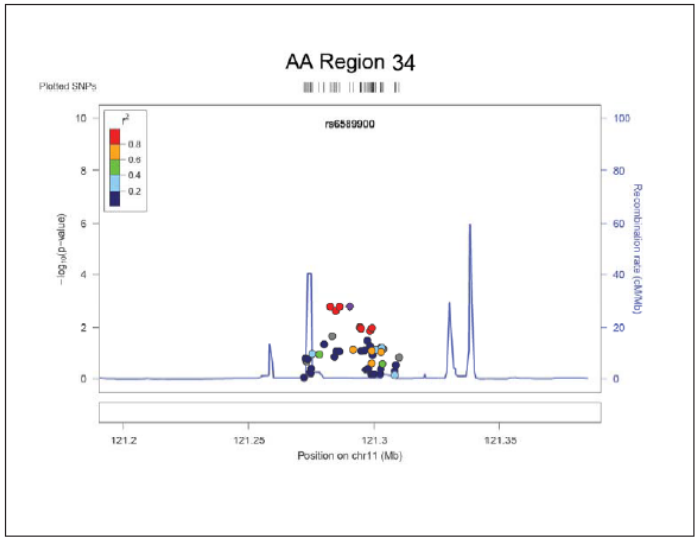


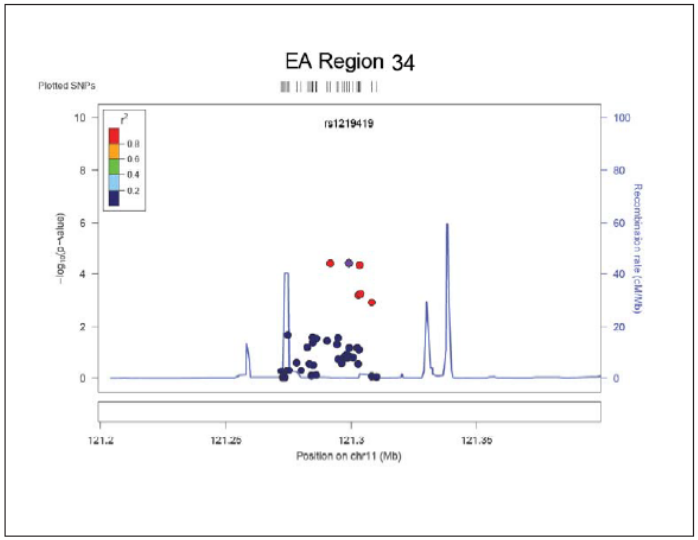


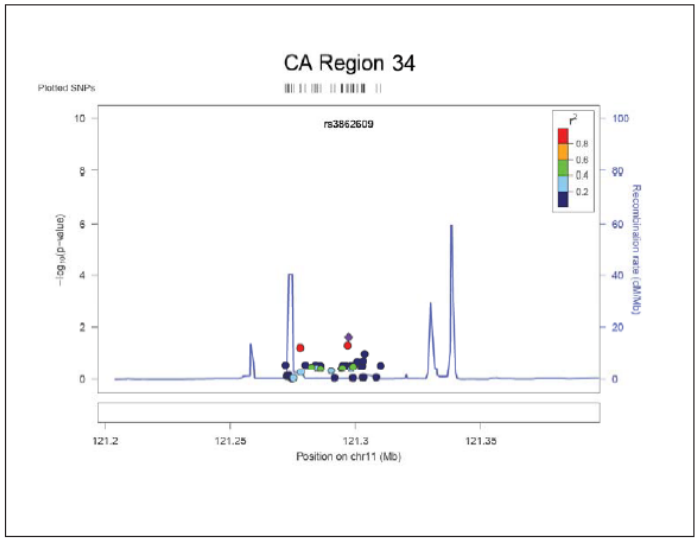


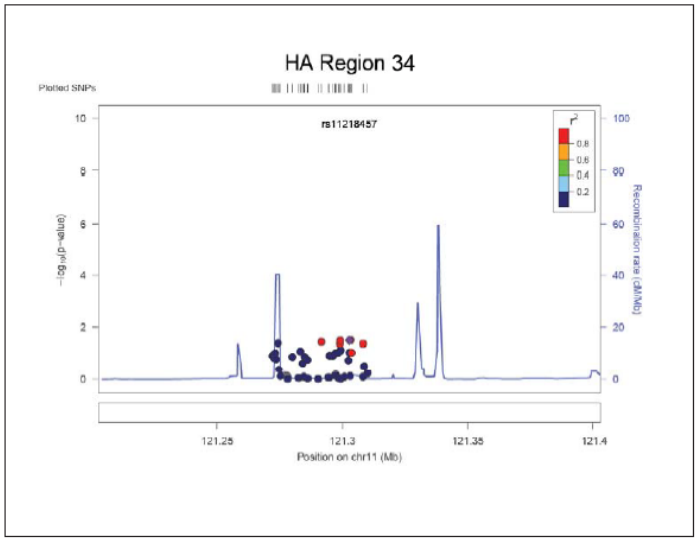


Appendix 54 LocusZoom plots for region 34, all ethnicities

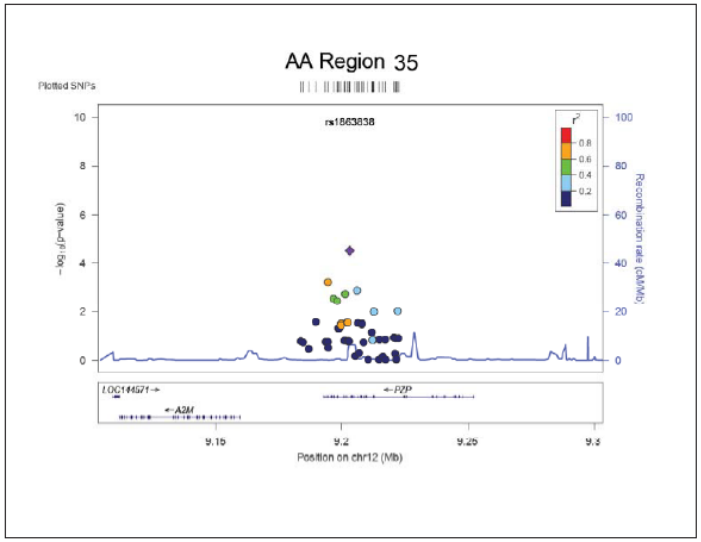


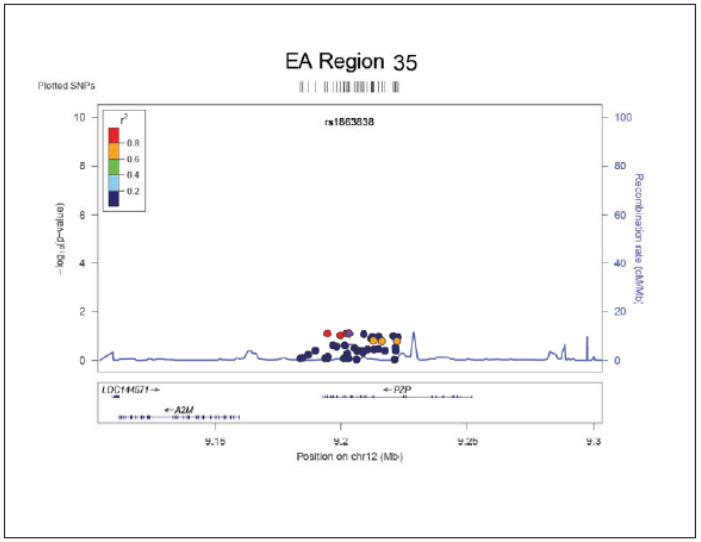


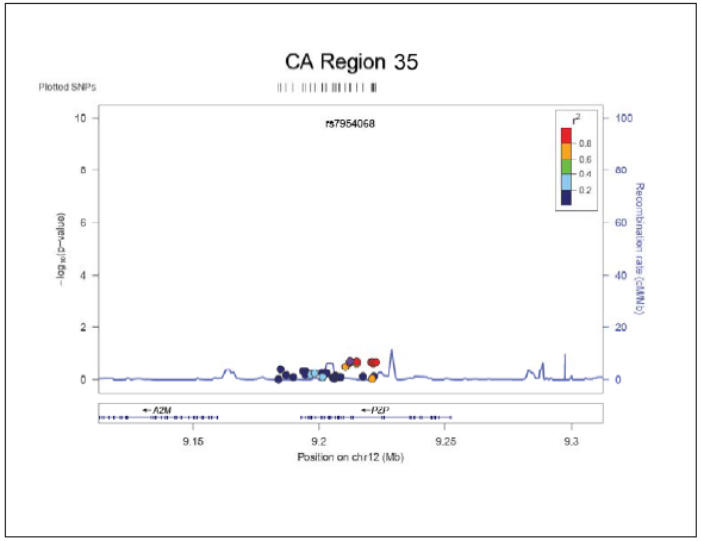


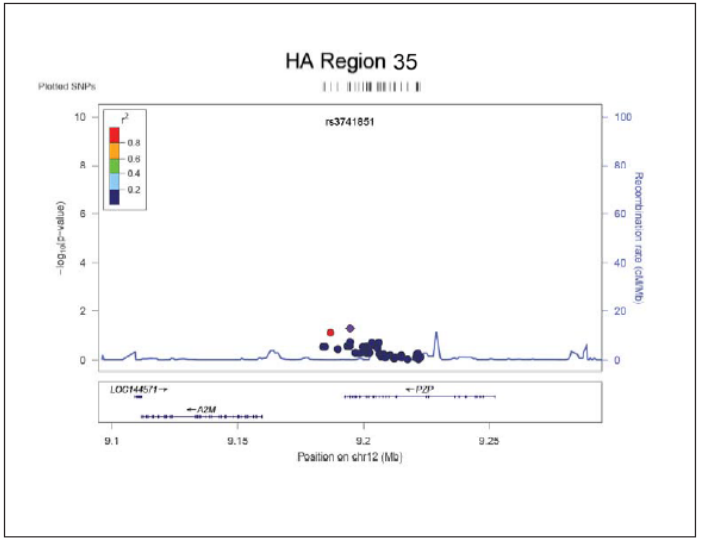


Appendix 55 LocusZoom plots for region 35, all ethnicities

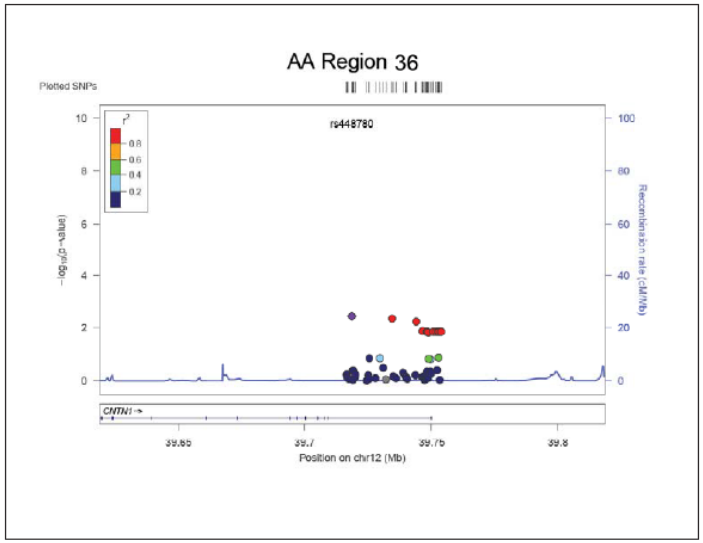


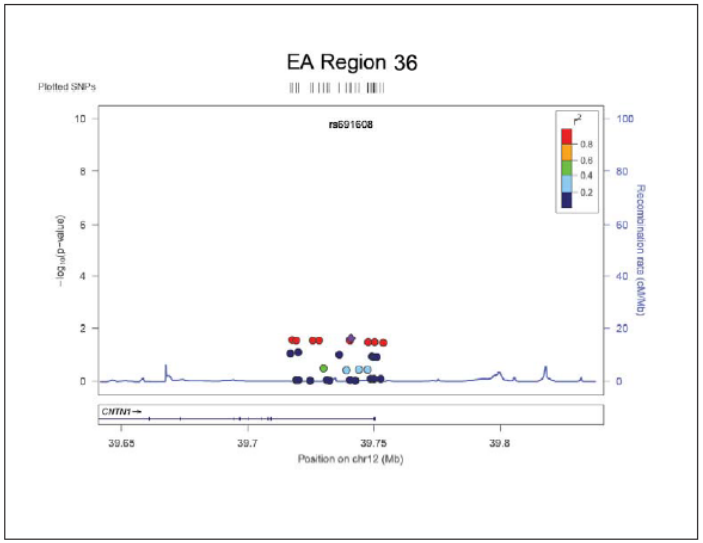


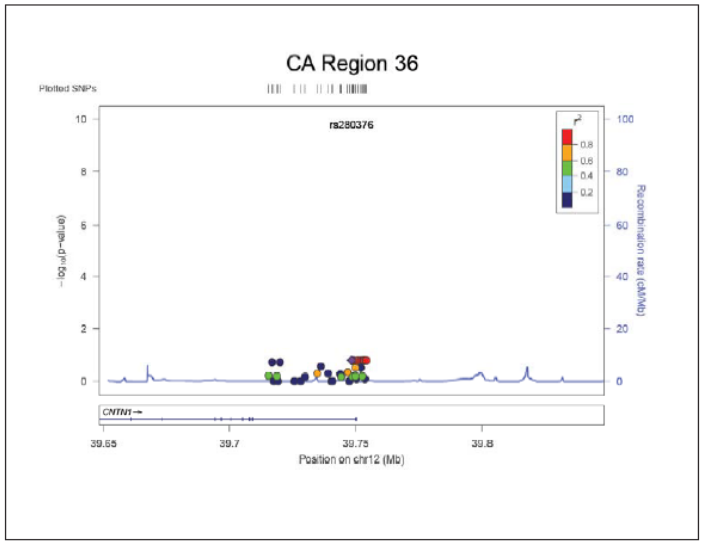


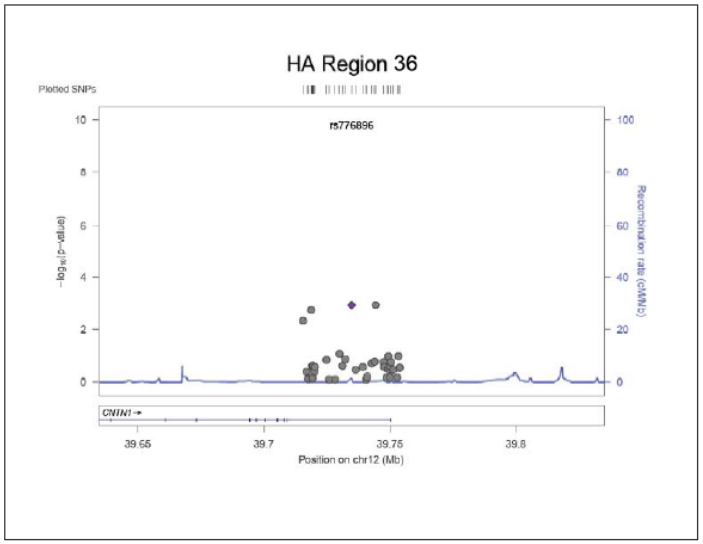


Appendix 56 LocusZoom plots for region 36, all ethnicities

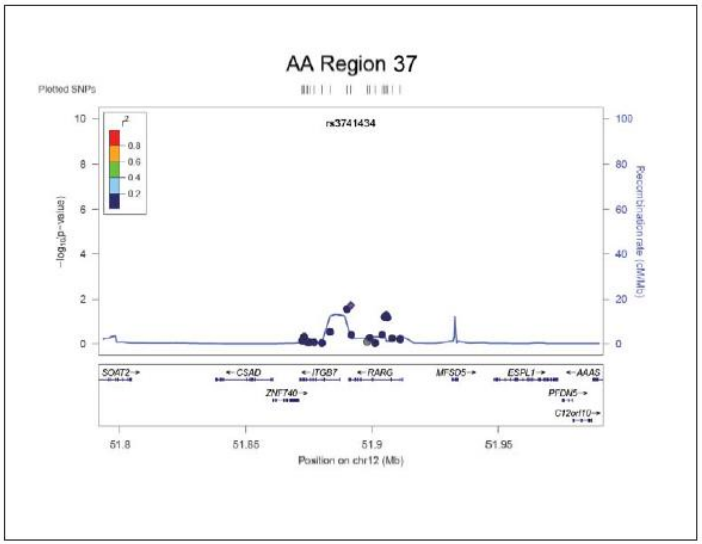


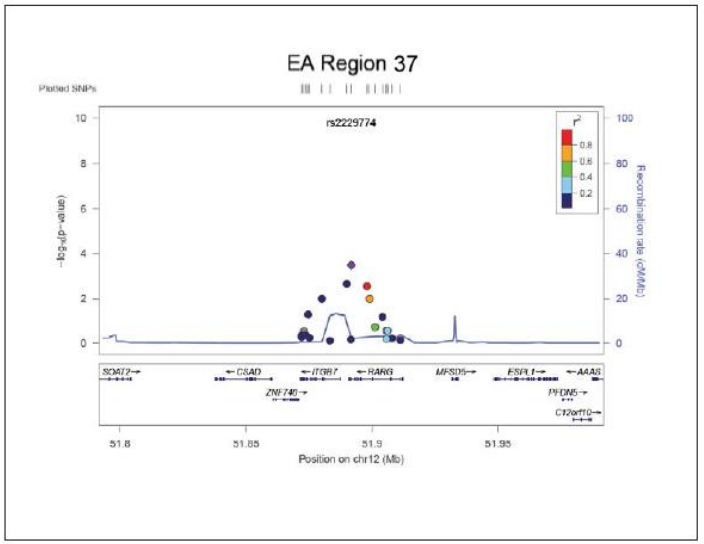


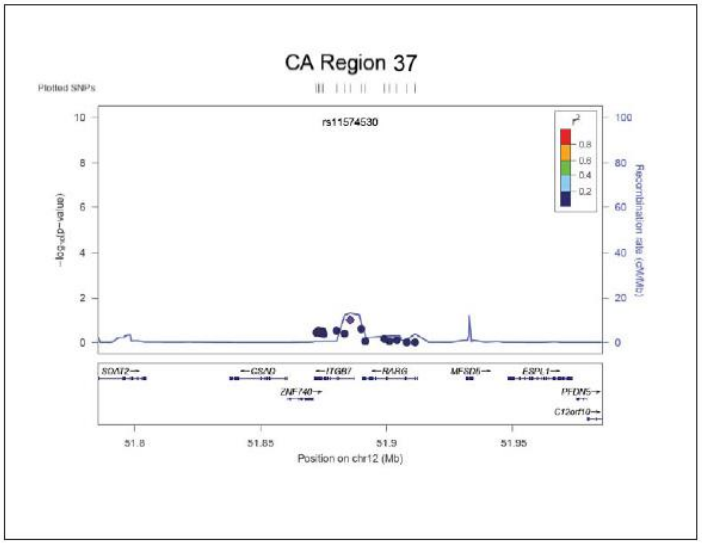


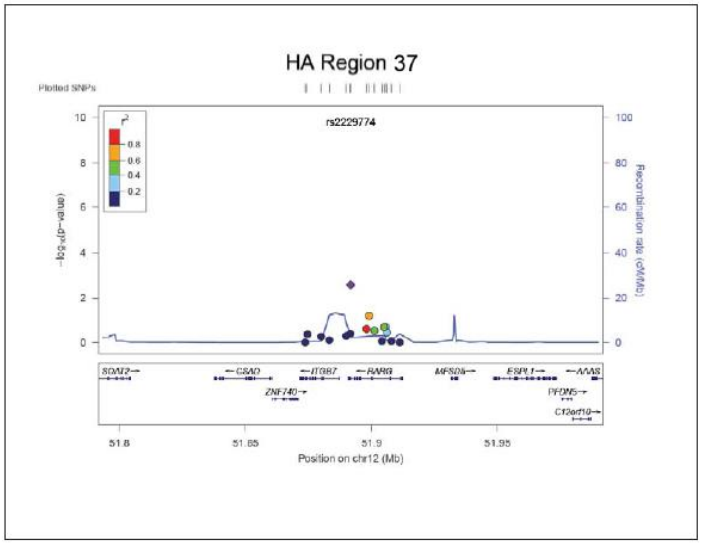


Appendix 57 LocusZoom plots for region 37, all ethnicities

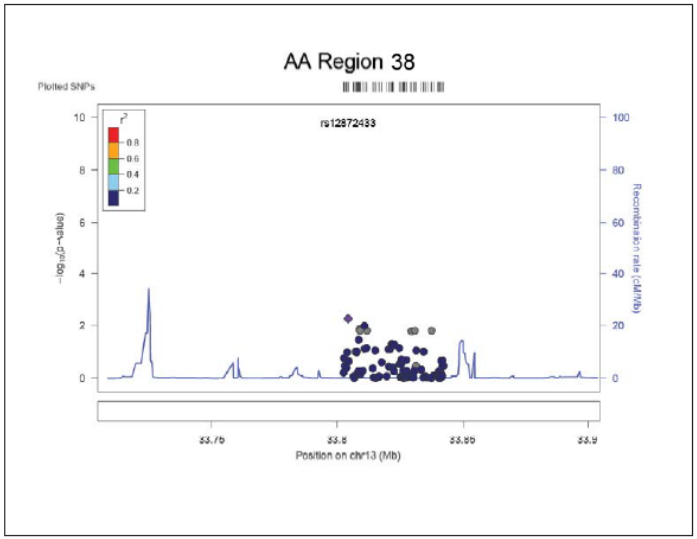


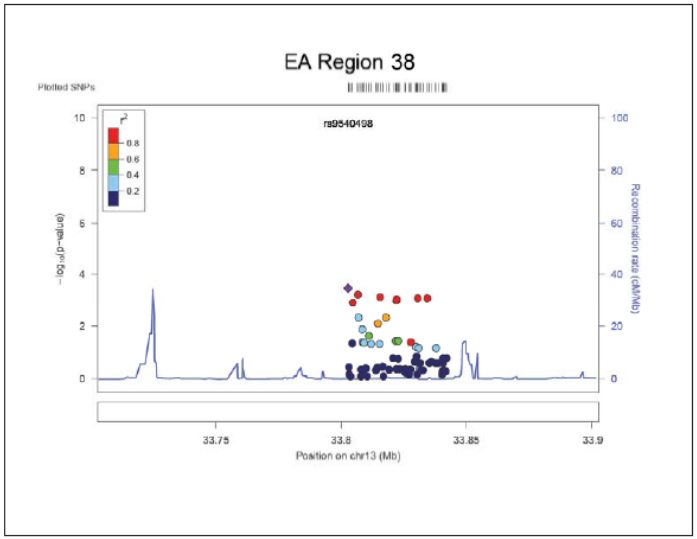


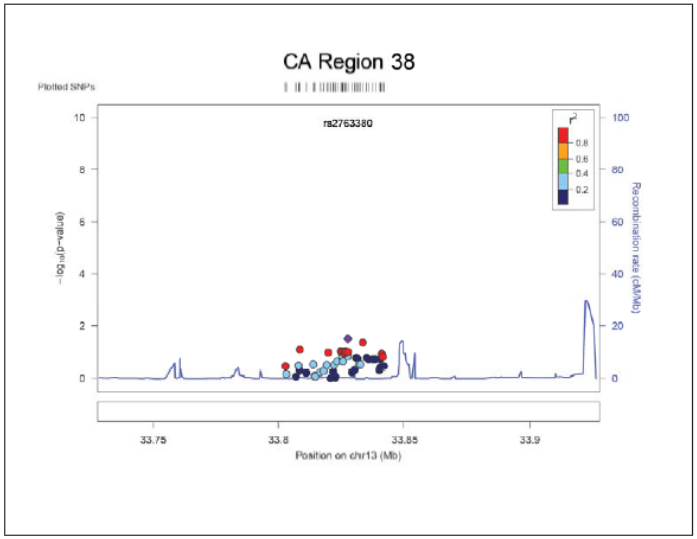


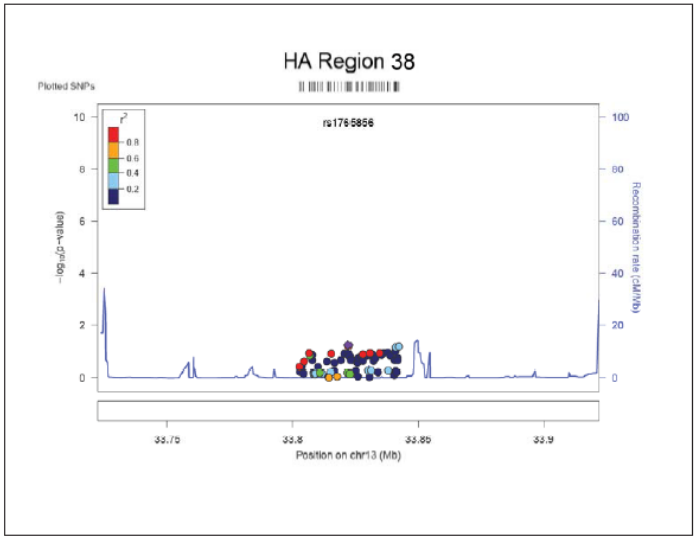


Appendix 58 LocusZoom plots for region 38, all ethnicities

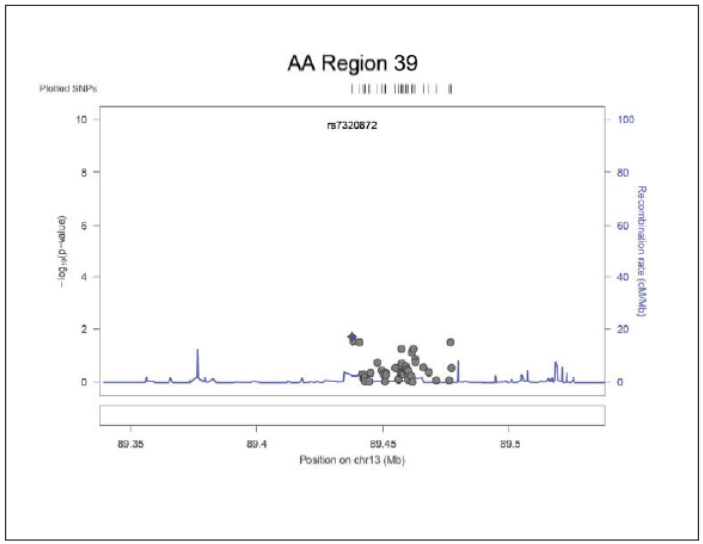


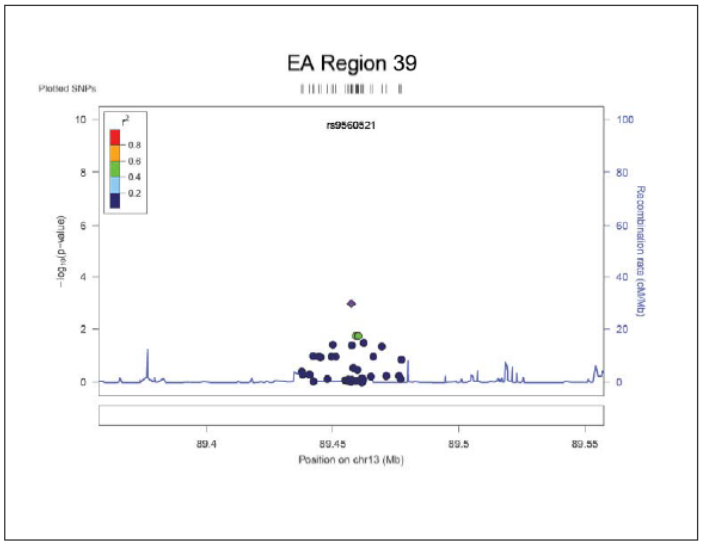


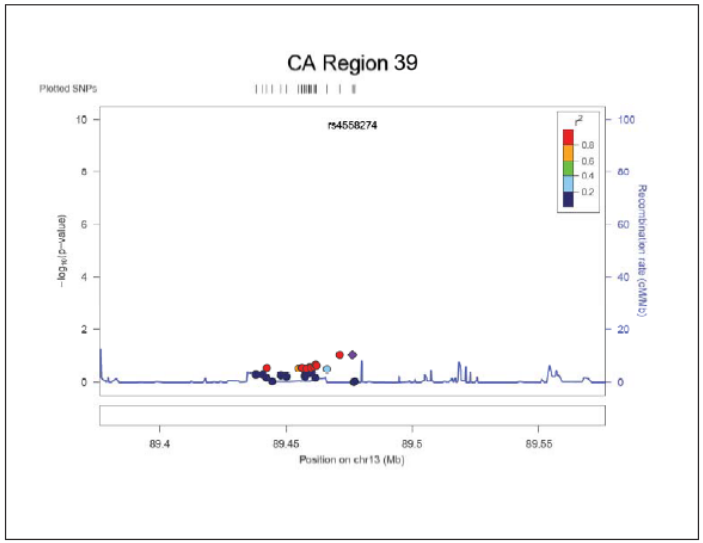


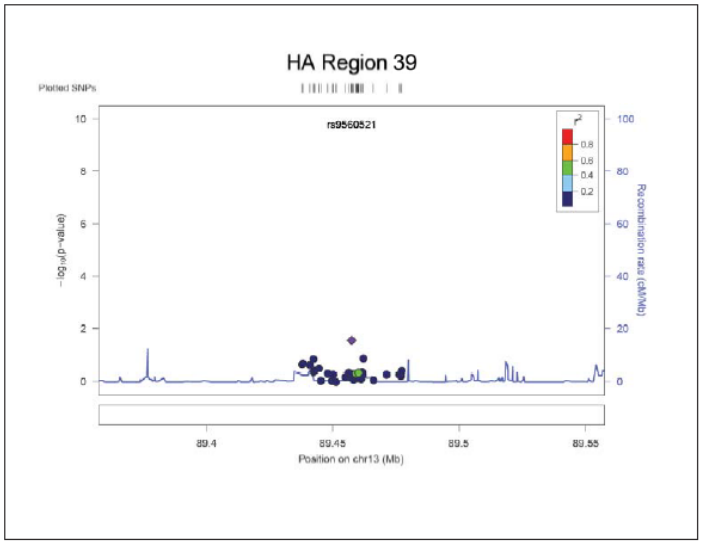


Appendix 59 LocusZoom plots for region 39, all ethnicities

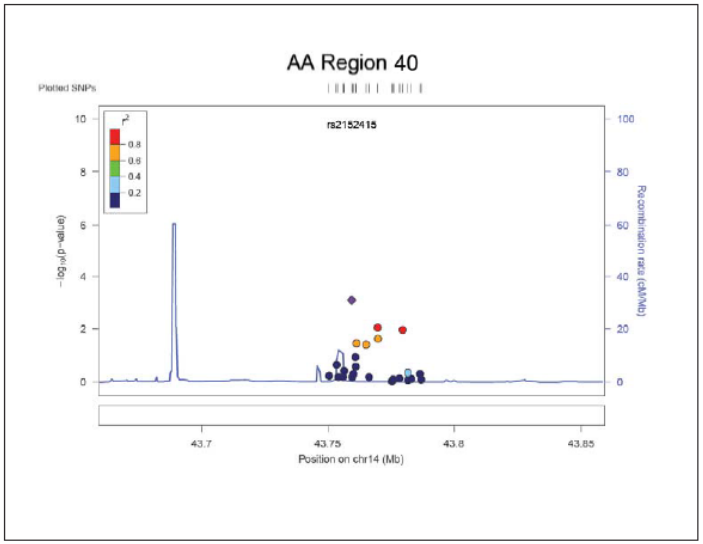


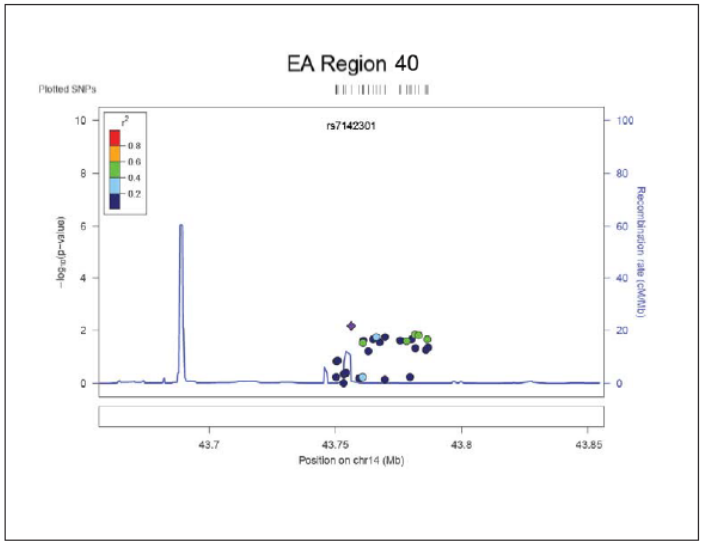


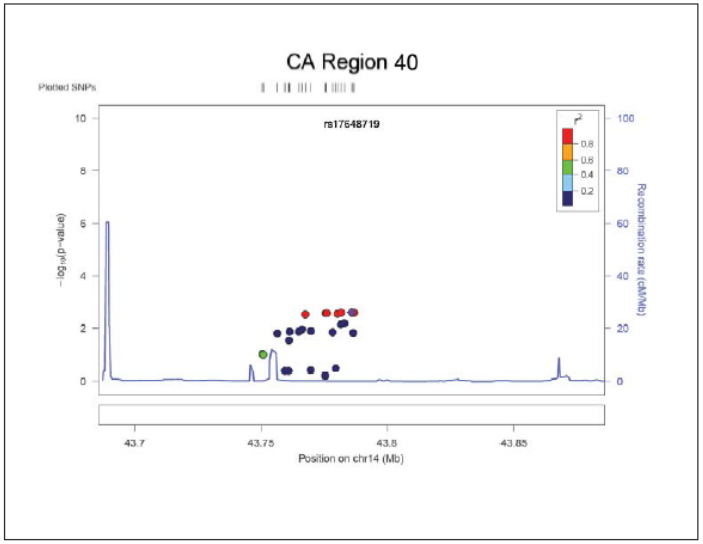


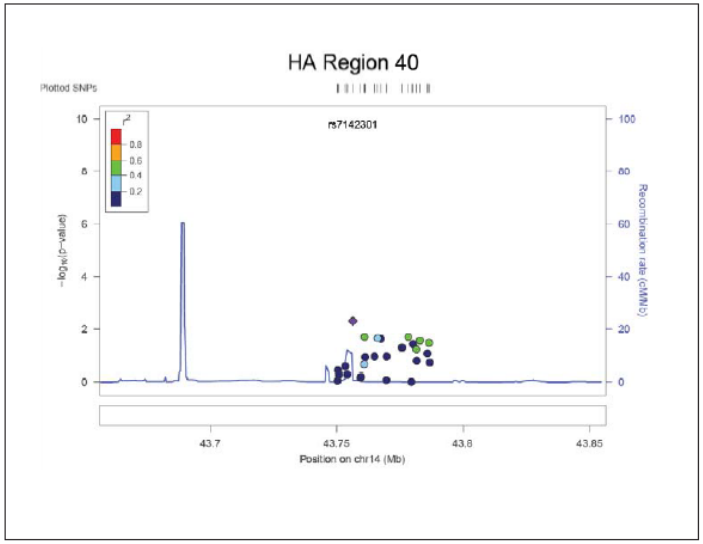


Appendix 60 LocusZoom plots for region 40, all ethnicities

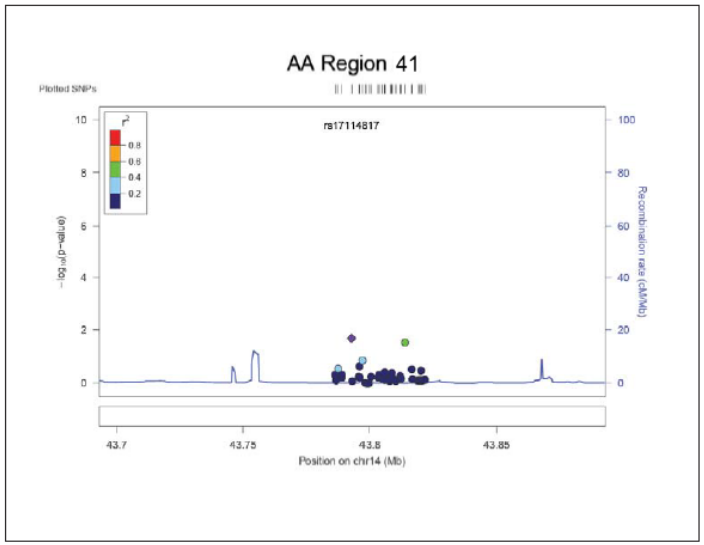


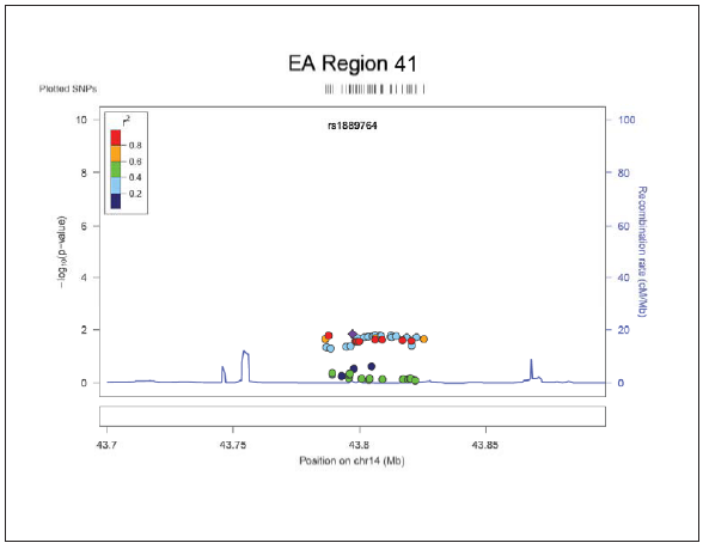


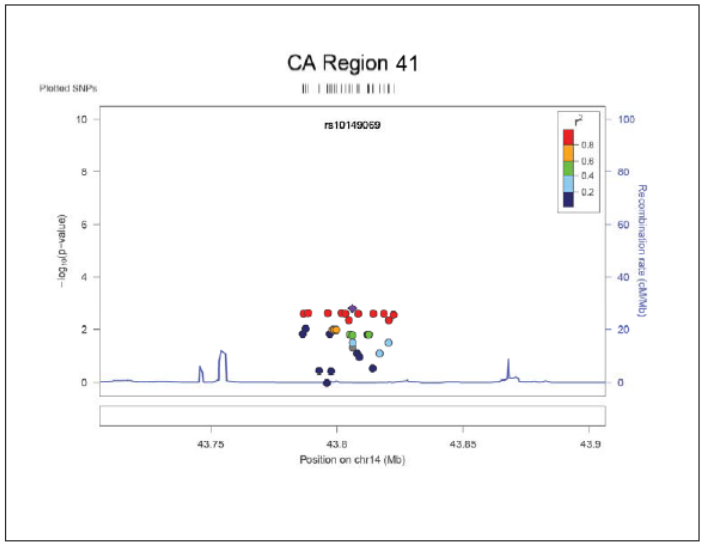


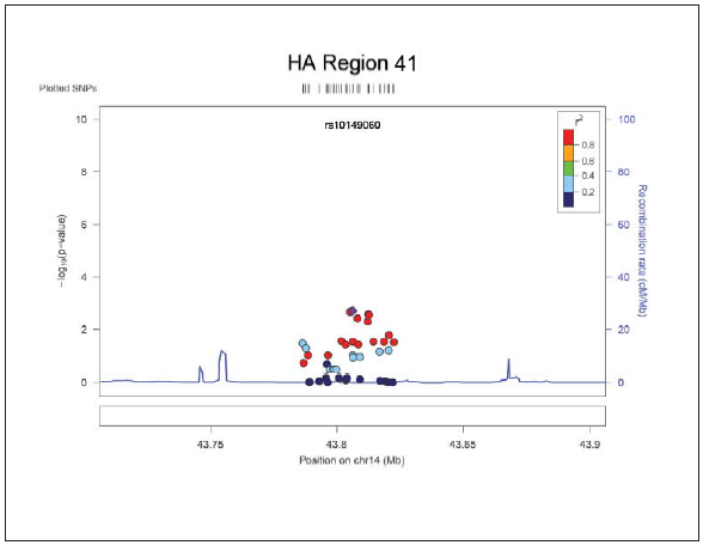


Appendix 61 LocusZoom plots for region 41, all ethnicities

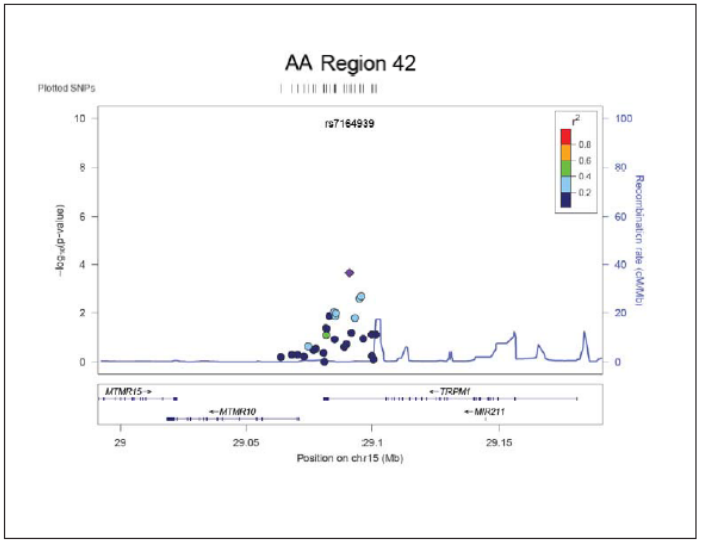


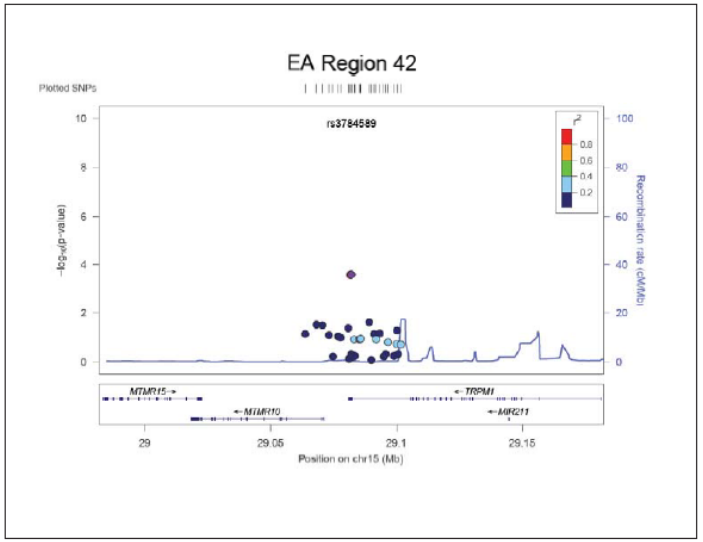


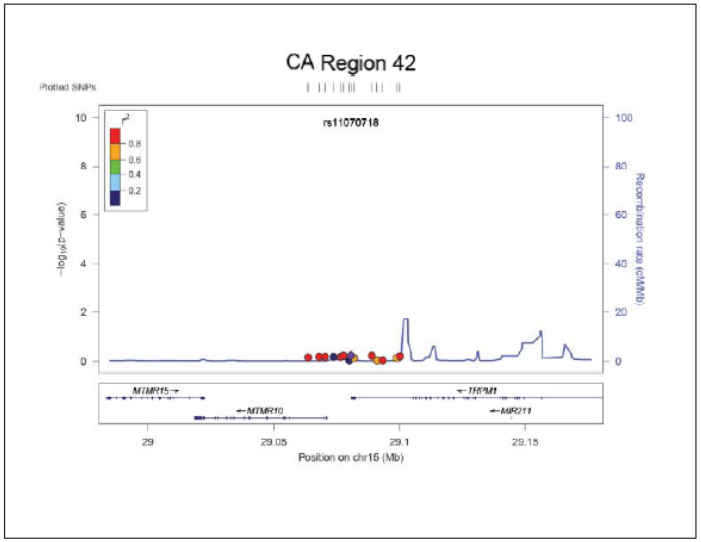


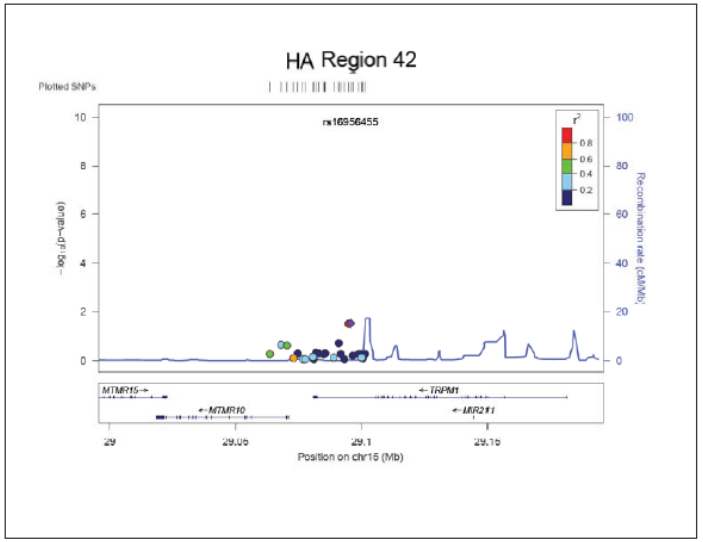


Appendix 62 LocusZoom plots for region 42, all ethnicities

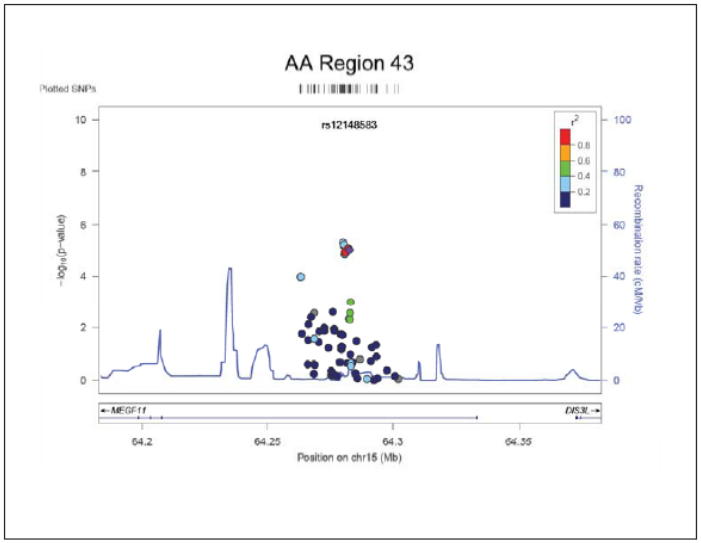


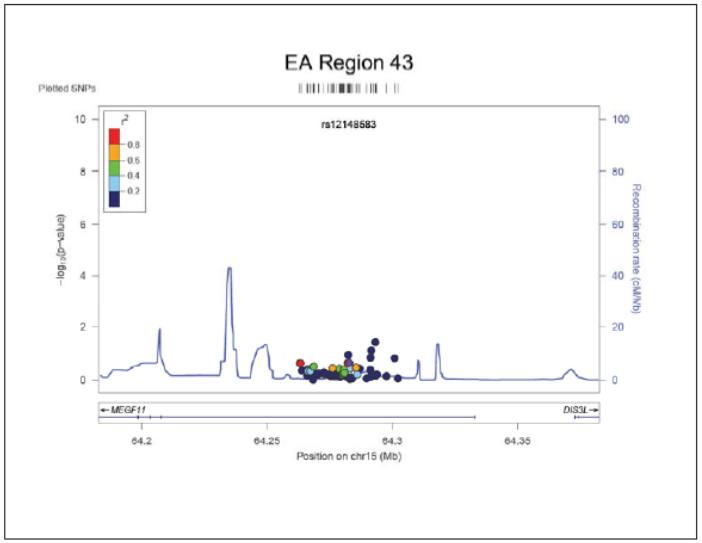


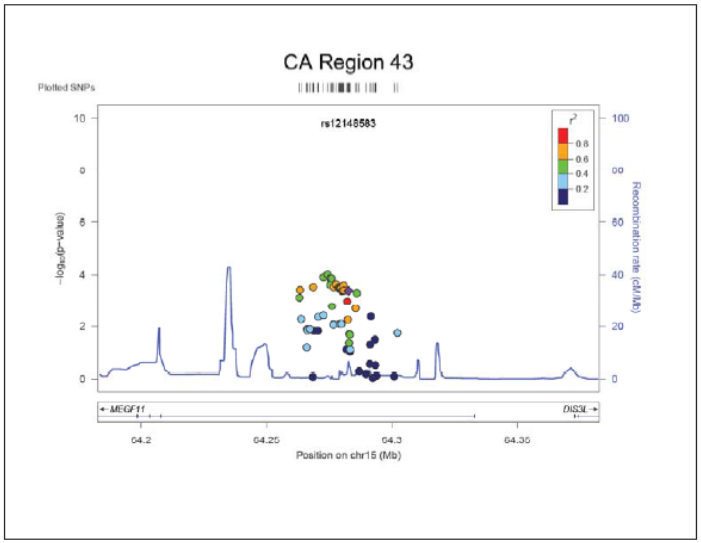


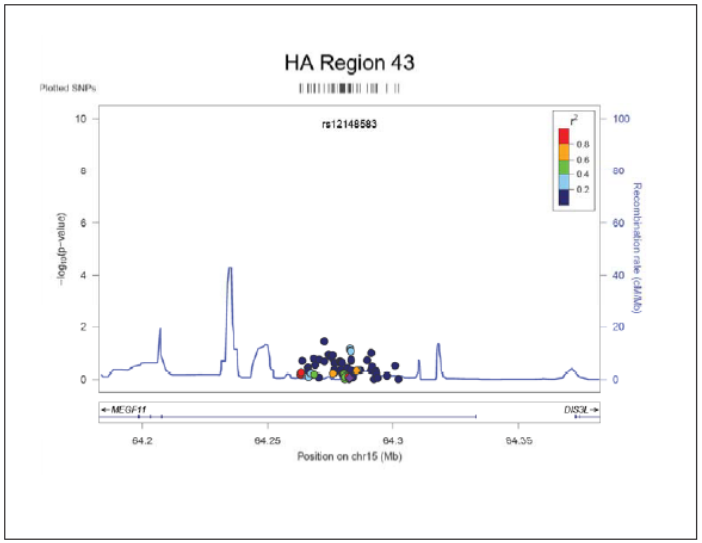


Appendix 63 LocusZoom plots for region 43, all ethnicities

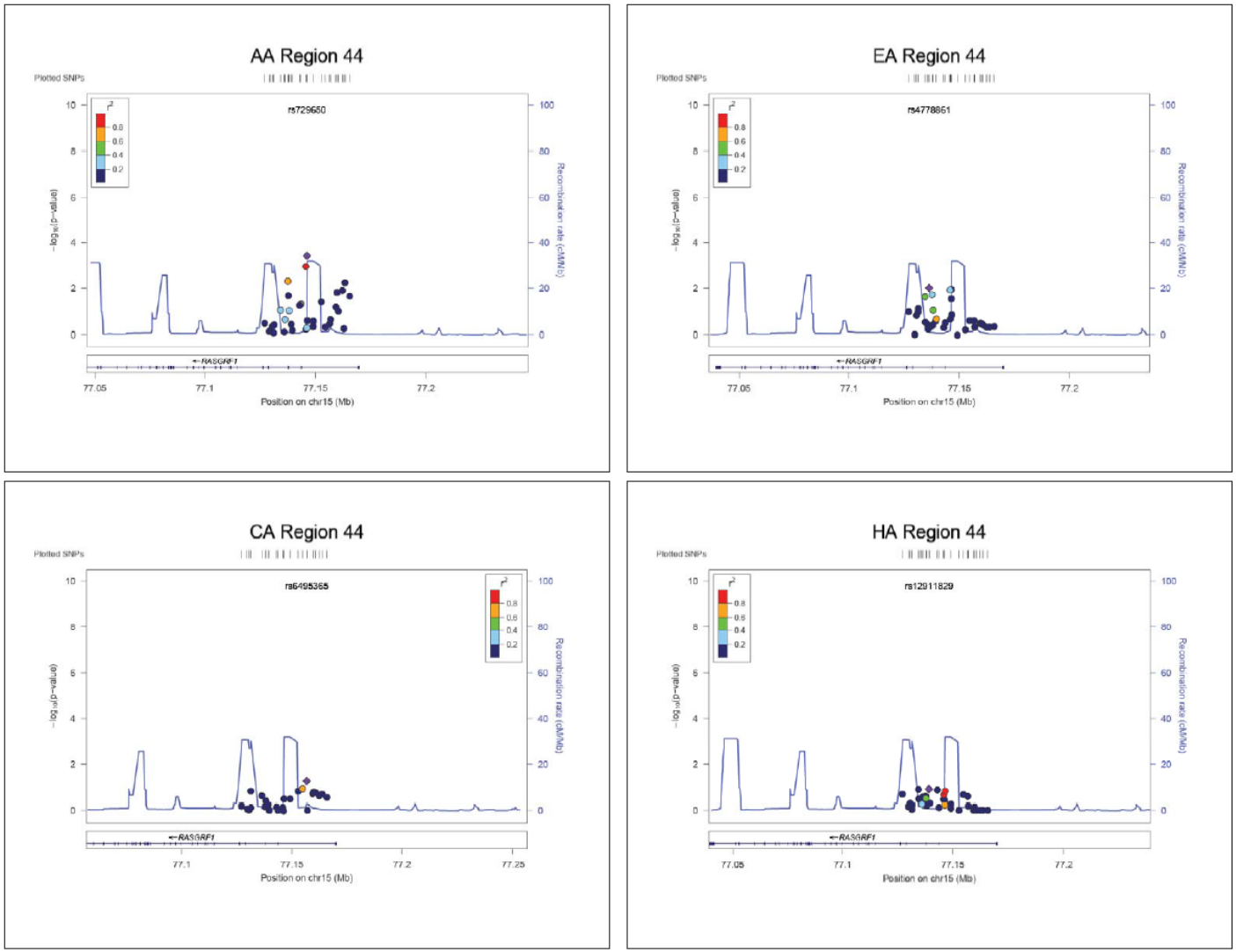




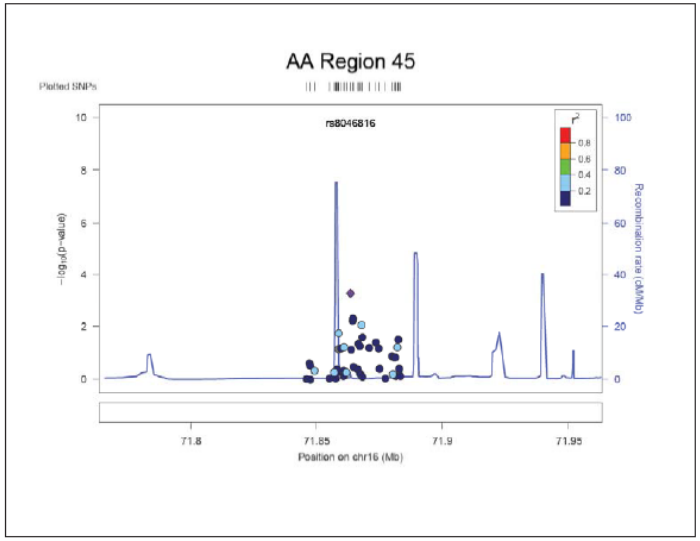


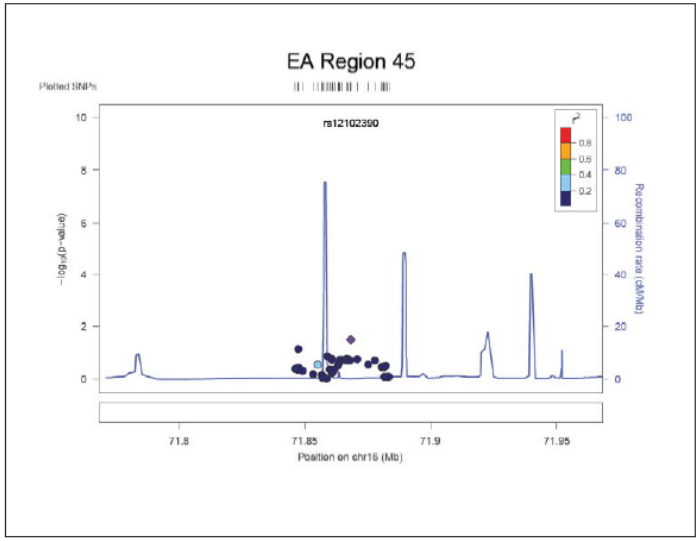


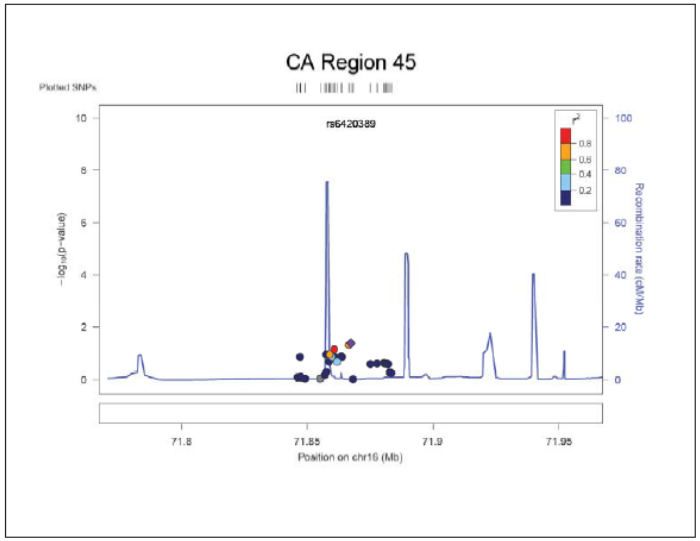
Appendix 64 LocusZoom plots for region 44, all ethnicities

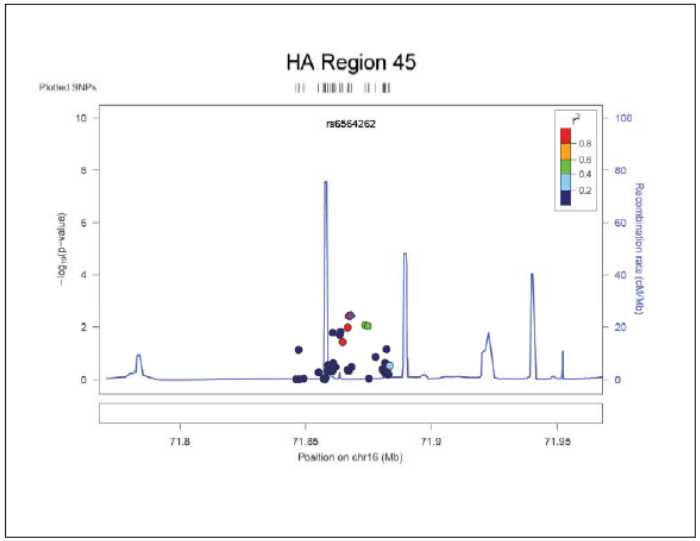


Appendix 65 LocusZoom plots for region 45, all ethnicities

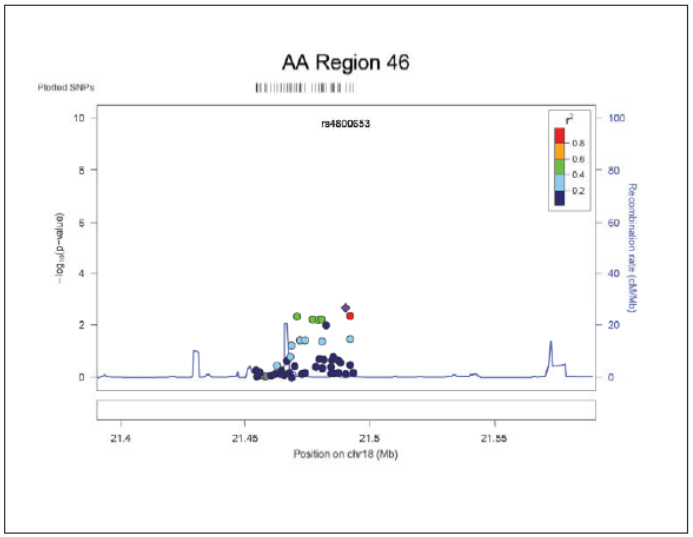


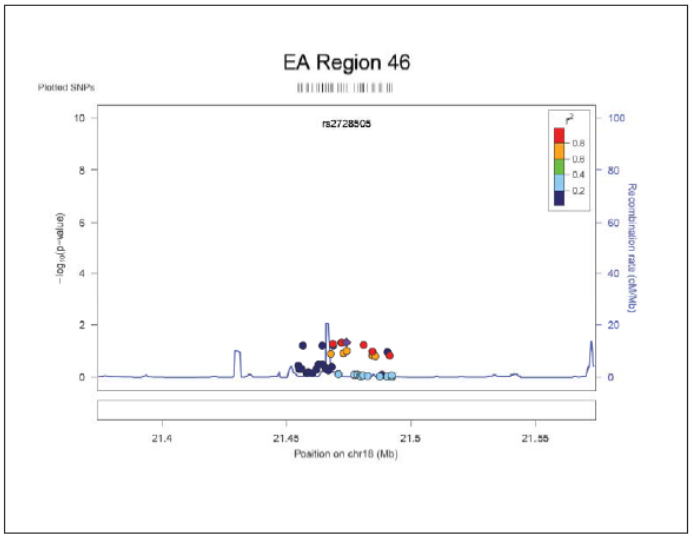


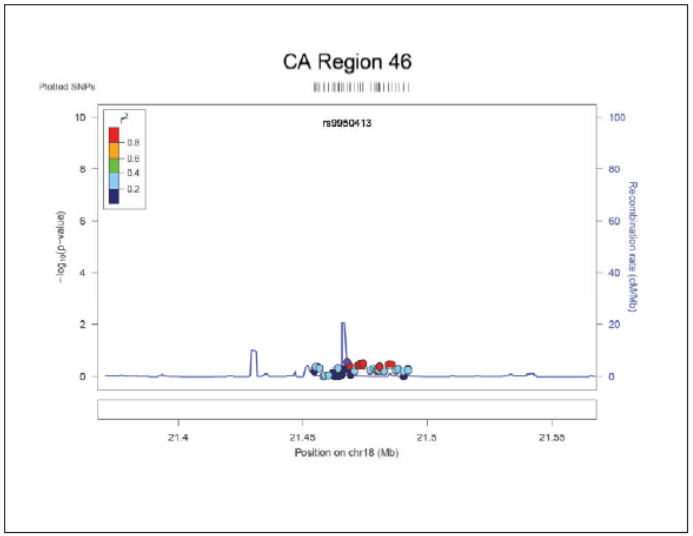


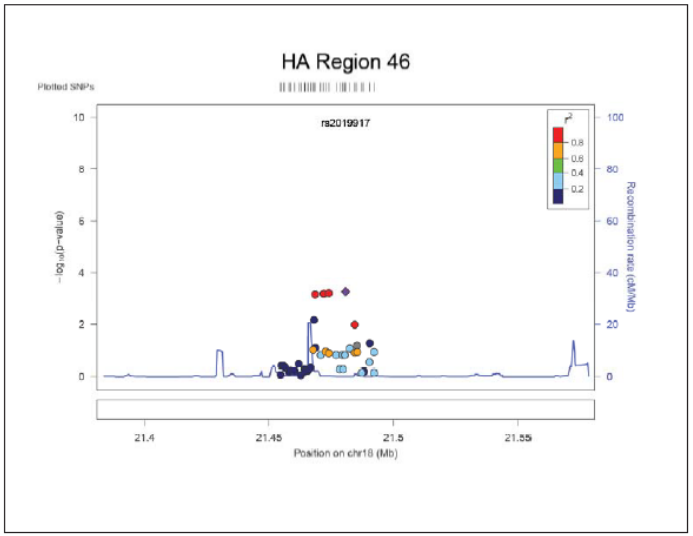


Appendix 66 LocusZoom plots for region 46, all ethnicities

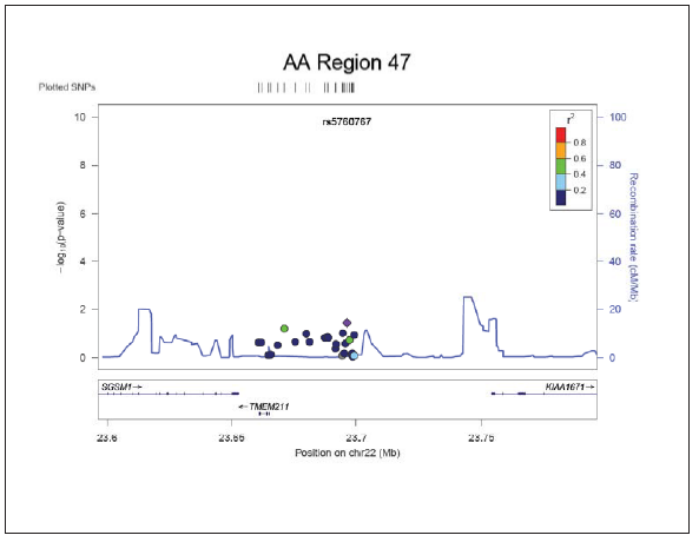


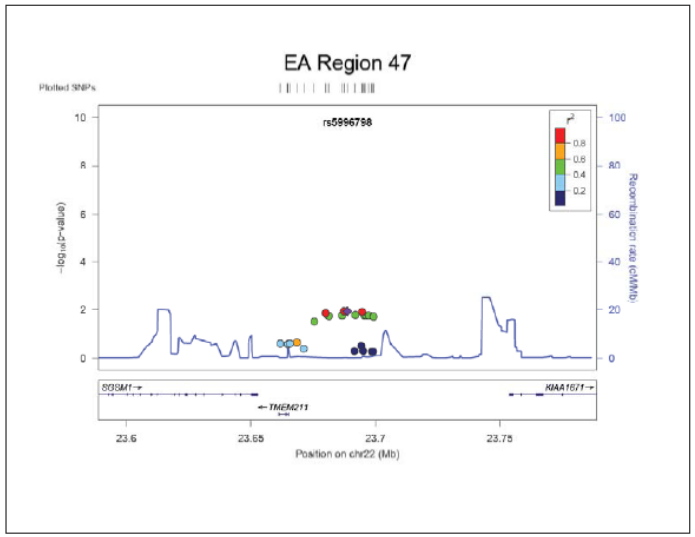


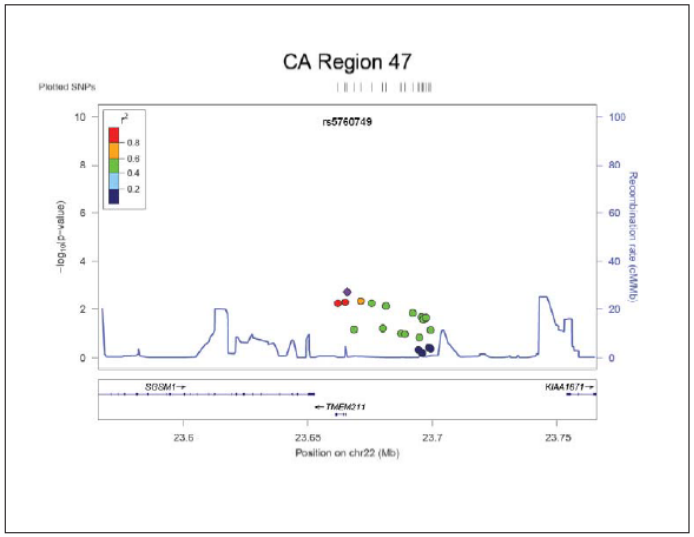


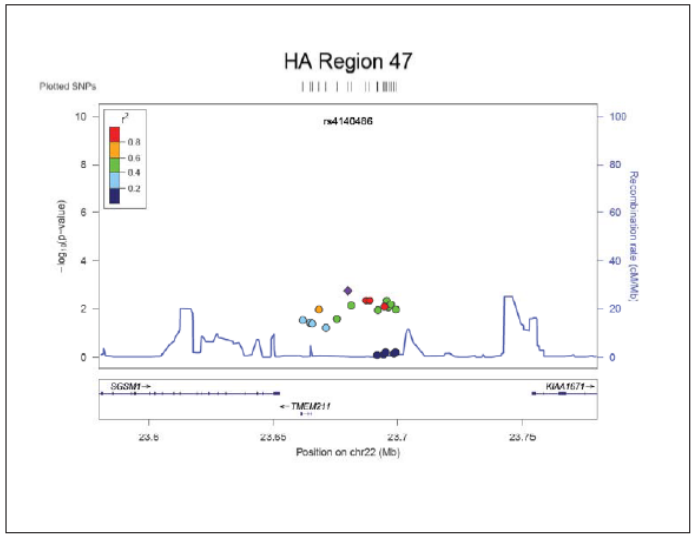


Appendix 67 LocusZoom plots for region 47, all ethnicities









Appendix 68 Fisher's combined p-values and ethnicity-specific p-values for each SNP in region 1

			AA		EA		CA		HA		Fisher's Summary Information		
SNP	BP	Coded Allele	n	p	n	p	n	p	n	p	X^2	df	X^2 pvalue
rs897630	2784009	A	1397	4.22E-01	2374	1.81E-03	747	1.19E-01	1379	5.89E-01	19.68	8	4.23E-03
rs897620	2777567	C	1396	9.84E-01	2374	1.41E-03	-	-	1379	-	13.15	4	4.59E-03
rs2842910	2779832	C	1397	8.46E-01	2374	3.59E-03	747	1.41E-01	1379	-	15.51	6	6.44E-03
rs2606406	2779766	A	1397	5.07E-01	2374	2.34E-03	747	1.88E-01	1378	5.38E-01	18.06	8	7.35E-03
rs2842914	2812006	A	1397	6.88E-01	2374	2.38E-03	746	1.24E-01	1379	5.99E-01	18.04	8	7.40E-03
rs750786	2795967	A	1397	9.97E-01	2374	2.34E-03	747	1.94E-01	1379	5.32E-01	16.67	8	1.16E-02
rs1456465	2783092	A	1397	8.34E-01	2374	3.59E-03	747	1.59E-01	1379	5.30E-01	16.57	8	1.20E-02
rs2842911	2799428	C	1397	6.45E-01	2374	3.59E-03	747	1.64E-01	1379	7.34E-01	16.37	8	1.27E-02
rs3100865	2807281	G	1397	7.41E-01	2374	1.86E-02	747	9.32E-01	1379	3.28E-02	15.55	8	1.65E-02
rs897631	2784330	C	1397	4.58E-01	2372	2.90E-02	747	1.34E-01	1376	-	12.67	6	1.78E-02
rs10909793	2787716	G	1396	4.46E-01	2374	4.32E-01	747	1.29E-02	1377	-	11.99	6	2.24E-02
rs880725	2786479	A	1397	4.54E-01	2374	3.54E-02	747	2.05E-01	1379	-	11.43	6	2.69E-02
rs11580768	2778968	C	1397	6.41E-01	2373	7.76E-01	747	1.38E-02	1377	-	9.96	6	4.26E-02
rs6676289	2810832	C	1397	5.79E-01	2374	-	747	4.53E-02	1379	-	7.28	4	4.78E-02
rs2445620	2791319	C	1397	7.88E-01	2370	1.54E-02	747	7.37E-01	1378	-	9.43	6	4.98E-02
rs1563474	2801674	A	1397	3.74E-01	2373	8.55E-01	746	2.97E-02	1378	-	9.31	6	5.15E-02
rs6673503	2808493	C	1395	9.05E-01	2374	5.26E-01	746	1.00E-02	1376	7.53E-01	11.25	8	5.35E-02
rs1563472	2796750	A	1397	8.36E-01	2373	8.55E-01	746	1.89E-02	1377	-	8.61	6	6.26E-02
rs10797342	2785861	C	1395	8.68E-01	2374	8.54E-01	744	2.05E-02	1376	-	8.37	6	6.67E-02
rs2168531	2788317	C	1397	7.90E-01	2374	8.33E-01	745	1.24E-02	1378	8.28E-01	10.00	8	7.02E-02
rs12091184	2813319	C	1397	7.62E-01	2373	7.02E-01	746	2.16E-01	1379	9.86E-01	4.34	8	9.72E-02
rs2045333	2776005	C	1397	7.25E-01	2373	4.77E-01	747	-	1379	-	2.12	6	9.75E-02
rs12063033	2813185	A	1387	8.68E-01	2336	9.10E-01	720	4.35E-02	1369	-	6.74	6	9.76E-02
rs897615	2787126	C	1396	6.70E-01	2374	9.99E-01	746	5.63E-02	1378	5.53E-01	7.74	8	1.01E-01
rs12035436	2789340	G	1396	7.52E-01	2373	7.02E-01	747	2.05E-01	1379	8.01E-01	4.89	8	1.06E-01
rs10910019	2784411	C	1397	5.16E-01	2365	9.60E-01	747	8.30E-02	1377	7.62E-01	6.93	8	1.08E-01
rs1563469	2812608	A	1396	3.64E-01	2374	8.84E-01	747	1.19E-01	1379	9.22E-01	6.69	8	1.10E-01
rs2124661	2788136	C	1397	7.33E-01	2374	5.47E-01	747	5.34E-01	1379	-	3.09	6	1.27E-01

Region 1 is located on Chromosome 1, $\alpha_{Bonferroni} = 0.001$, - indicates a model that did not converge

Only SNPs with ethnicity-specific minor allele frequency > 5% were included in the Fisher's combined analysis, SNP: single nucleotide polymorphism, BP: basepair position in kilobases, AA: African American, EA: European American, CA: Chinese American, HA: Hispanic American, n: sample size, p: p-value, df: degrees of freedom

Appendix 69 Fisher's combined p-values and ethnicity-specific p-values for each SNP in region 29

			AA		EA		CA		HA		Fisher's Summary Information		
SNP	BP	Coded Allele	n	p	n	p	n	p	n	p	X^2	df	X^2 pvalue
rs10086664	78211979	C	1402	3.55E-02	2376	4.57E-03	745	1.09E-01	1378	8.45E-01	22.23	8	8.27E-05
rs1452832	78236750	C	1402	1.85E-02	2376	1.15E-02	747	1.23E-01	1378	9.58E-01	21.19	8	1.33E-04
rs10093959	78213199	A	1402	5.48E-02	2376	5.07E-03	747	1.53E-01	1378	6.21E-01	21.09	8	1.39E-04
rs9657123	78243575	C	1400	2.43E-02	2376	1.57E-02	747	1.64E-01	1378	9.14E-01	19.53	8	2.80E-04
rs1993196	78211752	A	1402	2.91E-02	2376	1.61E-02	747	1.53E-01	1378	9.54E-01	19.18	8	3.28E-04
rs10102542	78243564	C	1402	3.19E-02	2376	1.57E-02	747	1.64E-01	1378	9.39E-01	18.94	8	3.65E-04
rs10504647	78220362	C	1402	3.19E-02	2376	1.57E-02	747	1.64E-01	1378	9.39E-01	18.94	8	3.65E-04
rs12680110	78225266	C	1402	3.19E-02	2376	1.57E-02	747	1.64E-01	1378	9.39E-01	18.94	8	3.65E-04
rs7016358	78246553	A	1402	3.19E-02	2376	1.57E-02	747	1.64E-01	1378	9.39E-01	18.94	8	3.65E-04
rs10957840	78235557	C	1402	3.33E-02	2376	1.61E-02	747	1.53E-01	1378	9.55E-01	18.91	8	3.70E-04
rs13250484	78245009	A	1402	3.33E-02	2376	1.61E-02	747	1.53E-01	1378	9.55E-01	18.91	8	3.70E-04
rs13269867	78219857	A	1402	3.33E-02	2376	1.61E-02	747	1.53E-01	1378	9.55E-01	18.91	8	3.70E-04
rs7821262	78208512	G	1402	3.33E-02	2376	1.61E-02	747	1.53E-01	1378	9.55E-01	18.91	8	3.70E-04
rs13439699	78220079	C	1402	3.70E-02	2376	7.14E-02	747	1.97E-01	1378	8.87E-01	15.36	8	1.77E-03
rs12056492	78225217	A	1402	4.36E-02	2376	6.26E-02	747	1.97E-01	1378	8.78E-01	15.32	8	1.81E-03
rs1377248	78232477	C	1402	3.70E-02	2376	6.99E-02	747	2.11E-01	1378	9.03E-01	15.23	8	1.88E-03
rs13259366	78225474	A	1402	4.16E-02	2376	7.14E-02	747	1.97E-01	1378	8.99E-01	15.1	8	1.99E-03
rs12056333	78212369	C	1402	4.76E-02	2376	7.14E-02	747	1.97E-01	1378	8.78E-01	14.88	8	2.18E-03
rs10282777	78237061	C	1402	2.22E-02	2376	5.96E-02	747	5.61E-01	1378	8.64E-01	14.7	8	2.36E-03
rs7832753	78242401	A	1402	1.19E-02	2376	1.35E-01	747	8.00E-01	1378	7.57E-01	13.86	8	3.39E-03
rs11996389	78226809	C	1402	3.85E-02	2376	1.42E-01	747	6.66E-01	1378	5.27E-01	12.51	8	6.01E-03
rs1377247	78222909	A	1402	2.25E-02	2376	1.83E-01	747	7.02E-01	1378	7.09E-01	12.39	8	6.32E-03
rs1377249	78232429	A	1402	2.25E-02	2376	1.83E-01	747	7.02E-01	1378	7.09E-01	12.39	8	6.32E-03
rs1545508	78208756	A	1402	3.82E-02	2376	1.92E-01	747	7.16E-01	1378	5.23E-01	11.8	8	8.08E-03
rs9298293	78211752	A	1402	4.38E-02	2376	1.83E-01	747	7.02E-01	1378	5.22E-01	11.67	8	8.53E-03
rs10504645	78230921	G	1402	4.32E-02	2376	1.83E-01	747	7.16E-01	1378	5.23E-01	11.65	8	8.60E-03
rs7831215	78236862	C	1402	4.32E-02	2376	1.83E-01	747	7.16E-01	1378	5.23E-01	11.65	8	8.60E-03
rs1452808	78228511	A	1402	4.75E-01	2376	6.99E-02	747	2.11E-01	1378	4.62E-01	11.47	8	9.26E-03
rs16939434	78227485	A	1402	1.63E-01	2376	2.63E-01	747	2.74E-01	1377	5.23E-01	10.19	8	1.56E-02
rs1840079	78211521	C	1402	7.99E-01	2376	6.68E-02	747	1.57E-01	1378	9.96E-01	9.57	8	2.00E-02

rs6991979	78243575	A	1402	8.76E-02	2376	8.25E-01	747	5.90E-01	1378	2.07E-01	9.46	8	2.09E-02
rs16939442	78242401	C	1393	8.11E-01	2369	1.67E-01	740	2.49E-01	1368	2.88E-01	9.26	8	2.26E-02
rs7836772	78221169	C	1402	9.92E-02	2376	1.83E-01	747	7.02E-01	1378	8.15E-01	9.14	8	2.37E-02
rs7842403	78211521	G	1402	9.92E-02	2376	1.83E-01	747	7.02E-01	1378	8.15E-01	9.14	8	2.37E-02
rs7833179	78226180	C	1402	4.53E-01	2376	6.91E-02	747	8.88E-01	1378	4.26E-01	8.87	8	2.63E-02
rs16939440	78208512	A	1402	8.27E-01	2376	2.29E-01	747	2.74E-01	1378	3.44E-01	8.05	8	3.60E-02
rs16939441	78236862	C	1402	8.27E-01	2376	2.29E-01	747	2.74E-01	1378	3.44E-01	8.05	8	3.60E-02
rs16939435	78230951	A	1402	8.27E-01	2376	2.29E-01	747	2.74E-01	1378	3.69E-01	7.91	8	3.79E-02
rs16939436	78209886	A	1402	8.27E-01	2376	2.29E-01	747	2.74E-01	1378	3.69E-01	7.91	8	3.79E-02
rs16939437	78213269	A	1402	8.27E-01	2376	2.29E-01	747	2.74E-01	1378	3.69E-01	7.91	8	3.79E-02
rs16939439	78239201	A	1402	8.27E-01	2376	2.29E-01	747	2.74E-01	1378	3.69E-01	7.91	8	3.79E-02
rs10095652	78208734	C	1402	7.70E-01	2376	2.32E-01	747	1.57E-01	1378	7.08E-01	7.83	8	3.90E-02
rs7015723	78248131	G	1402	7.74E-01	2376	2.32E-01	747	1.57E-01	1378	7.08E-01	7.82	8	3.92E-02
rs1470834	78231269	A	1402	8.57E-01	2376	2.23E-01	747	3.05E-01	1378	3.44E-01	7.82	8	3.92E-02
rs16939447	78234373	A	1402	8.57E-01	2376	2.23E-01	747	3.05E-01	1378	3.44E-01	7.82	8	3.92E-02
rs16939448	78243802	C	1402	8.57E-01	2376	2.23E-01	747	3.05E-01	1378	3.44E-01	7.82	8	3.92E-02
rs16939450	78226180	A	1402	8.57E-01	2376	2.23E-01	747	3.05E-01	1378	3.44E-01	7.82	8	3.92E-02
rs16939433	78214528	A	1402	8.74E-01	2376	2.29E-01	747	2.74E-01	1378	3.69E-01	7.8	8	3.95E-02
rs7813218	78234605	C	1402	9.71E-01	2376	2.23E-01	747	3.05E-01	1378	3.43E-01	7.57	8	4.30E-02
rs10097260	78224651	A	1399	3.44E-01	2376	2.00E-01	747	7.16E-01	1376	7.68E-01	6.55	8	6.19E-02
rs17378611	78221169	A	1401	6.98E-01	2375	5.05E-01	747	-	1378	8.13E-01	2.5	6	1.81E-01
rs10113852	78239301	C	1402	7.65E-01	2376	-	747	-	1378	5.58E-01	1.7	4	2.14E-01

Region 29 is located on Chromosome 8, - indicates a model that did not converge
Only SNPs with ethnicity-specific minor allele frequency > 5% were included in the Fisher's combined analysis
SNP: single nucleotide polymorphism, BP: basepair position in kilobases, AA: African American, EA: European American, CA: Chinese American, HA: Hispanic American, n: sample size, p: p-value, df: degrees of freedom
$\alpha_{Bonferroni} = 0.001$

Appendix 70 Fisher's combined p-values and ethnicity-specific p-values for each SNP in region 44

			AA		EA		CA		HA		Fisher's Summary Information		
SNP	BP	Coded Allele	n	p	n	p	n	p	n	p	X^2	df	X^2 pvalue
rs16970495	77131187	A	1397	1.73E-02	2373	4.27E-01	747	1.89E-01	1379	7.79E-02	18.25	8	6.90E-03
rs3816282	77129277	A	1396	5.62E-01	2374	1.86E-02	747	5.53E-02	1379	.	14.92	6	8.01E-03
rs7183818	77159468	C	1397	9.05E-02	2373	2.22E-01	747	4.75E-02	1379	2.64E-01	16.58	8	1.19E-02
rs12911829	77165876	C	1397	2.84E-01	2374	4.26E-03	747	5.01E-01	1379	4.27E-01	16.52	8	1.21E-02
rs744059	77146560	C	1397	2.28E-01	2374	2.89E-02	747	4.24E-01	1379	4.28E-01	13.47	8	3.03E-02
rs736827	77145921	A	1397	6.36E-01	2374	7.13E-03	747	4.86E-01	1379	5.64E-01	13.38	8	3.10E-02
rs6495367	77163186	A	1397	2.02E-01	2373	2.52E-01	747	1.51E-01	1377	2.49E-01	12.51	8	3.92E-02
rs6495366	77162402	C	1397	1.03E-01	2373	2.85E-01	747	1.29E-01	1379	6.35E-01	12.06	8	4.40E-02
rs2009197	77137895	C	1397	9.57E-01	2374	5.46E-01	747	9.77E-01	1379	.	1.34	6	5.74E-02
rs755362	77157055	A	1397	2.04E-01	2374	2.07E-01	747	1.00E-01	1379	9.95E-01	10.94	8	5.74E-02
rs4778879	77154848	A	1397	3.32E-01	2374	2.20E-01	745	8.92E-02	1379	7.48E-01	10.65	8	6.13E-02
rs12443101	77130589	A	1397	7.65E-01	2369	4.19E-01	746	4.04E-01	1371	4.02E-02	10.51	8	6.31E-02
rs13380104	77127099	C	1397	1.75E-01	2374	2.53E-01	747	1.22E-01	1379	9.78E-01	10.48	8	6.35E-02
rs744060	77146329	C	1397	6.48E-01	2374	9.11E-02	747	3.87E-01	1379	2.57E-01	10.28	8	6.63E-02
rs6495365	77160732	C	1397	3.74E-01	2374	8.23E-02	747	5.43E-01	1379	.	8.18	6	7.00E-02
rs6495364	77156751	C	1397	6.08E-01	2374	6.55E-02	747	5.35E-01	1379	.	7.70	6	7.89E-02
rs8030257	77138231	A	1397	6.23E-01	2373	5.86E-01	747	4.04E-02	1379	6.88E-01	9.18	8	8.18E-02
rs11072824	77131471	C	1397	7.45E-01	2374	1.34E-01	747	3.58E-01	1379	3.53E-01	8.75	8	8.78E-02
rs16970524	77145850	A	1397	8.41E-01	2374	4.58E-01	747	9.38E-01	1379	.	2.04	6	9.38E-02
rs11634225	77139747	A	1396	5.26E-01	2374	7.08E-01	747	9.61E-01	1379	.	2.05	6	9.42E-02
rs729650	77146365	C	1396	7.52E-01	2374	5.44E-01	746	8.73E-01	1379	.	2.06	6	9.47E-02
rs987057	77134426	C	1397	8.30E-01	2373	3.11E-01	747	7.11E-01	1379	5.71E-01	4.51	8	1.00E-01
rs11629697	77149214	A	1396	8.17E-01	2373	3.13E-01	746	1.49E-01	1378	.	6.54	6	1.02E-01
rs894784	77142996	G	1391	2.33E-01	2372	2.10E-01	747	8.48E-01	1377	.	6.37	6	1.05E-01
rs997285	77134521	G	1397	4.44E-01	2373	8.77E-01	747	7.94E-01	1379	.	2.34	6	1.06E-01
rs7174521	77149145	A	1397	9.52E-01	2374	7.69E-01	747	6.29E-02	1378	.	6.15	6	1.09E-01
rs12911414	77139194	A	1397	7.45E-01	2373	2.22E-01	747	5.69E-01	1379	4.28E-01	6.42	8	1.11E-01
rs12440502	77143686	G	1397	3.46E-01	2373	9.40E-01	747	2.80E-01	1379	6.66E-01	5.60	8	1.11E-01
rs7166032	77152835	A	1397	3.21E-01	2372	9.95E-01	747	2.66E-01	1379	7.09E-01	5.62	8	1.11E-01
rs7166598	77129724	C	1393	6.76E-01	2373	1.88E-01	747	8.01E-01	1378	5.74E-01	5.68	8	1.12E-01

rs16970502	77137679	A	1396	9.79E-02	2373	6.58E-01	747	8.43E-01	1378	.	5.83	6	1.15E-01
rs4778626	77135967	C	1396	3.31E-01	2374	1.70E-01	747	-	1378	.	5.76	6	1.16E-01
rs4778861	77159930	C	1397	4.14E-01	2374	3.62E-01	747	9.57E-01	1379	.	3.88	6	1.35E-01
rs747109	77163656	C	1397	6.69E-01	2374	3.35E-01	747	.	1379	.	2.99	4	1.68E-01
rs4778857	77136392	C	1391	7.57E-01	2367	3.16E-01	744	.	1378	.	2.86	4	1.71E-01
rs998031	77134521	C	1397	7.59E-01	2374	5.14E-01	747	.	1379	.	1.88	4	1.84E-01

Region 44 is located on Chromosome 15
Only SNPs with ethnicity-specific minor allele frequency > 5% were included in the Fisher's combined analysis
SNP: single nucleotide polymorphism, BP: basepair position in kilobases, AA: African American, EA: European American, CA: Chinese American, HA: Hispanic American,
n: sample size, p: p-value, df: degrees of freedom
$\alpha_{Bonferroni} = 0.001$

Appendix 71 Fisher's combined p-values and ethnicity-specific p-values for each SNP in region 46

			AA		EA		CA		HA		Fisher's Summary Information		
SNP	BP	Coded Allele	n	p	n	p	n	p	n	p	X^2	df	X^2 pvalue
rs4800653	21458098	A	1100	2.38E-02	1930	1.53E-02	538	2.30E-01	976	2.27E-03	30.95	8	1.47E-06
rs1840444	21472831	A	1100	3.33E-02	1930	1.21E-02	538	2.56E-01	976	1.09E-02	27.41	8	7.65E-06
rs2728504	21468660	A	1098	4.04E-02	1927	1.65E-02	538	2.50E-01	976	1.20E-02	26.24	8	1.32E-05
rs1811520	21485642	A	1100	4.92E-02	1930	1.55E-02	538	2.50E-01	976	1.20E-02	25.97	8	1.49E-05
rs2592062	21480858	G	1100	4.92E-02	1930	1.55E-02	538	2.50E-01	976	1.20E-02	25.97	8	1.49E-05
rs2728509	21471994	G	1100	4.92E-02	1930	1.55E-02	538	2.50E-01	976	1.20E-02	25.97	8	1.49E-05
rs1840445	21484484	C	1100	9.18E-01	1930	3.47E-03	538	2.30E-01	976	4.83E-03	25.1	8	2.22E-05
rs2568474	21492261	C	1099	3.38E-01	1930	9.78E-03	538	2.12E-01	976	1.60E-02	22.79	8	6.41E-05
rs2568476	21492275	C	1100	1.80E-01	1930	1.55E-02	538	1.97E-01	976	2.50E-02	22.39	8	7.69E-05
rs979166	21460370	A	1100	2.62E-01	1929	6.17E-03	538	2.16E-01	976	4.63E-02	22.06	8	8.94E-05
rs9950413	21462007	A	1100	9.44E-01	1929	1.04E-02	538	1.59E-01	974	1.68E-02	21.09	8	1.39E-04
rs4800652	21455858	A	1100	4.02E-01	1930	8.38E-03	538	2.52E-01	976	3.64E-02	20.77	8	1.60E-04
rs1455185	21493500	C	1100	9.92E-01	1930	1.26E-02	538	3.33E-01	976	1.41E-02	19.48	8	2.87E-04
rs1840439	21462799	A	1100	5.37E-01	1930	1.23E-02	538	2.12E-01	976	4.81E-02	19.21	8	3.24E-04
rs1840438	21480604	A	1100	9.74E-01	1930	1.26E-02	538	3.33E-01	976	1.69E-02	19.16	8	3.31E-04
rs2019917	21487395	C	1099	5.10E-01	1930	5.04E-02	538	3.89E-01	976	7.20E-03	19.07	8	3.45E-04
rs1840437	21464405	C	1099	7.30E-01	1930	4.76E-02	538	3.36E-01	976	7.68E-03	18.64	8	4.18E-04
rs2728505	21477104	A	1100	5.11E-01	1930	6.85E-02	538	4.00E-01	976	7.34E-03	18.37	8	4.71E-04
rs273770	21474070	A	1096	3.61E-02	1922	2.09E-01	538	2.86E-01	976	4.81E-02	18.35	8	4.75E-04
rs2604482	21478474	C	1100	9.90E-01	1929	2.06E-02	538	3.33E-01	976	1.74E-02	18.09	8	5.34E-04
rs2604480	21481587	C	1100	5.46E-01	1930	6.85E-02	538	3.90E-01	976	8.31E-03	18.03	8	5.48E-04
rs2604481	21479877	C	1100	5.46E-01	1930	6.85E-02	538	3.90E-01	976	8.31E-03	18.03	8	5.48E-04
rs1840435	21465750	A	1098	9.67E-01	1930	1.67E-02	538	3.24E-01	975	2.36E-02	17.99	8	5.58E-04
rs2592061	21468780	C	1100	8.92E-01	1930	6.85E-02	538	3.15E-01	976	9.07E-03	17.31	8	7.54E-04
rs274231	21455049	A	1099	2.28E-01	1925	2.58E-01	535	2.52E-01	970	3.72E-02	15	8	2.07E-03
rs8087975	21469911	A	1100	8.08E-03	1930	.	538	.	976	5.45E-01	10.85	4	2.20E-03
rs273772	21479486	A	1100	2.29E-01	1930	3.25E-01	538	2.74E-01	976	5.29E-02	13.66	8	3.69E-03
rs9965027	21492262	C	1100	2.19E-02	1930	.	538	.	976	3.69E-01	9.64	4	4.03E-03
rs1579854	21456605	C	1100	4.70E-02	1929	2.22E-01	538	9.02E-01	976	3.30E-01	11.55	8	8.96E-03
rs1584178	21464261	A	1100	4.70E-02	1930	2.24E-01	538	9.02E-01	976	3.30E-01	11.53	8	9.04E-03

rs11663205	21454353	A	1099	1.54E-01	1929	9.53E-01	538	1.68E-01	976	1.74E-01	10.91	8	1.17E-02
rs12458367	21481019	C	1099	3.91E-01	1928	1.74E-01	537	7.01E-01	976	1.21E-01	10.32	8	1.48E-02
rs9957023	21487592	A	1100	4.98E-02	1930	.	538	.	976	7.11E-01	6.68	4	1.77E-02
rs11873662	21466728	G	1099	5.36E-01	1926	8.93E-01	536	1.67E-02	974	9.88E-01	9.69	8	1.91E-02
rs12454499	21485419	A	1100	4.84E-01	1930	2.51E-01	538	6.00E-01	976	1.27E-01	9.37	8	2.16E-02
rs273768	21470808	A	1100	2.33E-01	1930	2.19E-01	538	5.93E-01	975	4.21E-01	8.73	8	2.77E-02
rs273766	21474112	A	1100	2.40E-01	1930	2.19E-01	538	5.93E-01	975	4.21E-01	8.67	8	2.84E-02
rs1947098	21484544	C	1100	8.02E-02	1930	5.57E-01	538	3.40E-01	976	9.24E-01	8.53	8	3.00E-02
rs9966851	21482557	A	1100	1.68E-01	1930	5.61E-01	538	3.21E-01	976	6.92E-01	7.72	8	4.07E-02
rs273773	21454669	A	1100	1.97E-01	1930	2.96E-01	538	9.68E-01	976	4.36E-01	7.41	8	4.56E-02
rs1823940	21465721	C	1100	1.71E-01	1930	4.55E-01	538	9.26E-01	976	4.71E-01	6.76	8	5.75E-02
rs273764	21472082	A	1100	6.82E-01	1930	5.08E-01	538	6.08E-01	976	5.70E-01	4.24	8	1.27E-01
rs1598310	21484743	G	1100	6.99E-01	1930	4.09E-01	538	8.90E-01	976	5.61E-01	3.89	8	1.39E-01
rs9952296	21458701	A	1100	6.66E-01	1930	4.72E-01	538	5.41E-01	976	9.28E-01	3.69	8	1.46E-01

Region 46 is located on Chromosome 18
Only SNPs with ethnicity-specific minor allele frequency > 5% were included in the Fisher's combined analysis
SNP: single nucleotide polymorphism, BP: basepair position in kilobases, AA: African American, EA: European American, CA: Chinese American, HA: Hispanic American,
n: sample size, p: p-value, df: degrees of freedom
$\alpha_{Bonferroni} = 0.001$

Appendix 72 Phylogenic conservation score from hg18 for Vertebrate Multiz Alignment & Conservation (17 Species) for each analyzed SNP within regions 1, 30 and 46

Region 1		Region 29				Region 46			
SNP	**PCS**	**SNP**	**PCS**	**SNP**	**PCS**	**SNP**	**PCS**	**SNP**	**PCS**
rs1563469[a]	**0.843**	rs7015723	0.000	rs12056492[d]	0.001	rs273764[e]	0.000	rs1840435	0.005
rs897615	0.000	rs10095652[b]	0.000	rs13250484[d]	0.001	rs273766[e]	0.000	rs2728505	0.000
rs897620[b]	0.000	rs1840079[b]	0.000	rs7821262	0.060	rs273768[d]	0.001	rs2604482	0.000
rs10910019	0.000	rs7832753[e]	0.000	rs10957840	0.003	rs12454499[e]	0.000	rs2592062	0.000
rs2124661	0.000	rs7833179	0.000	rs16939433[c]	0.000	rs273770[e]	0.004	rs2568476	0.002
rs2168531	0.004	rs10086664	0.000	rs1377249	0.007	**rs274231[e]**	**0.890**	rs2728509[e]	0.000
rs12063033	0.001	rs11996389	0.000	rs10282777[e]	0.000	**rs273772[e]**	**0.465**	rs2568474	0.000
rs6673503	0.000	rs10093959	0.000	rs1452832b	0.000	rs273773[e]	0.000	rs1811520[d]	0.001
rs6676289	0.007	rs1993196	0.000	rs1470834[e]	0.001	rs1823940	0.000	rs2019917[d]	0.001
rs10909793	0.000	rs1545508	0.000	rs1377248	0.000	rs12458367[e]	0.000	rs4800652[d]	0.000
rs10797342	0.000	rs9657121	0.000	**rs1377247**	**0.124**	rs1598310[e]	0.003	rs1840437[g]	0.002
rs1563472	0.001	rs9298291[c]	0.000	rs10504647	0.000	rs1579854[e]	0.001	rs1840438[d]	0.000
rs1563474	0.000	rs9298292	0.000	rs1452808	0.001	rs1584178[e]	0.000	rs11873662[d]	0.000
rs11580768	0.000	rs16939434	0.000	**rs7016358**	**0.142**	rs11663205	0.000	rs1455185	0.006
rs2045333	0.001	rs16939435	0.000	rs17378611	0.001	rs9950413	0.002	**rs1840439[e]**	**0.134**
rs3100865	0.000	rs16939436	0.000	rs10113852	0.000	rs9952296	0.001	rs9957023	0.000
rs2445620[b]	0.000	rs13259366	0.000	rs6991979[c]	0.001	rs2592061	0.000	rs979166	0.000
rs1456465	0.000	rs13269867	0.000	rs16939447[f]	0.006	rs1947098	0.000	**rs4800653**	**0.130**
rs2842910[c]	0.000	rs16939437	0.003	rs7813218	0.000	rs8087975[f]	0.001	rs9966851[f]	0.037
rs2842911[d]	0.000	rs10504645	0.001	rs10102542	0.001	rs2728504	0.000	rs1840444	0.000
rs2842914	0.003	**rs7831215**	**1.000**	rs7836772	0.000	rs2604480	0.001	rs9965027	0.000
rs2606406	0.003	**rs16939439**	**1.000**	rs9657123	0.000	rs2604481	0.001	rs1840445	0.005
rs12091184	0.000	rs13439699	0.013	rs16939448	0.000				
rs750786[e]	0.000	rs16939440[c]	0.044	rs12680110T[c]	0.000				
rs12035436	0.000	rs16939441	0.000	rs9298293	0.000				
rs897630[e]	0.000	rs10097260[e]	0.001	rs7842403[d]	0.000				
rs880725	0.000	rs12056333	0.000	**rs16939450**	**0.763**				
rs897631	0.000	rs16939442[d]	0.001						

SNPs with PCS greater than 0.1 are bolded.
SNP: single nucleotide polymorphism, PCS: Phylogenic Conservation Score – estimates the probability that each nucleotide belongs to a conserved element, based on the multiple alignments. [a]Another 2bp SNP overlaps this SNP (rs72543806), [b]SNP is in a SINE element, [c]SNP is in a DNA repeat element, [d]SNP is in a LTR,
[e]SNP is in a LINE element, [f]SNP is in a moderately well conserved region, [g]SNP is in a LTR region as well as a H3K4Me3 chip-associated region.[204, 270, 271]

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