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Investigating the Role of Genetic Variants and Environmental Factors in the Trajectory of Substance Use Disorders

Linda Johnson
Washington University in St. Louis

WASHINGTON UNIVERSITY IN ST. LOUIS

Division of Biology and Biomedical Sciences
Human and Statistical Genetics

Dissertation Examination Committee:
Laura J Bierut, Chair
Arpana Agrawal
Christina Gurnett
John Rice
Nancy Saccone

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by

Linda Johnson

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Linda Johnson

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Abstract

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Professor Laura J Bierut, Chair

Substance use disorders are a serious threat to public health with devastating social, economic, and health impacts on affected individuals and their families. This dissertation aims to improve understanding of substance use disorders in three parts: first, an epidemiological project examining smoking cessation and e-cigarette use at a population level; second, a genetic project examining the role of genetic variants on tobacco, alcohol, cannabis, and cocaine use; and finally, a review of the utility of polygenic risk scores in predicting risk of substance use behaviors.

Cigarette smoking is a leading cause of preventable death, accounting for over 480,000 deaths in the United States annually. Smoking is linked to heart disease, lung disease, and multiple cancers, and on average smokers die 10 years earlier than nonsmokers. In Chapter 2 I

demonstrate the trends in smoking prevalence and smoking behaviors since 2006 in two U.S. national surveys while accounting for demographic shifts in the population that influence smoking behaviors. In addition, I demonstrate the association of electronic cigarettes with past 12 month quit attempts and successful smoking cessation at the population level.

Numerous genome-wide association studies provide strong evidence for the genetic contribution to substance use disorders. Specifically, multiple studies have shown association between variants in the *CHRNA5-CHRNA3-CHRNB4* nicotinic receptor subunit gene cluster on chromosome 15 and nicotine use disorder in European ancestry populations. In Chapter 3 I demonstrate that this association is specific to nicotine use disorder and was not associated with alcohol, cannabis, and cocaine use disorders in our study population.

Substance use disorders are genetically complex, and there may be common underlying environmental and genetic risk factors that predispose to substance use behaviors. With the publication of large meta-analyses of genome-wide association studies, polygenic risk scores can now be used to measure genetic liability. Chapter 4 explores the utility of polygenic risk scores for exploring the genetic etiology of disease and the genetic correlations between them.

Overall, this dissertation advances our understanding of substance use disorders by incorporating population based evidence, genetic risk factors, and polygenic risk.

Chapter 1: Background and Introduction

1.1 The public health burden of substance abuse in United States

Substance use disorders are a leading cause of preventable death in both the United States and worldwide and pose a worldwide threat to public health and have a devastating social and economic impact on individuals and their families [1-9]. The World Health Organization has estimated that there are 2 billion alcohol users, 1.3 billion tobacco users, and 185 million illicit drug users worldwide[10].

1.1.1 Tobacco

Tobacco use disorder remains highly prevalent in the United States, accounting for over 480,000 deaths per year, making smoking cessation a public health priority[11]. Smoking harms nearly every organ of the body; it has been linked to heart disease, lung disease, and multiple cancers, and on average smokers die 10 years earlier than nonsmokers[11, 12]. Maternal smoking during pregnancy is also associated with several adverse outcomes in the fetus, including preterm delivery, spontaneous abortion, growth restriction, and increased risk of sudden infant death syndrome, as well as long-term behavioral and psychiatric disorders in children[13-18]. In addition to adverse effects on individual and population level health, smoking imposes an immense financial burden on society, with a cost to the U.S. economy of over $300 billion each year[19]. Moreover, the effects of tobacco use are not limited to the smoker, as secondhand smoke exposure can cause many diseases including lung cancer, heart disease, and stroke among adults, and respiratory illness, ear infections, and asthma attacks among children and infants[20-

25]. It has been estimated that exposure to secondhand smoke is responsible for 41,000 deaths among adults each year in the U.S.[11]

1.1.2 Alcohol

Each year, 3.3 million people die due to the harmful effects of alcohol, representing 5.9% of all deaths across the world[10]. Excessive alcohol consumption, the third leading cause of preventable death in the U.S., can cause damage to the central and peripheral nervous system, and to nearly every organ system in the body[26]. Alcohol consumption can have immediate health consequences such as unintentional injury, violent behavior, alcohol poisoning, and risky social behaviors[27]. Over time, excessive alcohol use can lead to numerous chronic diseases such as heart disease, diabetes, liver disease, cancer, and psychiatric disorders[27]. Alcohol use is also strongly correlated with alcohol use disorder, a disabling psychiatric disorder that affects approximately 12% of U.S. adults across their lifetime[28]. Alcohol use disorder is a common, complex disorder characterized by compulsive and uncontrolled alcohol consumption despite its negative effects on the user's health and social relationships[28].

1.1.3 Cannabis

Cannabis is the most widely consumed illegal substance across the world[29, 30]. The most common route of administration is inhalation. Cannabis use accounts for 75% of illicit drug use in the U.S. and continues to emerge as the most widely available illicit drug with the perception that it is relatively harmless[31]. In fact, similar to the use of other substances, cannabis has been linked to multiple short-term and long-term consequences including short-term memory, motor coordination, altered judgement, paranoia or psychosis, craving, use disorder, and drug-seeking

behavior[32-35]. The regular use of cannabis during adolescence is of particular concern, since regular use in this age group is associated with an increased likelihood of harmful consequences[36]. During developmental periods until the age of approximately 21 years, the brain is intrinsically more vulnerable than a mature brain to the adverse long-term effects of cannabis use, and frequent exposure to cannabis in adolescent have been associated with significant declines in IQ, possibly due to the prominent role in synapse formation the cannabinoid system holds during brain development[37, 38]. Furthermore, epidemiological and preclinical data suggest that cannabis use in adolescence could act as a gateway drug, influencing multiple addictive behaviors in adulthood[39-41]. Regular cannabis use is also associated with an increased risk of anxiety, depression, and psychoses, especially among individuals with a preexisting genetic vulnerability[42, 43].

1.1.4 Cocaine

Cocaine remains the second most commonly used and trafficked illicit drug in the world after cannabis[44]. Approximately 2.1% of U.S. residents have used cocaine in the past month, the second most prevalent nonmedical drug after cannabis[44, 45]. Cocaine is highly addicting, with 25-45% of past-year users meeting criteria for cocaine use disorder[46-48]. Cocaine use is costly to society, directly contributing to morbidity and medical costs, lost workdays, and other adverse individual, interpersonal, and societal effects[49, 50]. Cocaine use disorder is often associated with psychiatric comorbidity, e.g., other substance use disorder, anxiety disorders, major depressive disorder, bipolar disorders, and personality disorders[51, 52].

1.2 Genetic contribution to substance use disorders

Alcohol use disorder was the first substance use disorder to have validated genetic findings. In 1972, individuals of Asian descent were observed to have facial flushing and decreased alcohol tolerance as compared to individuals of European origin[53]. The observed flushing reaction to alcohol ingestion was determined to be due to a deficiency of aldehyde dehydrogenase (ADH), an enzyme involved in ethanol metabolism[54]. Alcohol is primarily metabolized in the liver, and the first step is the oxidation of ethanol to acetaldehyde, which is catalyzed by alcohol dehydrogenases. The enzyme encoded by the *ADH1B* gene on chromosome 4 has the highest concentration in the liver, and the *ADH1B* rs1229984 variant has a strong association for alcohol use disorder and related alcohol-induced diseases across different ancestry populations [55-58]. The minor A allele of variant rs1229984 results in an amino acid change that increases the rate of alcohol oxidation and causes transient increases in acetaldehyde, which leads to unpleasant symptoms such as nausea, dizziness, and tachycardia [56]. Given these toxicities of systemic acetaldehyde build-up, people with this *ADH1B* variant experience these negative effects when they consume alcohol, discouraging heavy drinking in this population. Several studies have identified multiple genetic variants of alcohol dehydrogenase genes that influence rates of alcohol metabolism and alcohol use disorder[55, 59, 60].

Studies in cell lines have demonstrated that nicotine and alcohol (ethanol) interact at nicotinic acetylcholine receptors, altering their expression levels and modulating their agonist response[61, 62]. Some studies have used lines of mice and rats selectively bred to be sensitive or resistant to the high-dose anesthetic effects of alcohol[63-65]. By analyzing the responses of these selected lines as well as a number of recombinant inbred strains developed from their

crosses genotype-specific responses to alcohol have been shown that suggest the role of *CHRNA4* in modulating ethanol sensitivity. Other studies support a common physiological basis for alcohol and nicotine's behavioral effects[66, 67]. In addition, recent pharmacological studies have implicated *CHRNA3* and *CHRNB4* in alcohol consumption and seeking in rats as well as in alcohol and nicotine co-dependencies[68, 69].

The development of all substance use disorders, including nicotine use disorder, requires initiation, conversion from occasional to daily use, and finally the development of advanced behaviors[70]. The first GWAS on nicotine use disorder demonstrated that the top genetic associations for nicotine use disorder are in genes encoding nicotinic receptor subunits[71]. Nicotine exerts its central and peripheral actions by binding to neuronal nicotinic acetylcholine receptors, a class of ligand-gated ion channels. The subunits are encoded by 9 α and 3 βsubunit genes, and the expression of different subunits in different anatomic areas results in functional specificity. Nicotine addiction is thought to arise from the interaction between dopaminergic and nicotinic neurons in the striatum involving subunits α4, α5, β2, and β3[45]. *CHRNA5*, *CHRNA3*, and *CHRNB4* are nicotinic receptor subunit genes adjacent to one another on chromosome 15, and SNPs in the 3 genes are in high linkage disequilibrium[45, 71, 72]. The strongest biologic associations with nicotine use disorder have been found in the a5 nicotinic receptor subunit gene CHRNA5 on chromosome 15[71, 73]. Specifically, the most compelling association with nicotine use disorder is in rs16969968, a nonsynonymous SNP in the α5 nicotinic receptor subunit gene CHRNA5 which causes an amino acid change of aspartic acid to asparagine[71]. SNPs in high linkage disequilibrium with rs16969968 span a large area, encompassing the genes *IREB2*, *PSMA4*, *CHRNA5*, *CHRNA3*, and *CHRNB4*[45]. In vitro functional studies have found that the amino acid substitution in α5 due to rs16969968 results in decreased response to

nicotine, suggesting that decreased nicotinic receptor function is associated with increased nicotine use disorder risk.

Race stratification is important because substance use behaviors and genetic allele frequencies differ substantially across populations. Differences in the genetic architecture of European Americans and African Americans indicate that distinct genetic factors differentially contribute to substance use disorders in these populations. These differences are highlighted by the fact that the well-established coding variant in *CHRNA5*, rs16969968, is more common in European (MAF=0.35) than African Americans (MAF=0.05). The contrasting genetic architecture in European Americans and African Americans can be leveraged to narrow down the potential functional source of the disease associations[74]. In the European ancestry population, many highly correlated SNPs often detect the same association signal, and determining which variants are causal is not straightforward. LD and allele frequencies can also differ between European and African populations. Therefore it is important to study associated variants that appear to be less common because of their potential to be higher frequency in certain geographic or racial groups, and therefore of higher impact on a population level. Comparing associations across European Americans and African Americans can help refine the region of association and point to variants more likely to have functional relevance[74, 75].

Another genetic factor in the development of smoking behaviors is variation in the *CYP2A6* gene on chromosome 19, which encodes a cytochrome P450 enzyme that metabolizes nicotine via the oxidation of nicotine to cotinine[76]. The *CYP2A6* gene locus is highly polymorphic, and variants with reduced function have been associated with slower rates of nicotine metabolism[77, 78]. Among smokers, the majority of studies demonstrate that genetically slower metabolizes smoke fewer cigarettes per day, which is hypothesized to be due to the fact that cigarettes

smokers naturally titrate their cigarette consumption in order to maintain steady nicotine levels[79, 80].

Cannabis use disorder tends to aggregate in families, and twin studies have attributed individual differences in cannabis involvement to both genetic and environmental factors[81-86]. However despite twin studies showing that 50-70% of variation in cannabis use disorder is attributable to heritable influences, only a few studies have identified genome-wide significant loci for cannabis use disorder: [29, 87, 88]. *CSMD1* may be a candidate gene that affects the risk for cannabis use disorder and other psychiatric disorders[29]. A recent meta-analyses demonstrated an association between lifetime cannabis use and four genes: *NCAM1, CAMD2, SCOC,* and *KCNT2[89]*. Approximately 9% of those who experiment with cannabis will become addicted, and the number goes up to about 1 in 6 among those who start using cannabis as teenagers and to 25-50% among those who smoke cannabis daily[90].

Similar to other substance use disorders, cocaine use disorder has a strong heritability component. Cocaine use disorder is understudied, particularly in relation to the extent of individual and societal problems it causes. Cocaine use disorder has a heritability of about 0.54 in females and 0.79 in males, and there have been numerous candidate gene association studies of cocaine use disorder traits[91-93]. Siblings of cocaine-dependent probands had an increased risk of developing cocaine use disorder compared with probands without cocaine-dependent siblings[82]. Previous work has demonstrated that the minor allele of rs16969968 is associated with decreased risk for cocaine use disorder, and a negative association was also observed in the *CHRNA5* SNP rs588765 and alcohol use disorder in a sample that was heavily comorbid with cocaine use disorder[94, 95]. GWAS studies have identified the SNP rs2629540 in the *FAM53B*

gene, which has a role in regulating cell proliferation, but additional work is needed to determine the relationship of this function to cocaine use disorder risk[49].

1.3 Objectives of the dissertation

This thesis is intended to improve our understanding of substance use disorders as a project in three independent parts. In the first part, presented in Chapter 2, I focus on nicotine use disorder, which is a leading cause of preventable death in the U.S. I use an epidemiologic approach using two population-based studies to examine changes in smoking behaviors over the past decade, during which e-cigarettes were introduced into the U.S. market. Specifically, I examine changes in current smoking, ever smoking, and former smoking from 2006-2016 while accounting for demographic shifts in the U.S. population that influence smoking behaviors. In addition, I sought to understand whether the current use of electronic cigarettes was associated with a change in past year quit attempts and successful smoking cessation at the population level.

Nicotine use disorder commonly co-occurs with polysubstance use and use disorder. The most robust genetic findings for nicotine use disorder and other smoking related traits and diseases have pinpointed a region on chromosome 15q25.1, which harbors the *CHRNA5-CHRNA3-CHRNAB4* gene cluster coding for α5, α3, and β4 nicotinic acetylcholine receptor (nAChR) subunits. Specifically, the most well-established locus within this region is tagged by the single nucleotide polymorphism (SNP) rs16969968 in *CHRNA5*, which is the strongest genetic risk factor for several smoking related traits. A few studies have reported association of this region with other substance related traits, with somewhat conflicting results. In the second part of this thesis, presented in Chapter 3, I examine whether this gene region represents a genetic risk

specific for nicotine use disorder, or whether this region also harbors genetic risk for other substance use, specifically alcohol, cannabis, and cocaine use disorder.

Genome-wide association studies have identified multiple variants associated with substance use disorder, however risk prediction is often limited by modest effect sizes based on single genetic variants. Polygenic risk scores can combine thousands of variants in order to achieve some predictive ability across a range of complex traits and diseases, including substance use disorders. In the final part of this thesis, presented in Chapter 4, I outline the role of using polygenic risk scores in predicting genetic liability to complex diseases.

Chapter 2: E-cigarette Usage Is Associated with Increased Past 12 Month Quit Attempts and Successful Smoking Cessation

2.1 Background

Cigarette smoking is a leading cause of preventable death, accounting for over 480,000 deaths in the United States annually.[96] Smoking is linked to heart disease, lung disease, and multiple cancers, and on average smokers die 10 years earlier than nonsmokers.[96, 97] In addition to harmful effects on health at both the individual and population levels, smoking imposes an immense financial burden on society, with a cost to the U.S. economy of over $300 billion each year.[96, 98]

In 2007 the Institute of Medicine (IOM) published "Ending the Tobacco Problem: A Blueprint for the Nation." Using data from the National Health Interview Survey (NHIS), the report described the smoking behavior of U.S. adults from 1965 to 2005.[99] During this 40-year period, the prevalence of current smoking declined from 42% to 21%. This dramatic decline was attributed to a decrease in ever smoking, defined as smoking 100 or more cigarettes lifetime, and to an increase in smoking cessation. In 1965, 56% of respondents reported ever smoking, compared to only 46% of respondents in 2005. The increase in smoking cessation was even more striking, with the percentage of ever smokers who reported now being former smokers rising from 24% in 1965 to 51% in 2005. Further examination revealed that most of the increase in cessation occurred between 1965 and 1991, when cessation almost doubled from 24% to 47%. It then gradually increased from 47% in 1991 to 51% in 2005.

The steep decline in ever smoking and current smoking and the increase in former smoking that occurred from 1965-2005 is not surprising given the numerous public health campaigns and interventions that were introduced during this period to discourage youth from initiating smoking and to encourage smokers to quit. Smoking has been broadly targeted by media campaigns, laws to restrict youth access to tobacco, increases on cigarette taxes, indoor smoking policies, and comprehensive smoke-free laws.[100, 101] Multiple efforts have also been made to help smokers quit. Prescription nicotine replacement therapy was introduced in the U.S. in 1984. In 1996, nicotine gum and nicotine patches were approved for over-the-counter sale, and utilization increased over 150% from 1996-1997.[102, 103] The first non-nicotine replacement medication for smoking cessation, bupropion, was approved by the Food and Drug Administration (FDA) in 1997, and the second, varenicline, was approved in 2006.[104-106] A total of three forms of nicotine replacement therapy (patch, gum and lozenge) are available to smokers over the counter, and two other forms of nicotine replacement (inhaler and nasal spray) are available by prescription, as are bupropion and varenicline, both non-nicotine replacement medications[107]. In 2010 the Affordable Care Act required non-grandfathered private insurance plans, Medicare, and all state Medicaid programs to cover tobacco cessation interventions[108-110].

In the past decade, another change in the smoking landscape has been the introduction of electronic cigarettes (e-cigarettes). They were first developed in China in 2003, introduced to the U.S. market in 2007, and quickly gained popularity in many countries[111-115]. E-cigarettes first became available to the U.S. market in 2007 and quickly gained popularity; they are particularly popular among smokers of combustible cigarettes, as many believe that e-cigarettes can help them quit.[116, 117] However, the scientific community has been divided on the use of e-cigarettes as a smoking cessation aid.[118, 119] Some argue that e-cigarettes are less harmful

than combustible cigarettes and have potential to help smokers quit,[120-123] while others express concern that smokers of conventional cigarettes will become dual users, thus delaying the cessation process.[124-126]

Although the debate continues, recent research suggests that e-cigarettes may be helping smokers quit. A study by Zhu et al.[127] found that e-cigarette use among smokers was associated with an increase in past year quit attempts and cessation at the population level. Using data from the Tobacco Use Supplement to the Current Population Survey (TUS-CPS) for the years 2014-2015 (the only years for which e-cigarette data were available), they found that past year quit attempts and cessation rates were significantly higher among smokers who also reported use of e-cigarettes in the past 12 months compared to smoker who did not.[127] When analyzing only smokers who did not use e-cigarettes, past year quit attempts and cessation remained flat across four survey periods ranging from 2001-2002 to 2010-2011. A significant increase in cessation at the population level was observed from 2010-2011 to 2014-2015 and this was associated with higher cessation among the e-cigarette users. The authors concluded that this represented the first significant increase in the population cessation rate in the U.S. in the past 15 years.

The prevalence of current smoking has continued to decline since the 2007 IOM report, with only 15.5% of US adults reporting current smoking in 2016.[128] With the current study, we sought to expand upon the work in the IOM report by using two national surveys, the NHIS and the TUS-CPS, to examine changes in multiple smoking behaviors from 2006 to 2016 while adjusting for changes in population demographics during this time. A second purpose of the current study was to use a second national sample, the NHIS, to extend the findings of the Zhu et al.[127] study, which suggested that increases in past year quit attempts and smoking cessation observed in the 2014-2015 TUS-CPS were associated with e-cigarette use.

2.2 Methods

2.2.1 Sample

Existing data were analyzed from two national surveys: the National Health Interview Survey (NHIS)[129] and the Tobacco Use Supplement to the Current Population Survey (TUS-CPS).[130-132] The NHIS and TUS-CPS datasets are coded and publically available through an open access policy.

The NHIS, administered by the Centers for Disease Control and Prevention, is an ongoing survey of a nationally representative sample of the noninstitutionalized U.S. population. The survey has assessed smoking behaviors annually since 1963; we used data from 2006 to 2016.[129] The survey is conducted annually in approximately 35,000 households using a multistage probability sampling design. A detailed description of the survey methodology can be found on the NHIS web site.[133]

The Tobacco Use Supplement to the Current Population Survey (TUS-CPS) is administered by the Census Bureau and is a nationally representative, cross-sectional survey of tobacco use.[134] The core CPS is a labor force survey conducted monthly in approximately 140,000 individuals living in 50,000 households using a multistage probability sampling design. In selected months, CPS sample households are also asked to complete the Tobacco Use Supplement. The TUS-CPS is funded by the National Cancer Institute, and it has been conducted approximately every 3-4 years since 1992. Data from the 2006-2007, 2010-2011, and 2014-2015 surveys were analyzed.[130-132] Additional details regarding methods and the sampling procedure are published elsewhere.[134]

Data were analyzed from respondents who participated in the NHIS 2006-2016 surveys or the TUS-CPS 2006-2007, 2010-2011, and 2014-2015 surveys, were 25-44 years old, and reported their smoking status. I focused on respondents who were aged 25-44 years at the time of survey to focus on a more homogeneous segment of the population and reduce potentially different smoking behaviors related to combustible cigarette and e-cigarette usage by age. Focusing on this age range also avoids bias among younger participants who may not have had sufficient time to develop their smoking behaviors. Limiting the sample to this age range also avoids bias among older participants who may have health comorbidities that influence their smoking status and eliminates potential for survivor bias because of increased mortality among those who smoke.[135, 136] In addition, this strategy provides a similarly aged comparison group over time given that the age distribution of the U.S. has changed over the last several decades.

2.2.2 Measures

In both datasets, current smokers were defined as individuals who had smoked at least 100 cigarettes lifetime and answered "every day" or "some days" to the question "Do you now smoke cigarettes every day, some days, or not at all?" The proportion of current smokers is reported as the number of current smokers out of the total sample. Ever smokers were defined as those who answered yes to the question "Have you smoked at least 100 cigarettes in your entire life?" The proportion of ever smokers is reported as the number of ever smokers out of the total sample. Former smokers were defined as ever smokers who answered "not at all" to the question "Do you now smoke cigarettes every day, some days, or not at all?" The proportion of former smokers is reported as the number of former smokers out of ever smokers. E-cigarette usage was queried in the most recent NHIS (2014-2016) and TUS-CPS (2014-2015) surveys. Current e-

cigarette users were those who answered “every day” or “some days” to the question “Do you now use an e-cigarette every day, some days, or not at all?”

Past 12 month quit attempts and smoking cessation were analyzed among respondents who were current smokers or reported quitting less than 12 months ago. In both the NHIS and the TUS-CPS, a quit attempt was defined as having tried to quit smoking and abstaining for at least 1 day in the past 12 months. Past 12 month cessation was defined as the percentage of smokers who had quit in the past 12 months and remained abstinent for at least three months at the time of the interview.

In addition, demographic characteristics that might affect smoking behaviors were considered in the logistic regression models, including sex, age, race/ethnicity, education, employment status, geographic region, and annual household income. For the TUS-CPS combustible cigarette smoking frequency (every day or some days) was also included as a covariate. Smoking frequency was not included as a covariate in the NHIS analyses because this information was only available for current smokers and not for former smokers.

2.2.3 Data Analysis

Data for the NHIS were accessed through the Integrated Public Use Microdata Series (IPUMS).[25] Data from the 2010-2011 and 2014-2015 TUS-CPS surveys were downloaded from the TUS-CPS website.[26,27] Data from the 2006-2007 TUS-CPS survey were requested from the United States Census Bureau.[28] All statistical analyses were performed using SAS 9.4, (Cary, NC, USA) using survey procedures and weighted to produce nationally representative estimates and to account for complex sample design. Self-response replicate weights, derived using balanced repeated replication (BRR), were used to obtain estimates of variance for TUS-CPS data

analyses. Multivariable logistic regression analyses were used to test whether past 12 month quit attempts and past 12 month cessation rates changed over time by entering survey year as a categorical variable and adjusting for sex, age, race/ethnicity, education, employment status, geographic region, and annual household income. Smoking frequency (every day versus some-day smoking) was also included for TUS-CPS analyses.

For the survey years in which e-cigarette data were available in the NHIS (2014-2016) and TUS-CPS (2014-2015), past 12 month quit attempts and cessation were stratified by e-cigarette use. E-cigarette usage was divided into two categories: (1) current e-cigarette use defined as e-cigarette use every day or some days at the time of the survey and (2) no current e-cigarette use, which included never use of an e-cigarette and previous e-cigarette use, but none at the time of the survey.

2.3 Results

2.3.1 Sample

The demographic characteristics of the samples analyzed from the NHIS (N=26,354) and the TUS-CPS (N=33,627) are presented in Table 1. Analyses of past 12 month quit attempts and smoking cessation focused on current smokers (NHIS N=24,027 and TUS-CPS N= 30,045) and recent former smokers (NHIS N=2,327 and TUS-CPS N=3,582).

Table 1. Respondent characteristics, adults aged 25-44 years in the NHIS (2006-2016) and TUS-CPS (2006-2007, 2010-2011, 2014-2015)

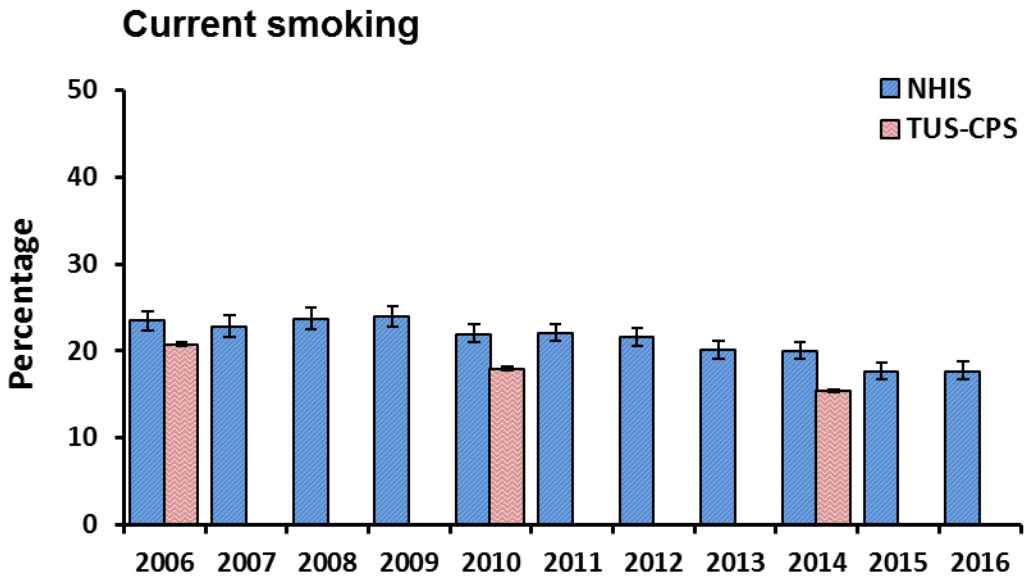
	NHIS			TUS-CPS		
Characteristic	**N**	**Weighted Percent**	**Standard Error**	**N**	**Weighted Percent**	**Standard Error**
Total	113,512			172,577		
Sex						
Male	51,189	47.3	0.2007	76,962	49.5	0.0045
Female	62,323	52.7	0.2007	95,615	50.5	0.0045
Age						
25-29	28,686	26.4	0.2217	40,225	25.6	0.0038
30-34	29,290	25.3	0.1565	42,660	24.5	0.0046
35-39	27,942	24.0	0.1608	43,950	24.4	0.004
40-44	27,594	24.3	0.1788	45,742	25.5	0.0043
Race/ethnicity						
White	60,580	62.5	0.3468	117,033	60.5	0.02
Hispanic or Latino	26,113	16.9	0.2679	25,030	18.9	0.0038
Black/African-American	17,071	13.7	0.2529	17,099	12.3	0.0152
Asian	8,206	5.7	0.1263	9,087	6.3	0.0158
Alaskan Native or American Indian	883	0.7	0.0543	1,708	0.6	0.0179
Multiple Race, No Primary Race Selected	340	0.3	0.0172	2,620	1.3	0.0153
Primary Race not releasable	319	0.2	0.0184	-	-	-
Educational attainment						
Bachelor's degree or higher	37,911	35.9	0.3518	62,717	36.1	0.1005
Some college	34,067	30.4	0.2273	49,954	28.1	0.0744
High school diploma or GED	25,353	22.0	0.2127	43,701	25.2	0.0746
Less than high school	15,656	11.3	0.1923	16,205	10.5	0.0578
Unknown	525	0.4	0.031	-	-	-
Employment Status						
Employed	87,848	78.8	0.1931	135,779	78.3	0.0537

Unemployed	6,903	5.7	0.0851	8,812	5.5	0.0341
Not in labor force	18,686	15.5	0.1641	27,986	16.2	0.0473
Unknown	75	0.1	0.0098	-	-	-
Geographic region						
Northeast	17,372	16.2	0.3384	30,697	18.2	0.0397
North Central/Midwest	24,036	23.9	0.4409	40,213	21.2	0.0378
South	41,208	37.0	0.4432	57,133	36.4	0.0527
West	30,896	22.9	0.3678	44,534	24.2	0.0405
Annual household income						
At or above poverty threshold	87,985	79.7	0.2214	-	-	-
Below poverty threshold	18,103	13.7	0.1769	-	-	-
<$25,000	-	-	-	33,108	19.8	0.0773
$25,000-$49,999	-	-	-	44,398	25.9	0.0797
$50,000 or more	-	-	-	89,484	50.9	0.0935
Unknown	7,424	6.6	0.1164	5,587	3.4	0.0345
Survey Year						
2006	8,974	9.5	0.1566	60,815	33.5	0.0052
2007	8,398	9.1	0.1314	-	-	-
2008	7,830	8.7	0.14	-	-	-
2009	9,917	9.3	0.138	-	-	-
2010	9,723	8.8	0.1292	58,369	33.0	0.0052
2011	11,724	9.4	0.1179	-	-	-
2012	11,786	8.9	0.1096	-	-	-
2013	11,931	9.2	0.1079	-	-	-
2014	12,321	9.1	0.1142	53,393	33.5	0.0049
2015	11,029	9	0.1336	-	-	-
2016	9,879	9	0.2178	-	-	-

2.3.2 Current Smoking 2006-2016

In the NHIS current smoking among 25-44 year olds decreased from 23.5% in 2006 to 17.7% in 2016 (Figure 1).

Figure 1. Prevalence of current smoking in 25-44 year olds, NHIS 2006-2016 and TUS-CPS 2006-2007, 2010-2011, and 2014-2015



After adjusting for demographic variables, there was no change in the odds of current smoking from 2006 to 2012 relative to the year 2006. Only the years 2013 and beyond showed a statistically significant decrease in current smoking compared to 2006 ($p<0.01$ for all comparisons; Table 2).

Table 2. Adjusted Odds Ratios for Current Smoking, Ever Smoking, and Former Smoking, NHIS 2006-2016

	Current Smoking			Ever Smoking			Former Smoking		
	aOR	95% CI	P-Value	aOR	95% CI	P-Value	aOR	95% CI	P-Value
Year									
2006	ref			ref			ref		
2007	0.99	0.90-1.09	0.8289	1.01	0.93-1.10	0.7678	1.05	0.91-1.22	0.4831
2008	1.05	0.96-1.16	0.2888	1.10	1.01-1.19	0.0273	1.04	0.91-1.19	0.5407
2009	1.04	0.95-1.13	0.369	1.08	1.00-1.17	0.0597	1.06	0.93-1.20	0.4262
2010	0.94	0.86-1.03	0.154	1.02	0.94-1.10	0.6715	1.19	1.05-1.35	0.0074
2011	0.93	0.85-1.02	0.1201	1.00	0.93-1.08	0.9208	1.17	1.03-1.32	0.014
2012	0.93	0.85-1.01	0.0856	1.00	0.92-1.09	0.9745	1.17	1.03-1.32	0.0128
2013	0.87	0.79-0.95	0.0015	1.01	0.93-1.10	0.7636	1.33	1.18-1.51	<.0001
2014	0.89	0.81-0.97	0.0064	1.01	0.93-1.09	0.9103	1.28	1.13-1.45	0.0001
2015	0.78	0.71-0.85	<.0001	0.94	0.87-1.02	0.144	1.51	1.32-1.73	<.0001
2016	0.79	0.71-0.87	<.0001	0.997	1.00-1.08	0.9366	1.52	1.34-1.74	<.0001
Sex									
Male	ref			ref			ref		
Female	0.71	0.69-0.74	<.0001	0.67	0.64-0.69	<.0001	1.01	0.95-1.06	0.8073
Age									
25-29	ref			ref			ref		
30-34	0.94	0.90-0.99	0.0258	1.17	1.11-1.22	<.0001	1.35	1.26-1.45	<.0001
35-39	0.86	0.81-0.90	<.0001	1.13	1.08-1.18	<.0001	1.53	1.41-1.66	<.0001
40-44	0.84	0.80-0.89	<.0001	1.11	1.06-1.16	<.0001	1.58	1.46-1.70	<.0001
Race/ethnicity									
White	ref			ref			ref		

Hispanic or Latino	0.28	0.26-0.30	<.0001	0.29	0.28-0.31	<.0001	1.51	1.39-1.65	<.0001
Black/African-American	0.55	0.52-0.58	<.0001	0.39	0.37-0.41	<.0001	0.64	0.58-0.70	<.0001
Asian	0.50	0.45-0.56	<.0001	0.43	0.39-0.46	<.0001	0.86	0.75-0.99	0.0293
Alaskan Native or American Indian	1.18	0.95-1.46	0.1289	1.28	1.03-1.61	0.0293	1.00	0.74-1.34	0.9736
Multiple Race, No Primary Race Selected	0.89	0.61-1.30	0.5509	1.02	0.76-1.39	0.8798	1.20	0.73-1.98	0.4665
Primary Race not releasable	0.85	0.55-1.32	0.4626	0.58	0.40-0.84	0.0042	0.56	0.28-1.11	0.0957
Educational attainment									
Bachelor's degree or higher	ref			ref			ref		
Some college	3.47	3.27-3.68	<.0001	2.70	2.57-2.82	<.0001	0.48	0.45-0.52	<.0001
High school diploma or GED	5.28	4.97-5.62	<.0001	3.44	3.27-3.61	<.0001	0.31	0.29-0.34	<.0001
Less than high school	6.62	6.14-7.13	<.0001	3.74	3.51-3.98	<.0001	0.24	0.22-0.27	<.0001
Unknown	2.85	2.03-4.01	<.0001	1.84	1.41-2.39	<.0001	0.38	0.23-0.63	0.0002
Employment Status									
Employed	ref			ref			ref		
Unemployed	1.86	1.73-2.00	<.0001	1.54	1.44-1.65	<.0001	0.54	0.49-0.60	<.0001
Not in labor force	1.07	1.01-1.13	0.016	1.02	0.97-1.07	0.4466	0.92	0.86-1.00	0.0413
Unknown	0.93	0.45-1.93	0.8424	0.96	0.50-1.84	0.9003	0.89	0.35-2.30	0.816
Geographic region									
Northeast	ref			ref			ref		
North Central/Midwest	1.14	1.05-1.23	0.0012	1.05	0.98-1.11	0.1586	0.85	0.78-0.93	0.0004
South	1.10	1.02-1.18	0.0109	0.98	0.92-1.04	0.4273	0.83	0.76-0.90	<.0001
West	0.83	0.77-0.91	<.0001	0.85	0.80-0.91	<.0001	1.13	1.03-1.24	0.0111
Annual household income									
At or above poverty threshold	ref			ref			ref		
Below poverty threshold	1.60	1.51-1.69	<.0001	1.36	1.29-1.43	<.0001	0.62	0.58-0.68	<.0001
Unknown	0.92	0.84-1.00	0.0436	0.82	0.76-0.88	<.0001	0.88	0.77-0.99	0.0311

aOR=Adjusted Odds Ratio

CI=Confidence Interval

A statistically significant decrease in current smoking was seen earlier in the TUS-CPS dataset, but the overall trend was the same. In 2006-2007, 20.8% of 25-44 year olds were current smokers, compared to only 15.4% in 2014-2015 (Figure 1). Using the 2006-2007 survey as a reference, the odds of current smoking significantly decreased in 2010-2011 (adjusted Odds Ratio aOR=0.80, 95% CI: 0.78-0.82, p<0.0001) and in 2014-2015 (aOR=0.75, 95% CI: 0.73-0.76, p<0.0001); Table 3).

Table 3. Adjusted Odds Ratios for Current Smoking, Ever Smoking, and Former Smoking, TUS-CPS 2006-2007, 2010-2011, 2014-2015

	Current Smoking			Ever Smoking			Former Smoking		
	aOR	95% CI	P-Value	aOR	95% CI	P-Value	aOR	95% CI	P-Value
Year									
2006-2007	ref			ref			ref		
2010-2011	0.80	0.78-0.82	<.0001	0.84	0.82-0.85	<.0001	1.18	1.15-1.21	<.0001
2014-2015	0.75	0.73-0.76	<.0001	0.84	0.83-0.86	<.0001	1.35	1.31-1.38	<.0001
Sex									
Male	ref			ref			ref		
Female	0.75	0.73-0.76	<.0001	0.71	0.71-0.72	<.0001	1.02	0.99-1.04	0.1633
Age									
25-29	ref			ref			ref		
30-34	1.01	0.99-1.03	0.4843	1.13	1.11-1.16	<.0001	1.16	1.12-1.20	<.0001
35-39	0.94	0.92-0.96	<.0001	1.12	1.10-1.14	<.0001	1.28	1.23-1.32	<.0001
40-44	0.93	0.91-0.95	<.0001	1.12	1.10-1.14	<.0001	1.34	1.30-1.39	<.0001
Race/ethnicity									
White	ref			ref			ref		
Hispanic or Latino	0.26	0.26-0.27	<.0001	0.28	0.27-0.28	<.0001	1.40	1.35-1.45	<.0001
Black/African-American	0.44	0.43-0.45	<.0001	0.34	0.33-0.34	<.0001	0.73	0.69-0.76	<.0001
Asian	0.54	0.52-0.57	<.0001	0.42	0.40-0.43	<.0001	0.65	0.61-0.70	<.0001
Alaskan Native or American Indian	1.04	0.95-1.14	0.4189	0.96	0.88-1.04	0.2813	0.85	0.76-0.95	0.004
Multiple Race, No Primary Race Selected	1.15	1.07-1.22	<.0001	1.12	1.07-1.18	<.0001	0.90	0.83-0.99	0.0235

Educational attainment									
Bachelor's degree or higher	ref			ref			ref		
Some college	3.05	2.98-3.11	<.0001	2.38	2.34-2.41	<.0001	0.52	0.51-0.54	<.0001
High school diploma or GED	4.34	4.24-4.44	<.0001	2.88	2.82-2.94	<.0001	0.35	0.34-0.36	<.0001
Less than high school	5.54	5.28-5.63	<.0001	3.27	3.18-3.35	<.0001	0.29	0.27-0.30	<.0001
Employment Status									
Employed	ref			ref			ref		
Unemployed	1.69	1.64-1.74	<.0001	1.56	1.52-1.61	<.0001	0.71	0.68-0.75	<.0001
Not in labor force	1.02	1.00-1.03	0.1429	0.97	0.96-0.99	0.001	0.96	0.93-0.99	0.0071
Geographic region									
Northeast	ref			ref			ref		
North Central/Midwest	1.14	1.11-1.17	<.0001	1.07	1.05-1.10	<.0001	0.90	0.87-0.92	<.0001
South	1.02	1.00-1.05	0.133	0.91	0.89-0.93	<.0001	0.84	0.82-0.87	<.0001
West	0.80	0.78-0.82	<.0001	0.84	0.82-0.85	<.0001	1.18	1.14-1.22	<.0001
Annual household income									
$50,000 or more	ref			ref			ref		
$25,000-$49,999	1.63	1.60-1.66	<.0001	1.31	1.28-1.33	<.0001	0.62	0.60-0.63	<.0001
<$25,000	2.25	2.20-2.31	<.0001	1.57	1.54-1.61	<.0001	0.42	0.40-0.43	<.0001
Unknown	1.11	1.06-1.15	<.0001	0.85	0.82-0.88	<.0001	0.67	0.63-0.72	<.0001

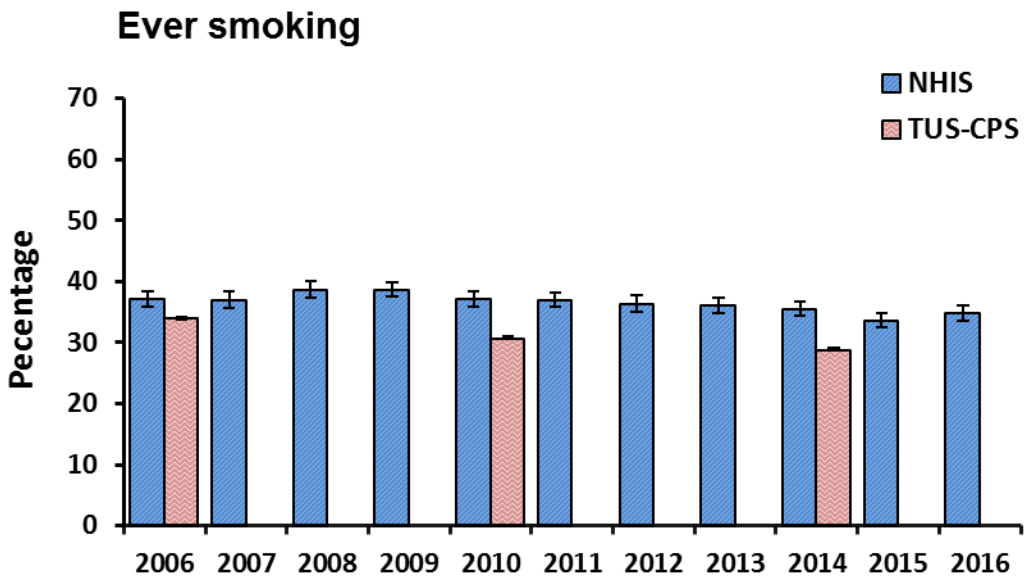
aOR=Adjusted Odds Ratio

CI=Confidence Interval

2.3.3 Ever Smoking 2006-2016

A decrease in ever smoking was also observed in the NHIS, starting at 37.2% of the sample in 2006 and falling to 31.4% in 2016 (Figure 2).

Figure 2. Prevalence of ever smoking (smoking 100 cigarettes lifetime) in 25-44 year olds, NHIS 2006-2016 and TUS-CPS 2006-2007, 2010-2011, and 2014-2015

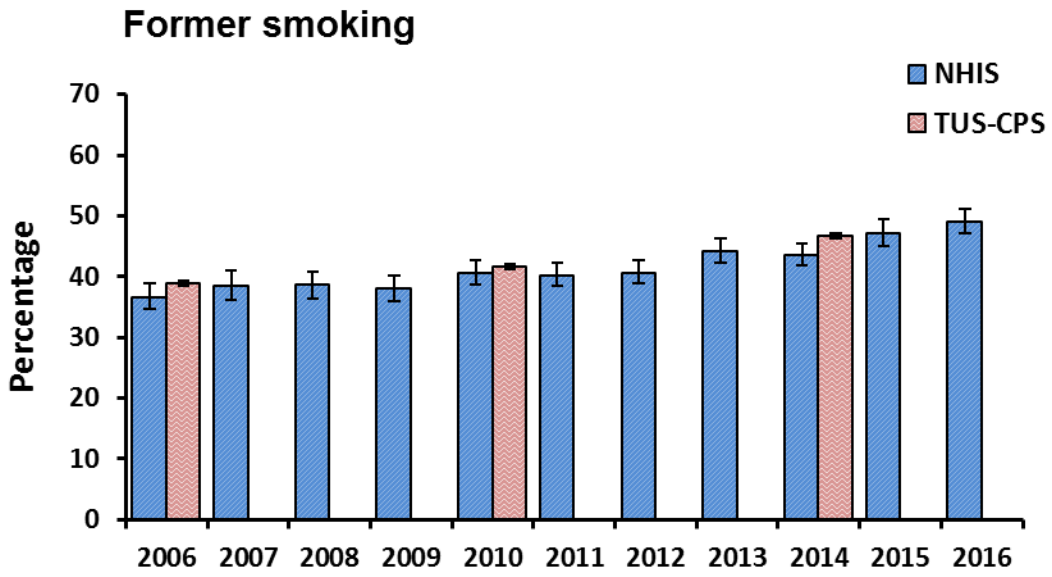


After adjusting for demographic variables, the only significant difference in ever smoking relative to 2006 was a slight increase in 2008 (aOR=1.10, 95% CI=1.01-1.19, p<0.03; Table 2). All other years from 2007 to 2016 showed no significant change in ever smoking compared to 2006. In the TUS-CPS sample, ever smoking fell from 34.0% in 2006-2007 to 28.8% in 2014-2015 (Figure 2). In contrast to the NHIS results, in multivariable logistic regression, relative to the 2006-2007 survey period, ever smoking significantly decreased in 2010-2011 (aOR=0.84, 95% CI: 0.82-0.85, p<0.0001) and in 2014-2015 (aOR=0.84, 95% CI: 0.83-0.86, p<0.0001; Table 3).

2.3.4 Former Smoking 2006-2016

In contrast to the decrease in current smoking and ever smoking, former smoking increased in both datasets from 2006 compared to 2016. In the NHIS sample, former smoking increased from 36.7% in 2006 to 49.1% in 2016 (Figure 3).

Figure 3. Prevalence of former smoking in 25-44 year olds, NHIS 2006-2016 and TUS-CPS 2006-2007, 2010-2011, and 2014-2015

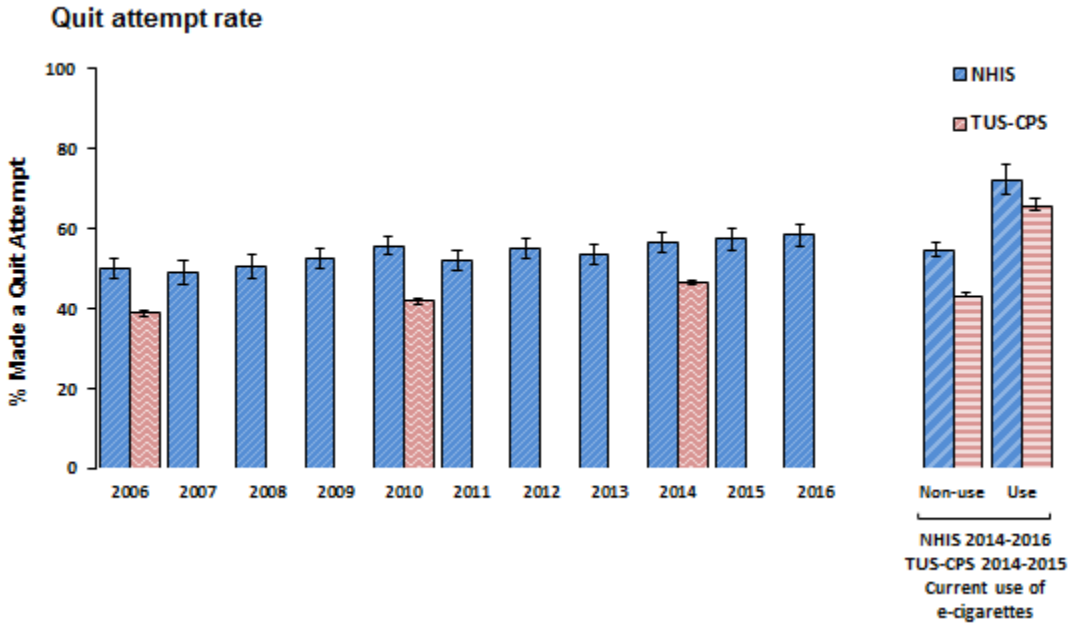


Multivariable logistic regression results for former smoking adjusted for demographic variables are shown in Table 2. Relative to 2006, former smoking significantly increased in years 2010 and beyond ($p<0.05$ for all comparisons). Likewise, in the TUS-CPS dataset, former smoking increased from 38.9% in 2006-2007 to 46.7% in 2014-2015 (Figure 3). Multivariable logistic regression results indicated that former smoking increased significantly in 2010-2011 (aOR=1.18, 95% CI: 1.15-1.21, $p<0.0001$) and 2014-2015 (aOR=1.35, 95% CI: 1.31-1.38, $p<0.0001$) relative to 2006-2007 (Table 3).

2.3.2 Past 12 Month Quit Attempts 2006-2016

In the NHIS dataset, past 12 month quit attempts increased from 49.9% in 2006 to 58.0% in 2016 (Figure 4).

Figure 4. Past 12 month quit attempts and smoking cessation in 25-44 year olds, NHIS and TUS-CPS



Quit attempt rate (defined as having tried to quit smoking and stopping smoking for at least 1 day during the past 12 months). NHIS data are shown for 2006-2016 and TUS-CPS data are shown for 2006-2007, 2010-2011, and 2014-2015. For survey years containing data on e-cigarette use (2014-2016 for NHIS; 2014-2015 for TUS-CPS), the quit attempt rate is stratified according to current e-cigarette use.

Multivariable regression analyses showed that past 12 month quit attempts increased significantly in 2014, 2015, and 2016 relative to the year 2006 ($p<0.05$ for all comparisons) (Table 4).

Table 4. Adjusted Odds Ratios for Past 12 Month Quit Attempts and Past 12 Month Smoking Cessation, NHIS 2006-2016

	Quit Attempt[1]			Smoking Cessation[2]		
	aOR	95% CI	P-Value	aOR	95% CI	P-Value
Year						
2006	ref			ref		
2007	0.92	0.79-1.06	0.25	1.16	0.79-1.70	0.44
2008	0.96	0.82-1.11	0.58	1.1	0.78-1.56	0.59
2009	1.04	0.90-1.20	0.62	1.17	0.85-1.61	0.33
2010	1.16	1.01-1.34	0.04	1.2	0.85-1.70	0.29
2011	1	0.87-1.14	0.98	1.21	0.89-1.65	0.23
2012	1.13	0.98-1.29	0.09	1.37	1.01-1.84	0.04
2013	1.07	0.94-1.23	0.31	1.33	0.97-1.81	0.08
2014	1.2	1.03-1.39	0.02	1.35	0.97-1.89	0.08
2015	1.26	1.08-1.46	0.003	1.39	0.99-1.95	0.05
2016	1.26	1.08-1.47	0.003	1.89	1.38-2.59	<0.0001
Sex						
Male	ref			ref		
Female	1.1	1.03-1.18	0.007	1.09	0.95-1.26	0.23
Age						
25-29	ref			ref		
30-34	1	0.92-1.09	0.99	1	0.83-1.20	0.97
35-39	0.88	0.80-0.96	0.005	0.71	0.59-0.85	0.0002
40-44	0.83	0.76-0.90	<0.0001	0.59	0.48-0.73	<0.0001
Race/ethnicity						
White	ref			ref		
Hispanic or Latino	1.36	1.24-1.49	<0.0001	1.26	1.02-1.57	0.03
Black/African-American	1.36	1.24-1.50	<0.0001	0.64	0.51-0.80	0.0001
Asian	1.22	1.03-1.44	0.02	1.07	0.75-1.51	0.72

Alaskan Native or American Indian	0.78	0.57-1.07	0.13	0.45	0.20-1.02	0.06
Multiple Race, No Primary Race Selected	0.91	0.53-1.56	0.74	1.15	0.43-3.07	0.79
Primary Race not releasable	0.62	0.32-1.19	0.15	0.17	0.04-0.79	0.02
Educational attainment						
Bachelor's degree or higher	ref			ref		
Some college	0.91	0.83-1.00	0.05	0.63	0.52-0.76	<0.0001
High school diploma or GED	0.67	0.61-0.74	<0.0001	0.46	0.38-0.56	<0.0001
Less than high school	0.6	0.54-0.68	<0.0001	0.4	0.30-0.53	<0.0001
Unknown	0.65	0.35-1.20	0.17	0.16	0.04-0.64	0.009
Employment Status						
Employed	ref			ref		
Unemployed	1.04	0.94-1.16	0.46	0.75	0.56-0.99	0.04
Not in labor force	0.93	0.85-1.02	0.13	1.03	0.85-1.26	0.75
Geographic region						
Northeast	ref			ref		
North Central/Midwest	0.89	0.80-0.99	0.03	0.84	0.67-1.07	0.16
South	0.82	0.74-0.91	<0.0001	0.9	0.72-1.13	0.36
West	1	0.89-1.12	0.97	1.2	0.96-1.51	0.11
Annual household income						
At or above poverty threshold	ref			ref		
Below poverty threshold	0.94	0.87-1.01	0.11	0.71	0.57-0.88	0.002
Unknown	0.69	0.60-0.79	<0.0001	0.82	0.59-1.13	0.22

In the TUS-CPS dataset, past 12 month quit attempts increased from 38.6% in 2006-2007 to 46.3% in 2014-2015 (Figure 4). Compared to 2006-2007, past 12 month quit attempts increased significantly in 2010-2011 (aOR=1.07, 95% CI: 1.04-1.11, p=0.0002) and in 2014-2015 (aOR=1.29, 95% CI: 1.24-1.34, p<0.0001) (Table 5).

Table 5. Adjusted Odds Ratios for Past 12 Month Quit Attempts and Past 12 Month Smoking Cessation, TUS-CPS 2006-2007, 2010-2011, 2014-2015

	Quit Attempts[1]			Smoking Cessation[2]		
	aOR	95% CI	P-Value	aOR	95% CI	P-Value
Year						
2006-2007	ref			ref		
2010-2011	1.07	1.04-1.11	0.0002	1.05	0.97-1.13	0.22
2014-2015	1.29	1.24-1.34	<0.0001	1.24	1.15-1.34	<0.0001
Smoking Frequency						
Some days	ref			ref		
Every day	0.89	0.86-0.92	<0.0001	0.82	0.76-0.87	<0.0001
Sex						
Male	ref			ref		
Female	1.09	1.06-1.12	<0.0001	1.07	1.01-1.13	0.03
Age						
25-29	ref			ref		
30-34	0.91	0.88-0.94	<0.0001	0.97	0.90-1.05	0.43
35-39	0.79	0.76-0.82	<0.0001	0.76	0.70-0.83	<0.0001
40-44	0.78	0.75-0.80	<0.0001	0.60	0.55-0.65	<0.0001
Race/ethnicity						
White	ref			ref		
Hispanic or Latino	1.04	0.99-1.09	0.12	1.34	1.22-1.48	<0.0001
Black/African-American	1.19	1.13-1.24	<0.0001	0.87	0.76-0.99	0.03

Asian	0.95	0.87-1.04	0.26	0.69	0.57-0.82	<0.0001
Alaskan Native or American Indian	1.13	1.02-1.26	0.02	0.70	0.51-0.98	0.04
Multiple Race, No Primary Race Selected	1.25	1.13-1.39	<0.0001	0.59	0.47-0.74	<0.0001
Educational attainment						
Bachelor's degree or higher	ref			ref		
Some college	1.04	0.99-1.09	0.15	0.76	0.71-0.83	<0.0001
High school diploma or GED	0.84	0.80-0.88	<0.0001	0.50	0.46-0.54	<0.0001
Less than high school	0.66	0.63-0.70	<0.0001	0.34	0.29-0.39	<0.0001
Employment Status						
Employed	ref			ref		
Unemployed	1.06	1.01-1.11	0.03	0.98	0.87-1.09	0.66
Not in labor force	1.03	0.99-1.07	0.11	1.17	1.07-1.29	0.0008
Geographic region						
Northeast	ref			ref		
North Central/Midwest	0.91	0.87-0.96	0.0003	0.95	0.86-1.05	0.31
South	0.84	0.81-0.87	<0.0001	0.85	0.77-0.94	0.0009
West	0.98	0.94-1.03	0.44	1.09	0.98-1.21	0.10
Annual household income						
$50,000 or more	ref			ref		
$25,000-$49,999	0.94	0.91-0.97	<0.0001	0.82	0.77-0.87	<0.0001
<$25,000	0.89	0.86-0.93	<0.0001	0.54	0.49-0.59	<0.0001
Unknown	0.59	0.54-0.64	<0.0001	0.56	0.45-0.71	<0.0001

aOR=Adjusted Odds Ratio

CI=Confidence Interval

[1]Defined as having tried to quit smoking and stopping smoking for at least 1 day during the past 12 months.

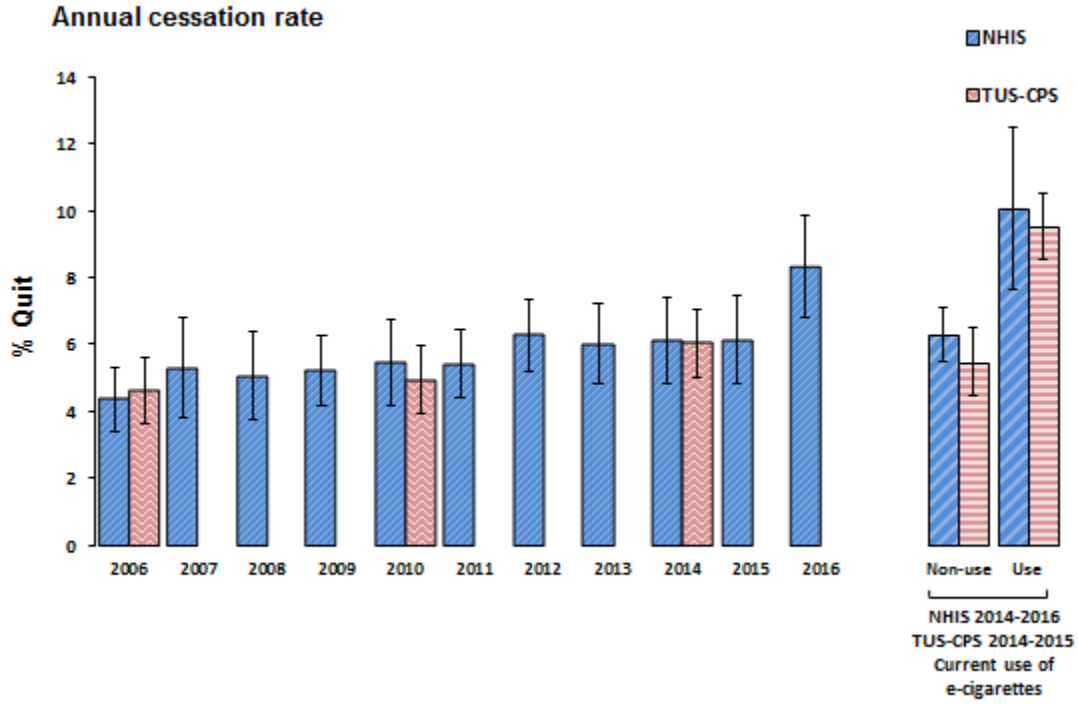
[2]Defined as quitting during the past 12 months and remaining abstinent for at least three months at the time of the interview.

In the TUS-CPS dataset, analyses included a comparison between every day smokers and some-day smokers. Those who smoked every day were significantly less likely to make a quit attempt in the past 12 months (aOR=0.89, 95% CI: 0.86-0.92, p<0.0001) compared to some-day smokers.

2.3.3 Past 12 Month Smoking Cessation 2006-2016

From 2006 to 2016, past 12 month smoking cessation increased from 4.4% to 8.3% in the NHIS sample (Figure 5).

Figure 5. Past 12 month quit attempts and smoking cessation in 25-44 year olds, NHIS and TUS-CPS



Annual cessation rate (defined as quitting during the past 12 months and remaining abstinent for at least 3 months at the time of interview). NHIS data are shown for 2006-2016 and TUS-CPS data are shown for 2006-2007, 2010-2011, and 2014-2015. For survey years containing data on

e-cigarette use (2014-2016 for NHIS; 2014-2015 for TUS-CPS), the cessation rate is stratified according to current e-cigarette use.

Multivariable logistic regression results indicated that the only significant increase in annual cessation occurred in 2016 relative to 2006 (aOR=1.89, 95% CI: 1.38-2.59, p<0.0001) (Table 2). Similar trends were seen in the TUS-CPS, with annual cessation increasing from 4.6% in 2006-2007 to 6.0% in 2014-2015 (Figure 6).

Compared to 2006-2007, the only significant increase in annual cessation was seen in the most recent survey period 2014-2015 (aOR=1.24, 95% CI: 1.15-1.34, p<0.0001) (Table 6). Every day smokers were less likely to report past 12 month cessation compared to some-day smokers (aOR=0.82, 95% CI: 0.76-0.87, p<0.0001).

Table 6. Adjusted Odds Ratios for Past 12 Month Quit Attempts and Past 12 Month Smoking Cessation, TUS-CPS 2006-2007, 2010-2011, 2014-2015

	Quit Attempts[1]			Smoking Cessation[2]		
	aOR	95% CI	P-Value	aOR	95% CI	P-Value
Year						
2006-2007	ref			ref		
2010-2011	1.07	1.04-1.11	0.0002	1.05	0.97-1.13	0.22
2014-2015	1.29	1.24-1.34	<0.0001	1.24	1.15-1.34	<0.0001
Smoking Frequency						
Some days	ref			ref		
Every day	0.89	0.86-0.92	<0.0001	0.82	0.76-0.87	<0.0001
Sex						
Male	ref			ref		
Female	1.09	1.06-1.12	<0.0001	1.07	1.01-1.13	0.03

Age						
25-29	ref			ref		
30-34	0.91	0.88-0.94	<0.0001	0.97	0.90-1.05	0.43
35-39	0.79	0.76-0.82	<0.0001	0.76	0.70-0.83	<0.0001
40-44	0.78	0.75-0.80	<0.0001	0.60	0.55-0.65	<0.0001
Race/ethnicity						
White	ref			ref		
Hispanic or Latino	1.04	0.99-1.09	0.12	1.34	1.22-1.48	<0.0001
Black/African-American	1.19	1.13-1.24	<0.0001	0.87	0.76-0.99	0.03
Asian	0.95	0.87-1.04	0.26	0.69	0.57-0.82	<0.0001
Alaskan Native or American Indian	1.13	1.02-1.26	0.02	0.70	0.51-0.98	0.04
Multiple Race, No Primary Race Selected	1.25	1.13-1.39	<0.0001	0.59	0.47-0.74	<0.0001
Educational attainment						
Bachelor's degree or higher	ref			ref		
Some college	1.04	0.99-1.09	0.15	0.76	0.71-0.83	<0.0001
High school diploma or GED	0.84	0.80-0.88	<0.0001	0.50	0.46-0.54	<0.0001
Less than high school	0.66	0.63-0.70	<0.0001	0.34	0.29-0.39	<0.0001
Employment Status						
Employed	ref			ref		
Unemployed	1.06	1.01-1.11	0.03	0.98	0.87-1.09	0.66
Not in labor force	1.03	0.99-1.07	0.11	1.17	1.07-1.29	0.0008
Geographic region						
Northeast	ref			ref		
North Central/Midwest	0.91	0.87-0.96	0.0003	0.95	0.86-1.05	0.31
South	0.84	0.81-0.87	<0.0001	0.85	0.77-0.94	0.0009
West	0.98	0.94-1.03	0.44	1.09	0.98-1.21	0.10
Annual household income						

$50,000 or more	ref			ref		
$25,000-$49,999	0.94	0.91-0.97	<0.0001	0.82	0.77-0.87	<0.0001
<$25,000	0.89	0.86-0.93	<0.0001	0.54	0.49-0.59	<0.0001
Unknown	0.59	0.54-0.64	<0.0001	0.56	0.45-0.71	<0.0001

aOR=Adjusted Odds Ratio

CI=Confidence Interval

[1]Defined as having tried to quit smoking and stopping smoking for at least 1 day during the past 12 months.

[2]Defined as quitting during the past 12 months and remaining abstinent for at least three months at the time of the interview.

2.3.4 Relation of Past 12 Month Quit Attempts and E-Cigarette Use 2014-2016

I further focused on the NHIS surveys from 2014-2016 to examine the relation of past 12 month quit attempts and e-cigarette use. Of these respondents, 15.2% (N=1,045) were current e-cigarette users. Past 12 month quit attempts for the years 2014-2016 for smokers who were not currently using e-cigarettes at the time of the survey (54.5%) were similar to those in previous years (50.0% in 2006 to 53.3% in 2013) (Figure 1). However, among those using e-cigarettes at the time of the survey, 72.0% reported making a quit attempt during the past 12 months. Multivariable regression analyses for the years 2014-2016 revealed that past 12 month quit attempts for current e-cigarette users were significantly higher than for non-users (aOR=2.29, 95% CI: 1.87-2.81, p<0.0001) (results of the full model are included in Table 7).

Table 7. Adjusted Odds Ratios for Past 12 Month Quit Attempts and Past 12 Month Smoking Cessation, NHIS 2014-2016

	Quit Attempt[1]			Smoking Cessation[2]		
	aOR	95% CI	P-Value	aOR	95% CI	P-Value
E-cigarettes						
No Current Use	ref			ref		
Current Use	2.29	1.87-2.81	<0.0001	1.64	1.21-2.21	0.001
Year						
2014	ref			ref		
2015	1.04	0.90-1.22	0.59	1.01	0.74-1.38	0.94
2016	1.09	0.94-1.28	0.26	1.44	1.06-1.94	0.02
Sex						
Male	ref			ref		
Female	1.12	0.98-1.27	0.11	1.12	0.85-1.46	0.42
Age						
25-29	ref			ref		
30-34	0.83	0.69-0.99	0.04	0.89	0.64-1.22	0.45
35-39	0.80	0.66-0.97	0.02	0.82	0.59-1.13	0.22
40-44	0.71	0.59-0.86	0.0003	0.62	0.43-0.88	0.008
Race/ethnicity						
White	ref			ref		
Hispanic or Latino	1.39	1.13-1.71	0.002	1.47	1.02-2.12	0.04
Black/African-American	1.61	1.31-1.97	<0.0001	0.43	0.26-0.70	0.0008
Asian	1.49	1.02-2.17	0.04	1.57	0.94-2.61	0.09
Alaskan Native or American Indian	0.44	0.23-0.82	0.01	0.16	0.05-0.52	0.002
Multiple Race, No Primary Race Selected	1.14	0.46-2.80	0.78	1.22	0.26-5.74	0.80
Primary Race not releasable	1.81	0.64-5.17	0.26	0.03	0.003-0.20	0.0005

Educational attainment						
Bachelor's degree or higher	ref			ref		
Some college	0.95	0.80-1.14	0.59	0.89	0.64-1.24	0.48
High school diploma or GED	0.80	0.65-0.97	0.03	0.52	0.36-0.76	0.0008
Less than high school	0.70	0.55-0.88	0.002	0.49	0.28-0.84	0.01
Unknown	0.31	0.10-0.97	0.04	0.18	0.02-1.44	0.11
Employment Status						
Employed	ref			ref		
Unemployed	0.88	0.69-1.11	0.27	0.77	0.48-1.24	0.27
Not in labor force	0.88	0.73-1.05	0.15	1.11	0.76-1.62	0.61
Geographic region						
Northeast	ref			ref		
North Central/Midwest	0.89	0.72-1.11	0.30	0.85	0.53-1.36	0.50
South	0.91	0.74-1.13	0.39	1.22	0.79-1.88	0.37
West	1.04	0.82-1.32	0.73	1.63	1.05-2.53	0.03
Annual household income						
At or above poverty threshold	ref			ref		
Below poverty threshold	0.98	0.83-1.16	0.85	0.64	0.42-0.98	0.04
Unknown	0.55	0.36-0.82	0.004	0.66	0.31-1.39	0.27

aOR=Adjusted Odds Ratio

CI=Confidence Interval

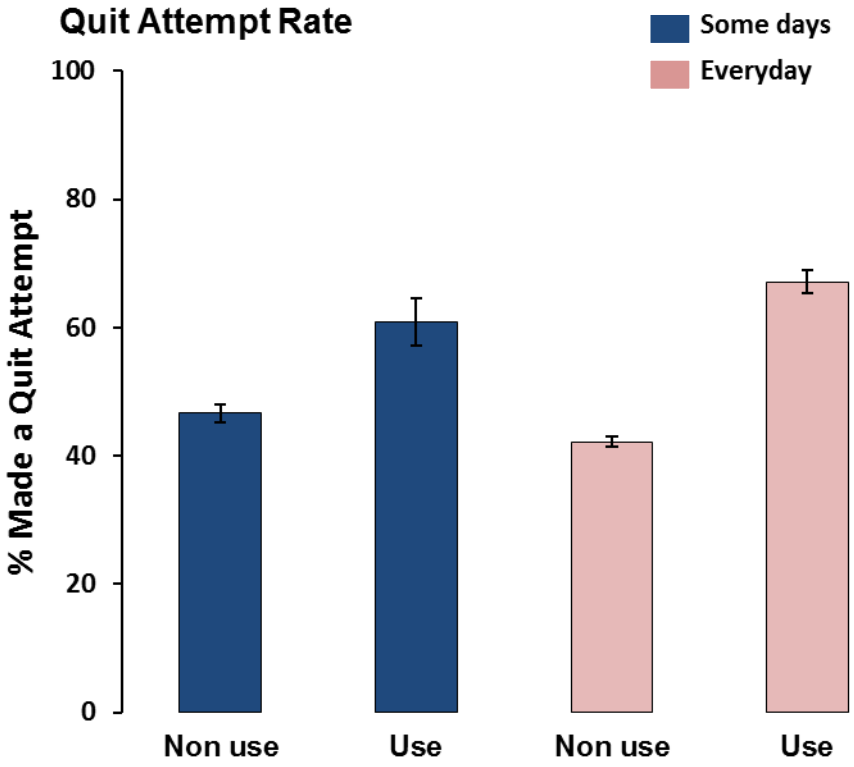
[1]Defined as having tried to quit smoking and stopping smoking for at least 1 day during the past 12 months.

[2]Defined as quitting during the past 12 months and remaining abstinent for at least three months at the time of the interview.

Similar findings were seen in the TUS-CPS dataset, with 13.5% (N=1,215) of respondents reporting current e-cigarette use. The prevalence of past 12 month quit attempts for smokers

who were not using e-cigarettes at the time of the 2014-2015 survey was 43.3%, which was similar to the prevalence in 2006-2007 (38.6%) and 2010-2011 (41.5%). Among smokers who were current e-cigarette users in 2014-2015, 65.8% had made a past 12 month quit attempt (Supplementary Figure 1a). The TUS-CPS dataset had additional information on smoking frequency (every day versus some days) in those who quit in the past 12 months. Combustible cigarette smokers were divided into those who smoked some days and those who smoked every day and examined past 12 month quit attempts and current e-cigarette use. Current e-cigarette use at the time of the survey was associated with increased past 12 month quit attempts in both some-day and every day combustible cigarette smokers. Among some-day smokers, 60.9% who currently used e-cigarettes reported making a quit attempt during the past 12 months compared to 46.7% of those who did not use e-cigarettes (Figure 6). Among every day smokers, a past 12 month quit attempt was made by 67.1% of those who used e-cigarettes and by 42.2% of those who did not use e-cigarettes (Figure 6).

Figure 6. Past 12 month quit attempts and smoking cessation in 25-44 year olds, by combustible cigarette smoking frequency and e-cigarette use, TUS-CPS 2014-2015



Quit attempt rate (defined as having tried to quit smoking and stopping smoking for at least 1 day during the past 12 months). Quit attempt rate is stratified according to current e-cigarette use.

A multivariable logistic regression of the TUS-CPS 2014-2015 data revealed a significant interaction between smoking frequency (every day and some-day smoking) and current e-cigarette use ($p<0.0001$) in influencing past 12 month quit attempts. Some-day smokers who were not current e-cigarette users served as the reference group. Some-day smokers who currently used e-cigarettes were more likely to make past 12 month quit attempts (aOR=1.70, 95% CI: 1.43-2.01, $p<0.0001$; Table 7) as were every day smokers who used e-cigarettes (aOR=2.42, 95% CI: 2.18-2.67, $p<0.0001$). Every day smokers who were not currently using e-

cigarettes were less likely to make a quit attempt in the past 12 months (aOR=0.86, 95% CI: 0.81-0.92, p<0.0001).

Table 8. Adjusted Odds Ratios for Past 12 Month Quit Attempts and Past 12 Month Smoking Cessation, TUS-CPS 2014-2015

	Quit Attempts[1]			Smoking Cessation[2]		
	aOR	95% CI	P-Value	aOR	95% CI	P-Value
Smoking Frequency x E-Cigarette Use						
Some day smoker, no e-cigarette use	ref			ref		
Some day smoker, e-cigarette use	1.70	1.43-2.01	<0.0001	0.74	0.54-1.02	0.06
Every day smoker, no e-cigarette use	0.86	0.81-0.92	<0.0001	0.86	0.75-0.98	0.03
Every day smoker, e-cigarette use	2.42	2.18-2.67	<0.0001	1.89	1.57-2.28	<0.0001
Sex						
Male	ref			ref		
Female	1.03	0.98-1.08	0.28	0.83	0.75-0.92	0.0005
Age						
25-29	ref			ref		
30-34	0.89	0.83-0.95	0.0007	1.00	0.86-1.16	0.98
35-39	0.79	0.74-0.85	<0.0001	0.77	0.66-0.90	0.001
40-44	0.84	0.78-0.91	<0.0001	0.74	0.64-0.85	<0.0001
Race/ethnicity						
White	ref			ref		
Hispanic or Latino	1.14	1.04-1.25	0.006	1.21	1.00-1.47	0.06
Black/African-American	1.19	1.10-1.29	<0.0001	0.93	0.76-1.14	0.50
Asian	0.85	0.74-0.99	0.03	0.67	0.49-0.93	0.02
Alaskan Native or American Indian	1.16	0.94-1.43	0.18	0.22	0.14-0.37	<0.0001
Multiple Race, No Primary Race Selected	1.32	1.11-1.57	0.002	0.28	0.17-0.47	<0.0001

Educational attainment						
Bachelor's degree or higher	ref			ref		
Some college	1.03	0.94-1.13	0.50	0.63	0.55-0.73	<0.0001
High school diploma or GED	0.85	0.78-0.93	0.0004	0.32	0.27-0.37	<0.0001
Less than high school	0.71	0.64-0.79	<0.0001	0.28	0.21-0.36	<0.0001
Employment Status						
Employed	ref			ref		
Unemployed	1.19	1.10-1.30	<0.0001	1.24	1.02-1.51	0.03
Not in labor force	1.05	0.98-1.12	0.16	1.35	1.13-1.60	0.0008
Geographic region						
Northeast	ref			ref		
North Central/Midwest	0.87	0.79-0.95	0.004	0.97	0.82-1.15	0.73
South	0.88	0.81-0.95	0.002	0.73	0.62-0.86	0.0002
West	0.82	0.74-0.90	<0.0001	0.95	0.78-1.14	0.56
Annual household income						
$50,000 or more	ref			ref		
$25,000-$49,999	1.01	0.95-1.08	0.79	0.87	0.77-1.00	0.04
<$25,000	0.96	0.89-1.03	0.22	0.68	0.58-0.78	<0.0001

aOR=Adjusted Odds Ratio

CI=Confidence Interval

[1]Defined as having tried to quit smoking and stopping smoking for at least 1 day during the past 12 months.

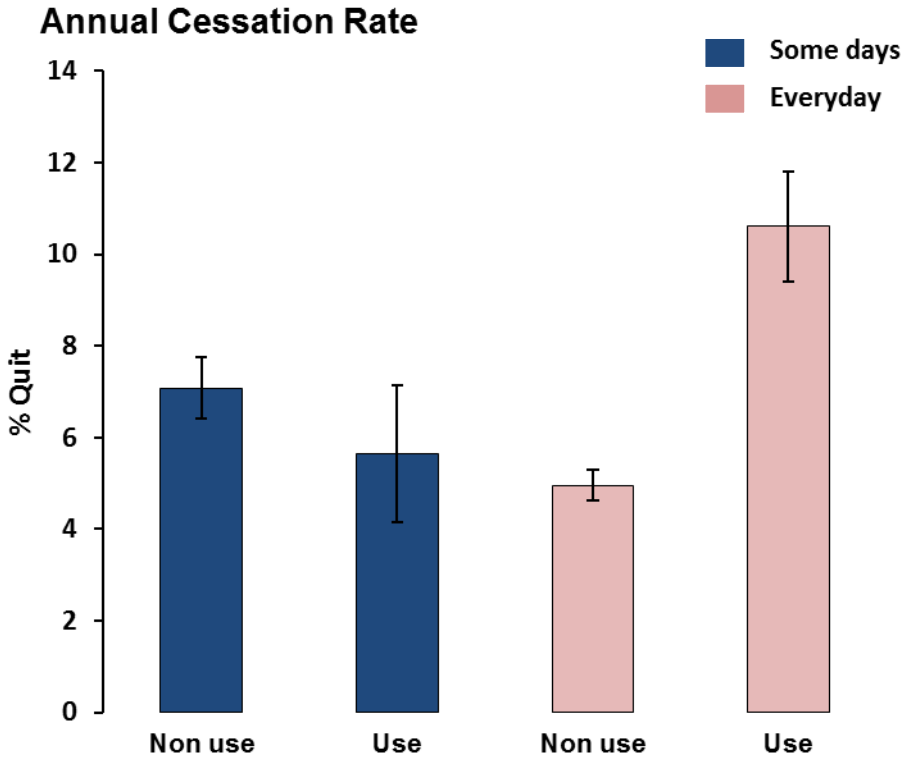
[2]Defined as quitting during the past 12 months and remaining abstinent for at least three months at the time of the interview

2.3.5 Relation of Past 12 Month Cessation and E-Cigarette Use 2014-2016

With respect to smoking cessation in the past 12 months, current e-cigarette users also had higher rates of smoking cessation than non-users in the NHIS dataset. For 2014-2016, 10.1% of those currently using e-cigarettes reported quitting in the past 12 months compared to 6.3% of those not currently using e-cigarettes (Figure 9). Multivariable logistic regression confirmed that current e-cigarette use was associated with significantly higher past 12 month cessation for 2014-2016 (aOR=1.64, 95% CI: 1.21-2.21, p=0.001) (results of the full model are included in Table 7).

Results were similar in the 2014-2015 TUS-CPS dataset, with a past 12 month cessation rate of 9.5% for current e-cigarette users compared to 5.5% for those not currently using e-cigarettes (Figure 9). Current e-cigarette use made no significant difference in past 12 month cessation among some-day smokers; 5.6% of those who currently used e-cigarettes reported quitting as did 7.1% of those who did not use e-cigarettes (Figure 7). Among every day smokers, however, current e-cigarette use made a significant difference in past 12 month cessation; 10.6% of every day smokers who used e-cigarettes reported quitting in the past 12 months as compared to 5.0% of every day smokers who did not use e-cigarettes (Figure 7).

Figure 7. Past 12 month quit attempts and smoking cessation in 25-44 year olds, by combustible cigarette smoking frequency and e-cigarette use, TUS-CPS 2014-2015



Annual cessation rate (defined as quitting during the past 12 months and remaining abstinent for at least 3 months at the time of interview). Cessation rate is stratified according to current e-cigarette use.

Multivariable logistic regression of TUS-CPS 2014-2015 data showed that current e-cigarette use was significantly associated with increased past 12 month smoking cessation, and yielded a significant interaction for smoking frequency (every day and some-day smoking) and current e-cigarette use ($p<0.0001$). Current e-cigarette use was not associated with combustible cigarette smoking cessation in the past 12 months among some-day smokers (aOR=0.74, 95% CI: 0.54-1.02, p=0.06; Supplementary Table 5). Every day smokers who were not currently

using e-cigarettes were less likely to have successfully quit smoking in the past 12 months (aOR=0.86, 95% CI: 0.75-0.98, p=0.03) compared to some-day smokers who were not current e-cigarette users. Every day smokers who currently used e-cigarettes were significantly more like to quit smoking during the past 12 months compared to the reference group of some-day smokers who did not currently use e-cigarettes (aOR=1.89, 95% CI: 1.57-2.28, p<0.0001).

2.3.6 Extension of Analyses to Other Age Groups

We repeated the analysis of past 12 month quit attempts and smoking cessation by current e-cigarette use for smokers aged 18-24 and those aged 45 and older. In keeping with the results for 25-44 year-olds, in 18-24 year-olds a significantly higher proportion of e-cigarette users reported past 12 month quit attempts in the NHIS (aOR=1.89, 95% CI: 1.22-2.91, p<0.005) and the TUS-CPS (aOR=1.75, 95% CI: 1.46-2.12, p<0.0001). The same was true for those aged 45 years and older in both the NHIS (aOR=2.12, 95% CI: 1.68-2.68, p<0.0001) and the TUS-CPS (aOR=2.21, 95% CI: 2.06-2.37, p<0.0001). There was no difference in past 12 month smoking cessation between current e-cigarette users and non-users among the 18-24 year-olds in either dataset. However, consistent with the results for 25-44 year-olds, among those aged 45 years and older, a significantly higher proportion of e-cigarette users reported past 12 month cessation than non-users in both the NHIS (aOR=2.05, 95% CI: 1.39-3.01, p=0.0003) and the TUS-CPS (aOR=1.76, 95% CI: 1.56-1.99, p<0.0001).

2.4 Discussion

2.4.1 Significance

Since the introduction of e-cigarettes to the U.S. market, there has been speculation that e-cigarettes will have a detrimental effect on smoking cessation by delaying quit attempts or reducing smoking cessation success among those who attempt to quit. In two large, nationally representative surveys, past 12 month quit attempts and successful smoking cessation significantly increased in the years of increased e-cigarette use as compared to 2006. Importantly, combustible cigarette smokers who reported current use of e-cigarettes at the time of the survey also reported significantly more past 12 month attempts to quit smoking and had higher rates of successful cessation in the past 12 months than did those who did not currently use e-cigarettes. These data are inconsistent with the hypothesis that e-cigarette usage has been detrimental to smoking cessation among established combustible cigarette smokers at a population-based level.

2.4.2 Strengths and Limitations

This study has several methodological strengths. Two large, nationally representative surveys of the U.S population were used to examine and confirm trends in past 12 month quit attempts and smoking cessation among adults. Demographic changes in the population during this period of time were also adjusted for while examining trends in smoking behavior. Furthermore, this study focused analyses on those aged 25-44, as this group has transitioned through the development of smoking behaviors and is less affected than older adults by health conditions that

may influence smoking status. I decided to analyze data from 2006-2016 because that period encompassed years that preceded and years that followed the introduction and widespread use of e-cigarettes into the U.S. market; this was intended to enhance detection of e-cigarette effects.

The findings of this study are limited by several factors. This study was cross-sectional, preventing the determination of temporal relations and stronger causal inference. Also, the current analyses focused on those who were currently using e-cigarettes; this may over-represent those who found e-cigarettes especially effective or satisfying. The surveys used relied on participant self-report, and questions about smoking behavior and cessation strategies were limited. Additionally, questions about the use of e-cigarettes were not introduced into the surveys until 2014, so the lack of information regarding use of e-cigarettes in earlier survey years prevented a fuller exploration the relationship between e-cigarette use and past 12 month quit attempts and smoking cessation.

2.4.3 Changing Population Demographics and Smoking Behaviors

The proportion of the population who currently smoke combustible cigarettes represents a dynamic balance between the number of individuals who initiate ever smoking (smoking at least 100 cigarettes lifetime) as well as those who successfully quit and become former smokers. I examined changes in multiple smoking behaviors over the last decade while adjusting for demographic changes in the population in order to better understand what contributes to potential changes in the proportion of current smoking. I used multivariable regression analyses that included race/ethnicity in addition to sex, age, education, employment status, geographic region, and annual household income—factors that have been shown to be associated with differences in smoking behaviors.

After adjusting for demographic characteristics in the population, I see an ongoing decrease in current smoking in the U.S. population in both large nationally representative datasets. Though I found no change in current smoking from 2006 to 2012 in the NHIS dataset, since 2013 has there been a decrease in current smoking. I saw a similar trend in the TUS-CPS dataset but with an earlier statistically significant decrease in current smoking in the TUS-CPS dataset. After adjusting for demographic variables in the TUS-CPS dataset, decreases in current smoking were seen in 2010-2011 and 2014-2015 compared to 2006-2007.

The findings of ever smoking, defined as smoking at least 100 cigarettes lifetime, differ in the NHIS and TUS-CPS datasets. A population level the proportion of the population that has smoked at least 100 cigarettes lifetime has decreased from 2006-2016 in the NHIS dataset, but this decrease can be explained by the demographic changes in the U.S. population. The percentages of U.S. adults who are non-White or of Hispanic ethnicity are increasing, and lower proportions of these groups have smoked at least 100 cigarettes lifetime as compared to Whites.[137, 138] In multivariable regression analyses of the NHIS data, there were no changes in ever smoking from 2006-2016 after adjusting for demographic characteristics in the population. The analysis of ever smoking in the TUS-CPS dataset yielded different results in that ever smoking was significantly lower in 2010-2011 and 2014-2015 compared to 2006-2007 in the multivariable logistic regression.

The proportion of ever smokers who reported their current smoking status as “former smoker” at the time of interview has increased in both surveys. After adjusting for demographic changes in the population, former smoking increased significantly in the NHIS for the years 2010-2016, as compared to 2006. Similarly, in the TUS-CPS former smoking increased in 2010-2011 and 2014-2015 compared to 2006-2007.

2.4.3 Association of Smoking Cessation and E-cigarette Use

Nonetheless, our findings show that among combustible cigarette smokers, past 12 month quit attempts and smoking cessation were not lower amongst those using e-cigarettes, and instead e-cigarette use may have contributed to the recent increase in past 12 month quit attempts and smoking cessation. Examining the period from 2014-2016, and after adjusting for many characteristics that influence past 12 month quit attempts and smoking cessation (sex, age, race, education, employment status, geographic region, and annual household income), smokers who used e-cigarettes at the time of the survey reported significantly higher past 12 month quit attempts and smoking cessation than did smokers who did not use e-cigarettes. This finding was seen across both population-based datasets and is in keeping with Zhu et al.[24] and other studies that found that e-cigarette use was positively associated with quit attempts and smoking cessation.[13,16,33-35] In contrast, these population-based results did not confirm the prospective cohort study by Weaver et al.[23] In that study of 858 combustible cigarette smokers who were followed over one year, those who used electronic nicotine devices (ENDs) were less likely to quit in the follow-up period. This study had just 248 ENDs users in the sample, which may not have captured the range of smoking behavior in a larger population-based study.

2.4.4 Extension of Association of E-cigarette Use and Smoking Cessation to Other Age Groups

The association between e-cigarette use and past 12 month quit attempts and smoking cessation also extends to other age groups. Past 12 month quit attempts were increased among those who currently used e-cigarettes in both younger (18-24 years) and older (45 years and older) age

groups. E-cigarette use was associated with increased past 12 month smoking cessation in the older group, but not among the group under 25 years of age.

2.4.5 Interplay between E-cigarette Use and Smoking Frequency

I was able to further examine past 12 month quit attempts and cessation according to smoking frequency (every day and some-day smoking) in the TUS-CPS dataset. I found a significant interaction between smoking frequency and current e-cigarette use for both past 12 month quit attempts and smoking cessation. E-cigarette use was associated with increased quit attempts and successful smoking cessation in the group who overall was less likely to quit. Among everyday smokers, current e-cigarette use was significantly associated with past 12 month quit attempts (67.1% versus 42.2%) and cessation (10.6% versus 5.0%) compared to non e-cigarette users. Among some-day smokers, current e-cigarette use was associated with increased past 12 month quit attempts (60.9% versus 46.7%), but did not alter past 12 month smoking cessation (5.6% versus 7.1%).

2.4.6 Balance between Smoking Initiation and Cessation

The proportion of the population who smoke combustible cigarettes represents a dynamic balance between the number of individuals who initiate smoking and those who successfully quit and become former smokers. Most individuals initiate smoking by age 18,[1] and our analyses focused on those aged 25-44 years from 2006 to 2016, which represented a population that established combustible cigarette smoking prior to the widespread entrance of e-cigarettes into the U.S. market. E-cigarettes may be beneficial to this group in reducing combustible cigarette

use; however, whether e-cigarettes contribute to greater initiation of combustible cigarette use among younger groups remains a public health concern and is not addressed in this study.

2.5 Conclusion

I examined past 12 month quit attempts and smoking cessation among adults aged 25-44 in the U.S. from 2006 to 2016. This is a particularly important period to analyze smoking behaviors given that it encompasses the introduction of e-cigarettes into the U.S. market in 2006 and their subsequent increased use. Notably, during the period of time when e-cigarette use was markedly increasing, the use of combustible cigarettes declined. Over the past decade, current smoking has continued to decrease. Though I find somewhat different findings for changes in ever smoking in this decade, what is clear is that ever smoking has not increased. Among ever smokers, the proportion of individuals who report that they no longer smoke has increased. Past 12 month quit attempts and cessation rates are on the rise and show significant increases in the most recent NHIS and TUS-CPS surveys. At the very least, these findings suggest that current e-cigarette use does not delay quit attempts or prevent smoking cessation in this age group. In fact, the strongest findings with respect to e-cigarette use and past 12 month cessation were seen among every day smokers – the group who has the most difficulty quitting. These findings clearly show that the introduction of e-cigarettes has not prevented a reduction of smoking in the population to date and may have encouraged it, though the use and effects of e-cigarettes may certainly change in the future. The current findings may be relevant to future tobacco control policies and interventions.

Chapter 3: Multiple CHRNA3-CHRNB4-CHRNA5 variants are genetic risk factors for nicotine use disorder in European Americans

3.1 Background

Substance use disorders are a leading cause of preventable death in both the United States and worldwide and pose a worldwide threat to public health and have a devastating social and economic impact on individuals and their families [1-8]. The World Health Organization has estimated that there are 2 billion alcohol users, 1.3 billion tobacco users, and 185 million illicit drug users worldwide[10]. Each year, 3.3 million people die due to the harmful effects of alcohol, representing 5.9% of all deaths across the world[10]. Tobacco smoking similarly causes nearly 6 million premature deaths per year, and is predicted cause more than 8 million deaths annually by 2030 if current smoking rates continue[11, 139]. Other psychoactive substances, such as cannabis and cocaine, also cause significant health and social problems for both the people who use them and their families. Major health consequences of illicit drug use include accidental and intended injury, drug-induced psychotic symptoms, and increased risk for heart, liver, and lung diseases [140]. A greater knowledge of the genetics underlying substance addiction therefore is crucial for the development of more effective treatments and public health interventions.

Multiple factors have long been recognized to influence the development of substance use disorders, including genetic and environmental characteristics, and one of the most important risk factors is a family history of substance use disorders. Evidence for genetic influence on substance use disorder has been provided by many family, twin, and adoption studies, and

multiple large-scale genome wide association studies have confirmed the contribution of specific genes to substance use [141-145]. Family members of individuals with alcohol use disorder have a higher probability of suffering from alcohol use disorder[146]. Similarly, siblings of probands with nicotine use disorder, cannabis use disorder, or cocaine use disorder were at increased risk of developing habitual smoking, cannabis use disorder, or cocaine use disorder compared with siblings of nondependent individuals [82]. Overall, twin studies estimate that the heritability of substance use disorder is about 50-60% [147-149].

Cigarette smoking commonly co-occurs with other substance use, suggesting that they may have a shared genetic predisposition [150]. Epidemiological and clinical studies have shown that many people subsequently use multiple drugs after the initiation of one drug [151, 152]. Twin studies have demonstrated the presence of shared environmental and genetic factors that contribute to substance use[153]. This shared environmental and genetic influence has a significant effect on smoking initiation, alcohol use, and other drug use [152]. Furthermore, tobacco, alcohol, marijuana, and cocaine use disorders frequently co-occur[154-158]. Several studies have found increased rates of drug use disorders in relatives of individuals dependent on opiates or cocaine compared with relatives of alcoholics or subjects from the general population. These family, adoption, and twin studies support the familial transmission of substance use disorders and implicate genetic factors [81, 82, 159, 160]. However, the molecular genetic basis for that shared genetic risk of substance use disorder is still largely unknown, and despite evidence for genetic overlap, few studies have examined whether genetic variants implicated for one substance also influence the other. Even though nicotine, alcohol, cannabis, and cocaine have unique pharmacological profiles, it is possible that pleiotropic effects may be responsible

for shared aspects of their use and use disorder, such as shared experience of reward or attenuation of negative mood states.

Genetic findings for nicotine use disorder and other smoking related traits and diseases have pinpointed a region on chromosome 15q25.1, which harbors the *CHRNA5-CHRNA3-CHRNAB4* gene cluster coding for a5, a3, and b4 nicotinic acetylcholine receptor (nAChR) subunits. Genetic variants in the CHRNA5-A3-B4 region are robustly associated with smoking behaviors such as nicotine use disorder and the number of cigarettes smoked per day among smokers, as well as risks for smoking related diseases such as lung cancer and chronic obstructive pulmonary disease[73, 79, 80, 161-164]. Evidence of association with smoking behavior and nicotine use disorder has been reported in a genome-wide association study (GWAS) and confirmed in large meta-analyses[71]. The most well-established locus within this region is tagged by the single nucleotide polymorphism (SNP) rs16969968[165]. Rs169969968 is a non-synonymous (D398N) SNP in exon 5 of *CHRNA5*, located in the cytoplasmic domain preceding the M4 transmembrane domain [166]. Asparagine (N) in the 398 position with its polar uncharged side chain lowers Ca^{2+} permeability and increases short-term desensitization [166]. When the function of this subunit is decreased, α5-containing nAChRs fail to trigger a normal inhibitory motivational signal that is supposed to limit nicotine intake[167]. Decreased function of the α5 subunit is thought to prevent negative feedback in response to cigarette smoking, resulting in heavy smoking behavior. Rs16969968 has also been shown to have a similar risk effect with nicotine use disorder and heaviness of smoking in African ancestry populations, where the minor allele is less common[168-170]. African Americans are a population that is often under-represented in genetic studies. In the present study, I investigated whether variants in *CHRNA5-CHRNA3-*

CHRNB4 alter risk for nicotine, alcohol, marijuana, and cocaine use disorders in European Americans and African Americans.

A few studies have reported association of this *CHRNA5-CHRNA3-CHRNB4* region with other substance related traits, with somewhat conflicting results. Chen et al found evidence of association between rs16969968 and symptoms of alcohol abuse or alcohol use disorder [171]. Studies by Wang et al and Hallfors et al found evidence of association between rs588765 and alcohol use disorder [94, 172]. Sherva et al detected association between alcohol use disorder and rs578776 and rs615470 in African Americans, but not European Americans [173]. Grucza et al found that the minor (A) allele of rs16969968, relative to the major G allele, may be a protective factor for cocaine use disorder [95]. The biological plausibility of such a bidirectional association stems from the involvement of nAChRs with both excitatory and inhibitory modulation of dopamine-mediated reward pathways. Additional distinct SNPs rs578776 and rs588765 in the 15q25.1 region have provided evidence of association with cigarettes per day [174].

In the present study, my first aim was to determine if the protective effect of rs16969968 for cocaine use disorder could be replicated in additional datasets and expanded to African Americans. The second aim was to determine if rs16969968 is associated with other substance use disorders, particularly alcohol, cannabis, and cocaine use disorders. Finally, the third aim was to study whether additional variants in the *CHRNA5-CHRNA3-CHRNB4* gene region affect risk of other substance use and to explore whether this role is independent of nicotine use disorder. To achieve these three aims, I examine meta-analyzed six independent studies of substance use disorder, which collectively included 4,108 individuals of European American descent and 6,090 individuals of African American descent. Each study comprehensively

assessed nicotine, alcohol, cannabis, and cocaine use disorder, which allowed me to examine whether the genetic association of this region is specific to nicotine use disorder or whether each variant tags a genetic susceptibility to other substances.

3.2 Methods

3.2.1 Sample

Subjects were recruited by six independent studies of addiction: the Genetic Study of Alcohol Use disorder in African Americans (ADAA), the Genetic Study of Nicotine Use disorder in African Americans (AAND), the Collaborative Study on the Genetics of Alcoholism (COGA), the Collaborative Genetic Study of Nicotine Use disorder (COGEND), the COGEND Biomarker Study, and the Family Study of Cocaine Use disorder (FSCD).

3.2.2 Genetic Study of Alcohol Use disorder in African Americans (ADAA)

The goal of ADAA was to identify and characterize genetic determinants of alcohol use disorder in an African American population. Alcohol-dependent probands were recruited from chemical dependency treatment centers in the greater St. Louis metropolitan area. Community-based comparison subjects were recruited using the Missouri Family Registry. All subjects had to self-identify as African American, be 18 years or older, and speak English. Alcohol dependent probands who met criteria for a lifetime diagnosis of DSM-IV alcohol use disorder were recruited from chemical dependency treatment units. Controls were matched to cases with alcohol use disorder based on age, gender, zip code, and education. Controls had to have consumed at least 12 alcoholic drinks in any one year of their life. Subjects could not have a condition that prevented them from providing informed consent or effectively participating in the

protocol (e.g., language difficulty, inability to understand the questions, or extremely poor health). Other substance use disorder or other psychopathology was not exclusionary criteria for alcohol dependent cases. Controls, however, could not be dependent on alcohol or tobacco.

3.2.3 Genetic Study of Nicotine Use disorder in African Americans (AAND)

The Genetic Study of Nicotine Use disorder in African Americans was initiated to identify and characterize genetic determinants of nicotine use disorder in a large African American population. Community-based recruitment was used to enroll nicotine dependent cases and non-dependent, smoking controls in the Chicago, IL area between 2010 and 2013. All participants had to be between the ages of 25-44 years, speak English, and self-identify as African American. Nicotine dependent cases were defined by a current score of 4 or greater on the Fagerström Test for Nicotine Use disorder (FTND)[175]. Control status was defined as smoking at least 40 cigarettes lifetime, but never being nicotine dependent (lifetime FTND score $\leq$ 1). Those who qualified as a nicotine dependent case or non-dependent control completed an in-person comprehensive interview and donated a blood sample for genetic analysis.

3.2.4 Collaborative Study on the Genetics of Alcoholism (COGA)

The Collaborative Study on the Genetics of Alcoholism was initiated in 1989 and is a large-scale family study that has had as its primary aim the identification of genes that contribute to alcoholism susceptibility and related characteristics. Subjects were recruited from 7 sites across the U.S. Alcohol dependent probands were recruited from treatment facilities and assessed by personal interview. After securing permission, other family members were also assessed. Assessment involved a comprehensive personal interview developed for this project, the Semi-

Structured Assessment for the Genetics of Alcoholism (SSAGA), which gathers detailed information on alcoholism related symptoms along with other drugs and psychiatric symptoms. Blood was obtained for genetic studies.

3.2.5 Collaborative Genetic Study of Nicotine Use disorder (COGEND)

The Collaborative Genetic Study of Nicotine Use disorder was initiated to detect and characterize genes that alter risk for heavy tobacco consumption, nicotine use disorder, and related phenotypes. Participants were recruited using the Missouri Family Registry in St. Louis and Health Maintenance Organizations in Detroit. Potential participants were screened to determine eligibility. All participants had to be between the ages of 25-44 years and speak English. Nicotine dependent cases were defined as current smokers with a Fagerström Test for Nicotine Use disorder (FTND) score of 4 or greater (Heatherton et al., 1991). Control status was defined as smoking at least 100 cigarettes lifetime, but never being nicotine dependent (lifetime FTND score $\leq$ 1). Other substance use disorder diagnoses or comorbid disorders were not exclusionary criteria. Those who qualified as a nicotine dependent case or non-dependent control completed an in-person comprehensive interview and donated a blood sample for genetic analysis.

3.2.6 COGEND Biomarker Study

The Collaborative Genetic Study of Nicotine Use disorder (COGEND) was initiated to detect and characterize genes that alter risk for heavy tobacco consumption, nicotine use disorder, and related phenotypes. We undertook a further extension to study biomarkers of smoking. Participants were recruited from the St. Louis metropolitan area between 2014 and 2015 via

internet advertising, Facebook, flyers, and word of mouth. All participants were current smokers as demonstrated by an exhaled carbon monoxide level $\geq$ 7 parts per million and self-reported smoking on $\geq$ 15 days during the past month. Participants were required to have smoked 100 cigarettes lifetime, be between the ages of 25-44 years and speak English. Those who qualified completed an in-person comprehensive interview, provided measures of exhaled carbon monoxide, and donated a saliva sample for genetic analysis. Following the informed consent process, participants were assessed for baseline demographics and substance use history using a modified Computer Assisted Personal Interview (CAPI). Healthcare literacy was assessed using the Rapid Estimate of Adult Literacy in Medicine [176]. Participants provided saliva samples for genetic analysis using 23andMe DNA collection kits. 23andMe is a privately held personal genomics and biotechnology company that produces high quality genetic data in CLIA-certified laboratories.

3.2.7 Family Study of Cocaine Use disorder (FSCD)

The Family Study of Cocaine Use disorder was initiated in 2000 as a case-control family study of cocaine use disorder. The primary goal of this case-control study was to increase the understanding of the familial and non-familial antecedents and consequences of cocaine use disorder. Subjects were systematically recruited from chemical dependency treatment units (both public and private; residential and outpatient) in the greater St. Louis metropolitan area to identify potential cocaine dependent individuals. Subjects were required to speak English and be over the age of 18 years. Subjects could not have a condition that prevented them from providing informed consent or effectively participating in the protocol (e.g., language difficulty, CNS damage, or extremely poor health). All subjects were assessed using the Semi-Structured

Assessment for Cocaine Use disorder (SSACD), a polydiagnostic instrument developed for this project and closely based on the SSAGA.

3.2.8 Assessment

Interview assessment for baseline demographics, psychiatric disorders, and substance use history for all studies was performed, modeled after the Semi-Structured Assessment for the Genetics of Alcoholism [177, 178]. The SSAGA is a validated instrument developed to provide a detailed evaluation of alcohol, nicotine and other substance use disorders. Lifetime history of use disorder on alcohol, cocaine, and cannabis was determined according to DSM-5 criteria. Although opioid use disorder was also assessed, these diagnoses were not included in this analysis because of the small number of subjects dependent on these substances and reduced power to detect association. Nicotine use disorder was also assessed using the Fagerström Test for Nicotine Use disorder [175].

3.2.10 Statistical Analyses

The primary analysis approach was a meta-analysis of odds ratios (ORs) using a consistently coded reference allele across the six studies in European Ancestry and African Ancestry strata to evaluate evidence for or against a consistent association across ancestral groups. This strategy maximized power to detect robust association while recognizing the potential impact of differences in ascertainment protocols and environmental differences among the studies. Analysis of the African American samples explored the consistency of signals in a distinct ancestral group.

Association analyses were conducted on all SNPs in the *CHRNA3-CHRNA5-CHRNB4* gene region. A regression model was implemented in the genomic analysis package PLINK [179]. The following steps were taken in our quality control analysis: (1) dropped SNPs with a minor allele frequency of ≤1%, (2) dropped SNPs and subjects with low genotyping efficiency (≤95%), and (3) dropped SNPs with a Hardy-Weinberg equilibrium $p \leq 1 \times 10^{-6}$. All calculations of ORs and standard errors for the studies were performed using plink software. We utilized a logistic regression method in which genotype (coded 0, 1, or 2 to represent the number of coded risk alleles) is expressed as the dependent outcome variable. In our logistic regression model, diagnoses for nicotine, alcohol, cannabis, and cocaine use disorders are expressed as the independent variables. In each analysis, the first 10 principal components and covariates representing sex and age [using quartiles, defined by 34 years and younger (reference), 35-39 years, 40-44 years, and 45 years and older] were included as categorical variables. Meta-analyses of the effect sizes of each study were performed using Metal software.

3.3 Results

3.3.1 Demographics

The demographic characteristics of the samples analyzed from the study participants are presented in Table 9. The sample consists of 33% European American and 67% African American subjects. Comorbid substance use disorders are common, with more than half the subjects meeting criteria for at least two substance use disorders.

Table 9. Demographics for the samples by ancestry group

Characteristic	AAND	ADAA	COGA	COGEND	FSCD	COGEND Biomarker
European American samples						
Sample size (n)	-	-	1308	1960	555	285
Sex, n (%)						
Males	-	-	701 (53.6%)	755 (38.5%)	276 (49.7%)	183 (64.2%)
Females	-	-	607 (46.4%)	1205 (61.5%)	279 (50.3%)	102 (35.8%)
Age, years						
Mean ± SD	-	-	** 41.0 ± 11.7	** 36.5 ± 5.5	** 33.4 ± 9.0	** 34.2 ± 5.5
Range	-	-	17-79	25-65	18-54	21-24
Diagnoses, n (%)						
Nicotine use disorder	-	-	488 (38.5%)	** 1251 (63.8%)	** 226 (40.7%)	258 (90.5%)
Alcohol use disorder	-	-	** 786 (60.1%)	447 (23.0%)	** 254 (45.8%)	128 (46.9%)
Cannabis use disorder	-	-	** 299 (22.9%)	** 184 (9.5%)	** 170 (30.6%)	88 (30.9%)
Cocaine use disorder	-	-	** 297 (22.7%)	** 128 (6.6%)	** 234 (42.2%)	** 41 (14.4%)
African American samples						
Sample size (n)	1723	1826	447	712	601	778
Sex, n (%)						
Males	730 (42.4%)	1017 (55.7%)	243 (54.4%)	448 (62.9%)	300 (49.9%)	528 (67.9%)
Females	993 (57.6%)	809 (44.3%)	204 (45.6%)	264 (37.1%)	301 (50.1%)	250 (32.1%)
Age, years						
Mean ± SD	35.4 ± 6.1	41.3 ± 10.0	** 44 ± 10.1	** 36.6 ± 5.8	** 40.3 ± 7.2	** 34.0 ± 5.5
Range	25-55	18-46	18-77	25-44	18-60	20-25
Diagnoses, n (%)						

Nicotine use disorder	1020 (59.3%)	692 (37.9%)	176 (40.3%)	** 537 (75.4%)	** 252 (41.9%)	699 (89.9%)
Alcohol use disorder	77 (5.5%)	888 (48.6%)	** 312 (69.8%)	142 (20.2%)	** 211 (35.1%)	304 (43.8%)
Cannabis use disorder	252 (14.7%)	582 (31.9%)	** 119 (26.6%)	** 118 (16.8%)	** 128 (21.3%)	284 (36.6%)
Cocaine use disorder	109 (6.3%)	681 (37.3%)	** 183 (40.9%)	** 84 (12.0%)	** 309 (51.4%)	** 75 (9.7%)

AAND= Genetic Study of Nicotine Use disorder in African Americans

ADAA= Genetic Study of Alcohol Use disorder in African Americans

COGA = Collaborative Study on the Genetics of Alcoholism

COGEND = Collaborative Genetic Study of Nicotine Use disorder

FSCD = Family Study of Cocaine Use disorder

SD = standard deviation

* and ** indicate variables with significant ($p < 0.05$) or highly significant differences ($p < 0.01$) between the European American and African American samples.

Rates of substance use and rates of DSM-5 substance use disorder among those who have used are shown in Tables 10 and 11. We define use of cigarettes as having smoked at least 100 lifetime cigarettes. For the other substances, use is defined as having tried it at least once. Each study's demographic characteristics are consistent with its recruitment design; for example the FSCD study has higher rates of cocaine use disorder due to its ascertainment for cocaine use disorder.

Table 10. Use prevalence

			Ever smoke			Ever drink			Ever cocaine			Ever cannabis		
Ancestry	**Study**	**N**	**YES**	**NO**	**%yes**	**YES**	**NO**	**%yes**	**YES**	**NO**	**%yes**	**YES**	**NO**	**%yes**
EA	COGA	1308	1038	128	89.00%	1308	0	100.00%	565	742	43.20%	870	354	66.60%
EA	COGEND	1960	1957	3	100.00%	1937	12	99.40%	670	1279	34.40%	1692	255	86.90%
EA	FSCDSAGE	555	433	122	78.00%	555	0	100.00%	292	263	52.60%	395	160	71.20%
EA	COGEND Biomarker	285	285	0	100.00%	275	10	96.49%	99	186	34.74%	173	112	60.70%
AA	AAND	1711	537	1174	31.39%	1662	61	96.46%	236	1485	13.71%	1277	443	74.24%
AA	ADAA	1826	1585	241	86.80%	1570	256	85.98%	834	992	45.67%	1539	287	84.28%
AA	COGA	447	371	42	89.83%	447	0	100.00%	261	186	58.40%	354	93	79.20%
AA	COGEND	712	712	0	99.90%	689	14	98.00%	173	530	24.60%	599	103	85.30%
AA	FSCDSAGE	601	479	122	79.70%	597	4	99.30%	347	254	57.74%	478	123	20.50%
AA	COGEND Biomarker	778	778	0	100.00%	725	53	93.19%	144	633	18.53%	529	247	68.17%

Table 11. Use disorder conditional on use

		Nicotine			Alcohol			Cocaine			Cannabis		
Ancestry	**Study**	Exposed	Dep	% dep	Exposed	Dep	% dep	Exposed	Dep	% dep	Exposed	Dep	% dep
EA	COGA	1038	488	47.01%	1308	786	60.09%	565	297	52.57%	870	299	34.37%
EA	COGEND	1957	1251	63.90%	1927	447	23.20%	670	128	19.10%	1692	184	10.90%
EA	FSCDSAGE	433	226	52.20%	555	254	45.80%	292	234	80.10%	395	170	43.00%
EA	COGEND Biomarker	285	258	90.53%	273	128	46.89%	99	41	41.41%	173	88	50.87%
AA	AAND	537	305	56.80%	1339	77	5.75%	236	109	46.19%	1277	252	19.73%
AA	ADAA	1585	692	43.66%	1570	888	56.56%	834	582	69.78%	1539	681	44.25%
AA	COGA	371	176	47.44%	447	312	39.80%	261	183	70.11%	354	119	33.62%
AA	COGEND	712	537	75.40%	687	142	20.70%	173	84	48.60%	599	118	19.70%
AA	FSCDSAGE	479	252	52.60%	597	211	35.30%	347	309	89.10%	478	128	26.78%
AA	COGEND Biomarker	778	699	89.85%	694	304	43.80%	144	75	52.08%	529	284	53.69%

3.3.1 Meta-Analysis

MAfter correcting for multiple testing using simpleM, the p-value threshold for statistical significance was set at 0.0005 based on the number of independent SNPs within the *CHRNA5-CHRNA3-CHRNB4* gene region[180, 181]. SimpleM is a multiple testing correction method for genetic associations studies for imputed SNPs[181]. Rs16969968 was statistically significant for nicotine use disorder (OR=1.13, p=0.0003372) in European Americans but was not significant for any other substances in European Americans and African Americans (Table 12).

Table 12. Association of substance use disorder and rs16969968

African Americans	Odds Ratio	Standard Error	p value
Alcohol	0.98906	0.0136	0.4182
Cannabis	0.993322	0.0082	0.4111
Cocaine	0.99551	0.0079	0.5653
Nicotine	1.00632	0.0084	0.4544

European Americans	Odds Ratio	Standard Error	p value
Alcohol	1.042061	0.0905	0.649
Cannabis	0.930066	0.0606	0.2317
Cocaine	0.996108	0.0281	0.8902
Nicotine	**1.13145**	0.0345	**0.00034**

31 variants were associated with nicotine use disorder in the European Ancestry group (Table 13). The point estimates of the ORs display a consistent direction of effect for the effect alleles across all three studies in all 31 variants, resulting in a highly significant association ($p<0.0005$) between the variants and nicotine use disorder after adjusting for comorbid alcohol, cannabis, and cocaine use disorders. The most significant variant was rs7172118, with an OR of 1.13 ($p=0.00001971$), however all of these 31 variants are in LD, with an r^2 approaching 1.0. In the African American sample, the results for all variants and nicotine use disorder were not significant after correcting for multiple testing.

Table 13. Results for genetic variants in the CHRNA5-CHRNA3-CHRNB4 region association results for nicotine use disorder in European Americans

SNP	Chr:Position	Gene	Allele 1	Allele 2	OR	P-value
rs7172118	15:78862453	CHRNA5	C	A	1.13	1.97E-05
rs17486195	15:78865197	CHRNA5	A	G	1.13	2.26E-05
rs140330585	15:78866445	CHRNA5	G	A	1.13	2.26E-05
rs11633958	15:78862064	CHRNA5	C	T	1.13	2.34E-05
rs55781567	15:78857986	CHRNA5	C	G	1.13	2.34E-05
rs945144020	15:78859605	CHRNB4	G	A	1.13	2.42E-05
rs56077333	15:78899003	CHRNA3	C	A	1.12	2.46E-05
rs72463576	15:78899560	CHRNA3	A	C	1.12	2.89E-05
rs72740964	15:78868636	CHRNA5	G	A	1.12	3.07E-05
rs114205691	15:78901113	CHRNA3	C	T	1.12	3.27E-05
rs7180002	15:78873993	CHRNA5	A	T	1.12	3.27E-05
rs141518190	15:78900647	CHRNA3	A	G	1.12	3.27E-05
rs147499554	15:78900650	CHRNA3	C	T	1.12	3.27E-05
rs138544659	15:78900701	CHRNA3	T	G	1.12	3.27E-05

rs951266	15:78878541	CHRNA5	G	A	1.12	3.70E-05
rs56390833	15:78877381	CHRNA5	C	T	1.12	3.94E-05
rs8192482	15:78886198	CHRNA3	G	A	1.12	4.01E-05
rs4887067	15:78886947	CHRNA3	C	G	1.12	4.25E-05
rs55676755	15:78898932	CHRNA3	C	T	1.12	4.59E-05
rs147144681	15:78900908	CHRNA3	A	T	1.12	4.69E-05
rs146009840	15:78906177	CHRNA3	T	C	1.12	5.43E-05
rs72743158	15:78926445	CHRNB4	G	C	1.12	6.98E-05
rs4243084	15:78911672	CHRNA3	G	A	1.12	9.17E-05
rs2869548	15:78922638	CHRNB4	T	G	1.12	9.66E-05
rs55853698	15:78857939	CHRNA5	T	G	1.12	0.0001004
rs149959208	15:78912710	CHRNA3	T	G	1.11	0.0001013
rs17486278	15:78867482	CHRNA5	A	C	1.12	0.0001119
rs1317286	15:78896129	CHRNA3	A	G	1.11	0.0003079
rs1051730	15:78894339	CHRNA3	C	T	1.13	0.0003269
rs16969968	15:78882925	CHRNA5	A	G	1.13	0.0003372
rs17487223	15:78923987	CHRNB4	C	T	1.11	0.0004077

None of the other substance use disorders displayed a statistically significant association with any variants in the CHRNA5/A3/B4 region in the European American subjects. Alcohol use disorder is common in both the European American and African American subjects across all datasets, and therefore we expect this meta-analysis to be well powered to detect association to this substance. Similarly, there is no clear genetic contribution to cocaine or cannabis use disorder, but the power to detect an association for these substances is reduced due to the smaller number of use disorder diagnoses.

Similar findings were observed in the African American analyses. Alcohol use disorder does not demonstrate a statistically significant independent association in these datasets. Neither cannabis nor cocaine use disorder were associated with any variants in this region either, although the power to detect association is reduced due to the smaller number of individuals dependent on these substances. The SNP with the lowest p-value for each substance is shown in table 4, with none achieving the significance threshold of $p<0.0005$.

Table 14. Top candidate SNPs in *CHRNA5-CHRNA3-CHRNB4* region and association with substance use disorder in African Americans and European Americans

	Chr:Position	Allele 1	Allele 2	Odds Ratio	p value
African Americans					
Alcohol	15:78861391	G	A	1.04	0.004751
Cannabis	15:78900813	G	C	1.02	0.003336
Cocaine	15:78927863	T	C	0.99	0.01598
Nicotine	15:78868294	T	C	1.03	0.005409
European Americans					
Alcohol	15:78911230	C	T	0.88	0.001554
Cannabis	15:78922486	T	C	2.19	0.007589
Cocaine	15:78882925	G	A	0.88	0.01268
Nicotine	15:78862453	C	A	1.13	1.97E-05

3.4 Discussion

Chromosome 15q25.1 harbors the well-established smoking locus within the CHRNA5-CHRNA3-CHRNB4 gene cluster, and within this gene cluster the functional SNP rs16969968 represents the strongest signal for nicotine use disorder and other smoking related phenotypes[79, 80, 162]. This study contributes several new findings about genetic risk for nicotine use disorder. First, the non-synonymous SNP rs16969968, which is unequivocally associated with nicotine use disorder, is specific for nicotine use disorder in individuals of European ancestry. We were unable to detect this association in the African American sample which we analyzed. Given that this is a low frequency variant in African Americans (5% minor allele frequency), power was low to detect this association. Second, rs16969968 does not represent a genetic risk for alcohol use disorder, cannabis use disorder, or cocaine use disorder in European Americans or African Americans, suggesting that this SNP does not represent a shared genetic risk for substance use disorder. Finally, expanding our analyses to other variants within the *CHRNA5-CHRNA3-CHRNB4* gene region demonstrate that 31 variants in this region are associated with an increased risk for nicotine use disorder in European Americans, there was no association with other substance use disorder in European Americans or African Americans, suggesting that the entire gene region is specific for nicotine use disorder. Based on my meta-analysis results, the *CHRNA5-CHRNA3-CHRNB4* region clearly plays a role in nicotine use disorder risk in European Americans.

First, rs16969968 is clearly implicated in risk for nicotine use disorder in individuals of European descent. This SNP is highly replicated, and in vitro studies demonstrate that nicotinic receptors with the risk variant at 16969968 exhibit lower maximal response to a nicotinic agonist than receptors with the other allele[72]. Rs16969968 is non-synonymous, functional, and a strong candidate to be a causal allele. This meta-analysis similarly found that rs16969968 is associated with an increased risk of nicotine use disorder in European Americans, although the effect size was modest across the six studies (OR=1.13, p=0.0003372). I also identified 30 additional variants in the *CHRNA5-CHRNA3-CHRNB4 r*egion that are associated with nicotine use disorder, however all 30 variants are in linkage disequilibrium with rs16969968, suggesting that these 30 variants do not represent independent risk factors for nicotine use disorder since they are highly correlated with each other.

This study had several methodological strengths. First, the meta-analysis of six independent studies allowed inclusion of over 10,000 individuals, increasing the power to detect small effect sizes in the sample. Second, I stratified the meta-analysis by race since allele frequencies of variants in the *CHRNA5-CHRNA3-CHRNB4* vary widely among European Americans and African Americans.

These findings also have limitations. The major limitation is that this analysis was restricted to a single gene region, however this method was chosen because it harbors the most replicated risk factors for nicotine use disorder. Another limitation is that each individual study was ascertained for use disorder on different substances (nicotine, alcohol, and cocaine), thus each study population represents different substance use prevalences in accordance with the study design.

Understanding the statistical power is especially critical when interpreting the null association results in our study. These negative results need to be interpreted in the context of sample size, frequency and hypothesized effect size of genotype, prevalence and relative risk of environmental factors, and type I error level. This study had limited statistical power because of the low disease allele frequency of many SNPs in this region. A post hoc power analysis for rs16969968 revealed that on the basis of a 0.650 reference allele frequency in European Americans and a 0.948 reference allele frequency in African Americans, an n of approximately 11,124 European Americans and 5,563 African Americans would be needed to obtain statistical power at the recommended 0.80 level assuming an effect size of 1.13.[182] Thus the null results in this study should be interpreted with caution, and extremely large sample sizes, in the order of tens of thousands of individuals, will be needed to detect a modest moderation effect in multiple substances if it is present.

Overall, this meta-analysis demonstrates that the CHRNA5-CHRNA3-CHRNB4 gene region is associated with nicotine use disorder risk and therefore also associated with risk for other diseases in which smoking is a major contributing factor. These findings are supported by multiple other studies demonstrating the association of this gene region and nicotine use disorder, but contradict some studies demonstrating risk in this region with other substances and larger meta-analyses are needed[72, 94, 95, 169, 174]. Given the public health burden of smoking related illnesses, variants in this gene region must be considered in prognostic risk stratification and treatment considerations for these diseases.

Chapter 4: Polygenic Liability to Substance Use disorder

4.1 Background

The advent of genome-wide association studies (GWAS) has revolutionized the identification of genetic variants contributing to substance use disorders and has been particularly successful in uncovering new genes and pathways of diseases[183]. GWAS technology can examine many genetic variants involved in disease etiology and pathophysiology in the genome simultaneously, without an a priori hypothesis[184]. GWAS targets millions of SNPs, common variants of small effects[184]. For substance use disorders, it has become evident that thousands of variants confer small effects to the overall risk of substance use disorders[185, 186]. GWAS results are robust, however GWAS require a large sample size for optimal statistical power, mainly to identify common variants with small effects. Researchers have begun to create composite genetic risk scores to investigate the polygenic manner of the genetic variants[187, 188].

The approach to using the polygenic risk score (PRS), introduced by the International Schizophrenia Consortium has facilitated the creation of genomic profiles which combine the effects of many associated genetic variants to predict risk of disease[189]. The genetic architecture of common adult-onset diseases is likely a continuum of common low-risk to rare high-risk genetic variants that can act cumulatively to drive overall risk in any single individual[190]. Since complex traits such as substance use behaviors and substance use disorders are thought to be highly polygenic in nature with hundreds of effects, each SNP has a small individual effect contributing to the development of the phenotype, even when the sample

sizes are large. Therefore, modeling the additive or cumulative effects of associated variants has the potential to explain a higher proportion of variation in a trait relative to a single variant.

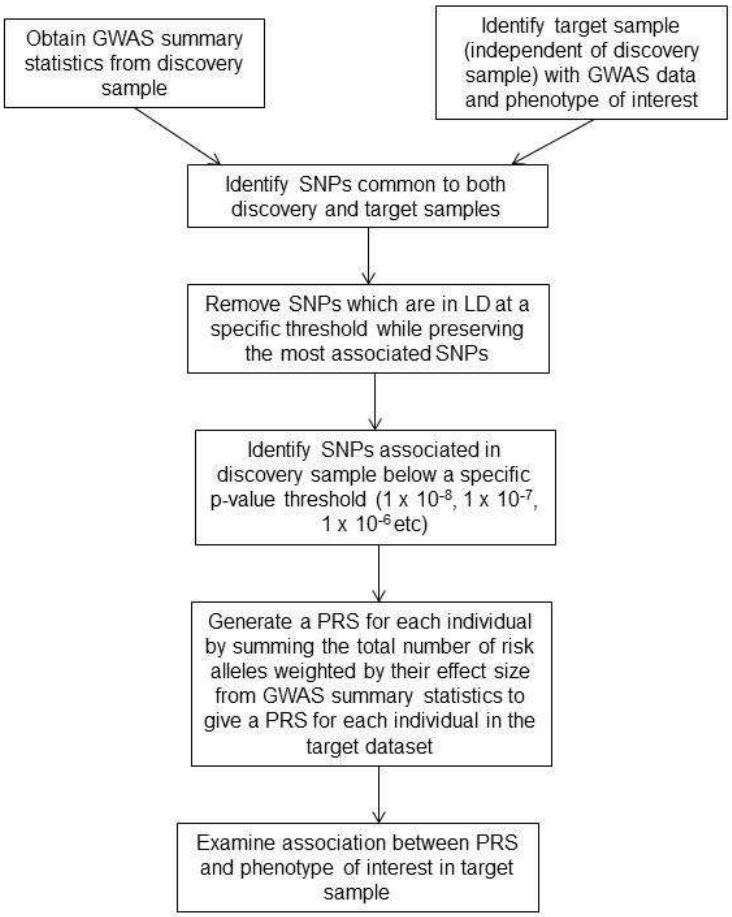
Complex psychiatric disorders, including substance use disorders, have a significant risk component, in which genetic liability is conferred by the combined additive effects of large numbers of variants with small effect sizes[191]. Polygenic risk score (PRS) analysis allows for polygenicity to be assessed from genomic data because it calculates the collective contribution of common SNPs that show disease association but fail to meet the accepted p-value threshold for genome-wide significance. It was initially introduced as a summary score of the gene variants that are less significant than the GWAS-significant threshold value, but the score has also been shown to be valuable when including variants that surpass the GWAS threshold. The PRS was conceptualized based on the number of risk alleles at each loci (0, 1, or 2) and weighted by the odds ratio log from a discovery sample. Polygenic risk can be characterized in a GWAS by creating a summary score of weighted allelic dosage across all SNPs associated with the outcome at a series of pre-specified significance thresholds, often from $1x10^{-8}$ to 0.5[179, 192]. To improve risk prediction, a Bayes approach can be used to enable an automatic PRS weighting scheme without the need of choosing p-value thresholds[192].

4.2 Methods

In an independent sample with GWAS data (the target sample), the PRS is calculated for each individual in the dataset by adding up the risk alleles weighted by their odds ratios from the discovery sample. Variants that are in LD are removed while preserving the most associated SNPs, but some methods instead propose keeping these variants in the model but re-weighting to account for LD[193]. Once variants are selected, the number of risk alleles at each marker are

tallied, weighted by their respective regression beta weight, and summed to create a risk score for each individual in an independent replication sample. It is then possible to evaluate the prediction value of PRS using the coefficient of determination from the regression analyses, expressed as r^2. A trans-ethnic approach assessing the genetic risk of complex traits in minority populations may improve predictive accuracy by including partial genetic overlap in genetic architectures across populations, without assuming transferability of all trait loci between populations[194]. These risk scores are then used to determine the proportion of variation in the trait that can be explained by their cumulative effects. The methodology of creating a single PRS is shown in Figure 8. This can be done using different p-value significance thresholds from the discovery sample, thereby testing whether including more SNPs increases the power of prediction. In order to achieve successful construction of the PRS, the target and discovery samples must be large[195, 196]. If the genetic architecture of a disease consists of many low frequency SNPs with small effect sizes, as opposed to a genetic architecture with SNPs with relatively high effect sizes, a larger discovery sample may be necessary. To achieve sufficient power in smaller target samples of interest, large collaborations may be required. With this score, it is possible to either verify the consistency between the discovery and target sample (for example, to the same disease) as well as to use this score as a variable to correlate with other clinical traits. The potential of PRS is very broad, and may become a fundamental step for any study involving genetic risk.

Figure 8. Basic steps for constructing a typical p-value threshold PRS



4.3 Discussion

There have been numerous applications of PRSs to psychiatric phenotypes thus far, and polygenic risk scores are an emerging method of studying substance use disorders[197-200]. A study by Vink et al calculated risk scores from the Tobacco and Genetics Consortium and generated polygenic risk scores for subjects in an independent target sample from the Netherlands Twin Register and demonstrated that smoking, alcohol, and cannabis use are influenced by aggregated genetic risk factors shared between these substances[187]. Polygenic risk scores can also be utilized in longitudinal studies to assess genetic risks that disrupt the

developmental progression of smoking behaviors. Belsky et al utilized a multilocus genetic risk score unique from a traditional PRS approach which was composed of SNPs identified in 3 meta-analyses of GWAS of smoking phenotypes and found that genetic risk score was unrelated to smoking initiation but that individuals at higher genetic risk were more likely to convert to daily smoking as teenagers, progress more rapidly from smoking initiation to heavy smoking, and were more likely to fail in their cessation attempts[201]. Gene x environment interactions can also be studied using polygenic risk scores. Meyers et. al. calculated genetic risk scores in the Detroit Neighborhood Health Study and found that the association between genetic risk and smoking was greater among individuals who had experienced an increased number of traumatic events in their lifetimes, and diminished among individuals who lived in a neighborhood characterized by greater social cohesion[202]. These studies demonstrate the potential of PRS approaches to increase our understanding of how genetic influences contribute to risk for individual substance use phenotypes, as well as how these genetic influences might contribute to shared risk across substances. Future PRS data will likely continue discovering additional traits that can benefit from a PRS approach as well as incorporating environmental variables as well. As these PRS scores become more predictive, polygenic scores are likely to play a large part in the future of clinical practice.

4.4 Conclusion

Over the past 10 years large-scale GWAS have been highly successful in advancing the field of genetics of substance use disorders, both by revealing new genes and molecular pathways in the pathogenesis of these disorders, although genetic mechanisms remain unknown in many instances[203]. PRS analysis allows the identification of population groups and lowest and highest risks of developing substance use disorders. Distinguishing individuals at high risk for

substance use disorders will prove important for prognosis and early intervention. Additionally, identifying these individuals would benefit study recruitment into clinical trials and longitudinal epidemiological cohorts and could facilitate a better understanding of how gene-gene and gene-environment interactions increase the risk for substance use disorders. Furthermore, it may be possible to capture the polygenic signal for specific biological systems relevant to substance use disorders and utilize these to further elucidate the role of a particular system in the disease process of substance use disorders. A recent paper by Boyle et al proposed the omnigenic model of complex traits, which suggests that all genes affect every complex trait such that interpretation of PRS data may require an understanding of the core genes at the center of a disease process in order to broaden understanding of how peripheral genes contribute to the development of a disorder[204]. In conclusion, PRS analyses have shown successful progress in the research of psychiatric disorders and may inform addiction research to understand more about the genetic underpinnings of substance use disorders. In this way PRS may be useful in the investigation of shared genetic risk with comorbidities and personalizing substance use disorder treatment. Individuals with high genetic risk scores may be able to attenuate their risk with lifestyle modifications or pharmacologic therapies, for which the benefit of treatment may be greater in those at highest genetic risk. Overall, the work described in this chapter shows the complexity of substance use disorder genetics, and further und future genetic studies allowing for more effective integration of PRS data may help bridge the current body of knowledge on genetics and clinical practice.

Chapter 5: Summary and Future Directions

5.1 E-Cigarettes

In Chapter 2, I demonstrated that past 12 month quit attempts and smoking cessation increased among U.S. adults aged 25-44 in recent years compared to 2006. Notably, over the past decade when e-cigarette use was markedly increasing, current use of combustible cigarettes declined. Though we find somewhat different findings for changes in ever smoking in this decade, what is clear is that ever smoking has not increased. Among ever smokers, the proportion of individuals who report that they no longer smoke has increased. Current e-cigarette use was associated with increased past 12 month quit attempts and successful smoking cessation among established smokers. These trends are inconsistent with the hypothesis that e-cigarette use is delaying quit attempts and leading to decreased smoking cessation. In contrast, current e-cigarette use was associated with significantly higher past 12 month quit attempts and past 12 month cessation. These findings suggest that e-cigarette use contributes to a reduction in combustible cigarette use among established smokers. These findings reassure us that the introduction of e-cigarettes has not hampered the ongoing reduction of smoking in the population to date – though we must be vigilant to a reversal of these trends. At this time, e-cigarettes are relevant to future tobacco control policies and interventions and should be considered in these efforts.

Of course, this also raises the question of how e-cigarettes affect combustible cigarette smoking in young adults (<25 years). In this study I found that the association between e-cigarette use and past 12 month quit attempts also extended younger individuals aged 18-24 years but that e-cigarette use was not associated with past 12 month smoking cessation in this younger age group.

Whether e-cigarettes contribute to greater initiation of combustible cigarette use among younger age groups remains a serious public health concern and future studies in this area are warranted.

Finally, although this work examined two independent population-based samples, this study was cross-sectional. In order to determine temporal relationships in smoking behaviors and e-cigarette usage, future studies should investigate the longitudinal effects of e-cigarettes on smoking combustible cigarettes, quit attempts, and smoking cessation in the form of a prospective cohort study or a randomized clinical trial. Randomized clinical trials were once the gold standard in clinical research, however large sample sizes are often needed and validity may require multiple sites, which increases the cost of the study. Furthermore, studying the long term effect of e-cigarettes on smoking behaviors would require long trial run times, which may result in loss of relevance as products and policies may have changed by the time the trial is published. Therefore, future research efforts should focus on smaller, more focused clinical trials involving fewer patients, which would take less time and would focus enrollment on targeted individuals.

5.2 *CHRNA5-CHRNA3-CHRNB4* and Substance Use disorder

In Chapter 3, I demonstrated that the CHRNA5-CHRNA3-CHRNB4 gene cluster on chromosome 15, long been proven in its robust association with smoking behaviors, is specific in its genetic risk for nicotine use disorder in European Americans and was not extended to African Americans. I further demonstrated that this region does not represent a genetic risk for other substance use disorders, specifically alcohol use disorder, cannabis use disorder, and cocaine use disorder in either European Americans or African Americans. Importantly, the functional SNP

rs16969968 (*CHRNA5*) was specific in its increased risk of nicotine use disorder in European Americans and did not have a statistically significant effect on nicotine use disorder in African Americans as well as alcohol, cannabis, and cocaine use disorders in individuals of European and African ancestry. These findings are inconsistent with the hypothesis that this SNP and possibly the entire *CHRNA5-CHRNA3-CHRNB4* gene region may represent a shared genetic risk for substance use disorder other than nicotine. These findings suggest that while nicotine use disorder often co-occurs with other substance use disorders, this observed comorbidity may represent shared environmental risk factors increasing polysubstance use or other shared genetic risk outside of this gene region. Substance use disorders are a leading cause of preventable death worldwide and future risk stratification and treatment protocols should encompass these and other genetic findings on the risk of multiple substance use disorders.

This study provides new evidence for the specificity in the role of the *CHRNA5-CHRNA3-CHRNB4* gene region for nicotine use disorder. Although this meta-analysis incorporated six independent samples, additional larger samples could help to verify all of the association findings in this study. Replication of these findings in additional samples using a variety of different ancestry populations would provide additional evidence supporting the robustness and generalizability of my conclusions.

Association findings from these analyses have led to new hypotheses about the role of shared genetic variation in the development of substance use disorder, and future work should test these hypotheses *in vitro*. Previous experimental studies of rs16969968 have demonstrated that the risk A allele causes a lowered response to nicotine agonists in cell culture experiments and decreased calcium ion permeability in *Xenopus laevis* oocytes[72, 166]. Similar cell experiments could be used in the future to examine the functional effects of the *CHRNA5-CHRNA3-CHRNB4* variants

identified in this study. These biological experiments could improve our understanding of the cellular mechanisms by which these genetic variants affect risk for nicotine use disorder. In this study, I examined the association between the *CHRNA5-CHRNA3-CHRNB4* gene region and substance use disorder and demonstrated that this region is specific for nicotine use disorder in European Americans. A critical next step will be to extend these findings to long-term disease risk and therapeutic response. Tobacco use is associated with an increased risk of numerous chronic diseases, and it will be important to understand how these observed genetic associations with nicotine use disorder translate to overall disease risk and response to treatment. Previous studies have shown that rs16969968 is the strongest genetic risk factor for lung cancer and chronic obstructive pulmonary disease and influences individual response to various smoking cessation therapies, so the logical next step in this work would be to test whether the additional variants in the *CHRNA5-CHRNA3-CHRNB4* region significant for nicotine use disorder in this study similarly increase the risk of smoking-related illnesses and response to smoking cessation treatment[161, 205, 206]. Implementation of these gene-based risk stratification and tailored treatment could improve personalized medical care of nicotine dependent individuals.

5.2 Polygenic Risk of Substance Use Disorders

In Chapter 4, I outline the utility of future studies to expand on these individual gene findings to create polygenic risk scores that predict the development of substance use disorders based on multiple genetic factors. Genome-wide association studies have made enormous strides in identifying common variants associated with substance use disorders, but polygenic risk scores allow the combination of thousands of variants in order to capture an individual's risk of developing complex traits and diseases. Environmental risk factors can also be added to the

genetic predictors in order to make an improved prediction of the disease phenotype. As the U.S. health care system is increasingly technologically driven, polygenic risk scores will need to be used in risk prediction, as a prognostic indicator, or for stratification of therapeutic benefit.

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