GENETIC DETERMINANTS OF SMOKING CESSATION

by

Mindi Annette Styn

BS, Westminster College, 1997

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This dissertation was presented

by

Mindi Annette Styn

It was defended on

May 5, 2006

and approved by

Dissertation Advisor: Joel Weissfeld, MD, MPH
Associate Professor
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Committee Member: Stephanie Land, PhD
Research Assistant Professor
Department of Biostatistics
Graduate School of Public Health
University of Pittsburgh

Committee Member: Kenneth Perkins, PhD
Professor
Department of Psychiatry
School of Medicine
University of Pittsburgh

Committee Member: Marjorie Romkes, PhD
Associate Professor
Department of Medicine
School of Medicine
University of Pittsburgh

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GENETIC DETERMINANTS OF SMOKING CESSATION

Mindi Annette Styn, PhD

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Current findings related to nicotine addiction and related physiologic-metabolic processes create a biological basis to consider the role of interindividual genetic differences governing smoking behavior.

This study examined associations between smoking cessation and a set of potential risk factors measured in a group of adult cigarette smokers participating in a computed tomography (CT) lung cancer screening program. The investigation of non-genetic factors focused on the relationship between CT results and smoking cessation. The investigation of genetic factors attempted to determine genetic influences on the relationship between the dopamine pathway and smoking cessation by examining genetic variation in the dopamine receptor 2 (*DRD2*: *TaqIA*, *TaqIB*, *C957T*, *–141C Ins/Del*) and dopamine transporter (*SLC6A3*).

Participants were part of the Pittsburgh Lung Screening Study (PLuSS), a research based low-dose CT screening program containing current and former cigarette smokers, ages 50 to 79. These analyses were restricted to baseline smokers who indicated their smoking status at follow-up. Non-genetic factors were assessed for all eligible members of the cohort; genetic factors were assessed for a subset.

A CT scan of the lungs that resulted in a referral was significantly associated with abstinence (for more than 30 days) at one year. The relative risk of being abstinent at one year after receiving a CT referral was 1.39 (95% Confidence Interval (CI): 1.14-1.70). After controlling for the matching variables and other genotypes, the *DRD2 TaqIA* polymorphism was significantly associated with being abstinent at one year (p=0.01). Compared to participants with the *A2A2* genotype, participants who carried at least one variant allele (*A1*) were less likely to be abstinent (Odds Ratio: 0.47, 95% CI: 0.24-0.94). *SLC6A3* genotype was not associated with abstinence at one-year (p=0.757). No significant gene-gene interaction with *TaqIA* was observed.

CT screening can create a "teachable moment" for smoking interventions. The association between *TaqIA* and abstinence at one year supports the hypothesis that genetic variation in the dopamine pathway influences smoking cessation.

Public Health Significance: Smoking is the leading preventable cause of death in the United States. Identifying genetic variations that influence smoking behaviors could enhance treatment options for smoking cessation. This dissertation identified both non-genetic and genetic influences on smoking cessation. Consideration of those influences in the selection of quitting regimens may improve success rates thereby reducing the morbidity and mortality due to continued cigarette smoking.

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The primary analyses used in this dissertation project focused on individuals in PLuSS who were smoking cigarettes at baseline (baseline smokers). At the annual study update, approximately one year after enrollment into PLuSS, participants provided information on their cigarette smoking status. This information was used to define groups for the analyses.

A "quitter" was defined as a baseline smoker, who one year later, reported having tried to quit smoking over the preceding year.

A "successful quitter" was defined as a baseline smoker, who one year later, reported not smoking cigarettes for more than 30 days. In this dissertation the terms "abstinent at one year" and "quit at one year" are synonymous with the primary term "successful quitter."

An "unsuccessful quitter" was defined as a baseline smoker who reported current cigarette smoking one year later despite an attempt to quit smoking over the preceding year. In this dissertation the terms "attempted to quit, but not abstinent at one year" is synonymous with the term "unsuccessful quitter."

A "nonquitter" is defined as a cigarette smoker who, one year later, reported not having tried to quit smoking over the preceding year.

1.0 INTRODUCTION

Current findings related to nicotine addiction and related physiologic-metabolic processes create a strong biological basis to consider the role of interindividual genetic differences governing cigarette smoking behavior. The capability to identify and to understand interindividual differences could be translated into more effective potentially pharmacologically based approaches to smoking prevention and treatment. This PhD dissertation paper presents primary results related to smoking cessation and genetic variability in two genes (*DRD2* and *SLC6A3*) involved in dopamine pathways. To justify the investigation of the possible role of *DRD2* and *SLC6A3* in smoking cessation, the following text briefly summarizes current scientific knowledge regarding the genetic basis of cigarette smoking, with particular emphasis on dopamine-related genes.

1.1 BACKGROUND AND SIGNIFICANCE

The link between cigarette smoking and chronic disease is well established. The Center for Disease Control and Prevention (CDC) indicates that cigarette smoking is the leading preventable cause of death in the United States. Over 400,000 premature deaths occur annually because of cigarette smoking. Mortality rates for lung cancer, the leading cause of cancer death, are increased more than 10-fold for current smokers.[1]

Cigarette addiction is a significant public health problem. Almost half of all adults have smoked in their lifetime and, of those adults who have smoked, over half continue to smoke. Although the prevalence of cigarette smoking has decreased over the past few decades in reaction to public health campaigns, many smokers are still unable to quit. According to the

National Health Interview Survey, 70% of smokers indicated that they wanted to quit; however, only 4.7% could abstain from smoking for at least three months.[2]

Despite the various behavioral and pharmacological smoking cessation aides that are currently available, the vast majority (over 90%) of individuals who quit successfully do so without any assistance.[1] The limited effectiveness of cessation aides in the population may be due to interindividual differences in various aspects of smoking behavior including both the strength of addiction and the burden of withdrawal symptoms. These interindividual differences are partially dictated by genetics. Therefore, identification of and consideration of genetic influences on smoking behavior in the selection of quitting regimens may improve success rates.

1.2 LITERATURE REVIEW

1.2.1 Biological Plausibility of Genetic Influences on Smoking Behavior.

Nicotine is considered the main addictive component of cigarettes. One way that nicotine induces addiction is by creating feelings of pleasure in smokers. The main reward pathway acted upon by nicotine is the dopamine pathway, which originates in the ventral tegmental area of the midbrain and extends into the forebrain, most significantly to the nucleus accumbens. [3, 4] Nicotinic acetylcholine receptors (nAChRs) on dopamine neurons trigger the release of dopamine into the synaptic cleft. The dopamine then activates dopamine receptors on the postsynaptic neurons to create the feelings of pleasure that are associated with smoking. Neurotransmission ends when dopamine is depleted from the synapse. Dopamine transporters on the presynaptic neuron ends are responsible for dopamine re-uptake. Dopamine in the synapse is also depleted when it is metabolized either to homovanillic acid by catechol-o-methyl transferase (COMT), monoamine oxidase A and B (MAO A, MAO B), and aldehyde dehydrogenase (ALD) or to norepinephrine by dopamine β-hydroxylase (DBH).[3-5]

Smokers drive the dopamine reward pathway by making nicotine available in their bodies. However, variations along this pathway dictate the amount of nicotine a smoker needs. For example, the density and sensitivity of nAChRs vary among individuals and are altered with long-term exposure to nicotine. The receptors become desensitized leading to nicotine tolerance.

Ultimately these desensitized receptors become inactive and turnover more slowly. This slow turnover leads to increased receptor density. However, after an extended period of time without nicotine exposure, the inactive receptors become active again. Since these receptors are also involved in non-reward pathways, the activation of a large number of receptors may lead to the unpleasant symptoms of withdrawal and may explain why many addicted smokers report that their first cigarette of the day is their most enjoyable. [4]

The efficiency of the pathway is also dictated by the amount of dopamine that is available to activate the dopamine receptors. Dopamine synthesis starts when the amino acid tyrosine enters the neuron and is converted to dihydroxyphenylalaine (L-DOPA) by tyrosine hydroxlyase (TH). [3, 5] Since this is considered the rate-limiting step in dopamine synthesis, variations in the *TH* gene may play a role in the effects of nicotine on the body and on smoking behaviors.

The availability of dopamine is not solely influenced by the amount of dopamine created; it is also influenced by the rates of re-uptake and metabolism that remove dopamine from the synapse. Genetic variations in the dopamine transporter gene (*SLC6A3*), *MAO A* and *B*, and the *COMT* gene may influence the rates of reuptake and metabolism of dopamine.[5, 6]

Activation of dopamine receptors causes the feelings of pleasure that smokers get from nicotine. The amount of activation is not only influenced by the amount of dopamine available, but also by the number, or density, of receptors. Five types of dopamine receptors, designated D1 through D5, have been identified. Each of the dopamine receptor genes (*DRD1* through *DRD5*) is polymorphic, and some of these polymorphisms such as the *TaqIA* polymorphism on *DRD2* have been shown to decrease receptor density.[7] Polymorphisms in the *DRD2* gene have been implicated in studies of alcoholism and, thus, have been the most scrutinized in studies of other addictions including nicotine addiction. [3]

To sustain the pleasurable effects of nicotine on the body, smokers must regulate nicotine levels in their bodies. This desire to maintain a steady state of nicotine dictates individual smoking behaviors such as how frequently a smoker smokes and how deeply the smoker inhales. When nicotine levels or the effects of nicotine on the body are artificially manipulated, smokers modify their smoking behaviors. For example, nicotine replacement therapy can successfully be used to reduce the number of cigarettes consumed by increasing the amount of nicotine available in the body. Alternatively, the drug mecamylamine, which blocks central nicotinic receptors and reduces the effects of nicotine, causes an increase in the number of cigarettes consumed.[8]

Nicotine levels in the body are drained by metabolism and elimination of nicotine. When cigarette smoke is inhaled, nicotine is absorbed through the lung epithelium and travels to the brain where it crosses the blood-brain barrier. Nicotine can be metabolized through three different pathways: C-oxidation, N-oxidation, and N-methylation. [6]

The primary pathway for the metabolism of nicotine is C-oxidation in the liver by the cytochrome P450 enzyme CYP2A6, which produces cotinine.[5] CYP2A6 metabolizes 60 to 80 percent of nicotine to cotinine.[6] Phenotyping studies done with the probe drug coumarin show interindividual variability in CYP2A6 activity in vivo.[9] A 30-fold variation in nicotine-to-cotinine Vmax was found when CYP2A6 levels and activity were studied in human liver microsomes.[9, 10] Since CYP2A6 is the only enzyme that is able to produce the 7-hydroxy metabolite of coumarin, studies of the metabolite directly reflect CYP2A6 enzyme activity. [11]

Based on our extensive knowledge of the physiology of nicotine addiction, we hypothesize that genetic variation, involving the genes mentioned above (*COMT, MAO A, MAO B, ALD, DBH, TH, SLC6A3, CYP2A6, DRD1* through *DRD5*), might partially determine interindividual differences in smoking. This list is not exhaustive. Other investigators have hypothesized roles for additional genes including *CYP2D6*, *CYP2A13*, *CYP2B6*, *orosomucoid 1* (*ORM1*), *alcohol dehydrogenase 3* (*ADH3*), opioid receptors *OPRD1* and *OPRM1*, serotonin transporter (*SLC6A4*), serotonin receptors hydroxytryptamine 1D (*HTR1D*) and *HTR1B*, tryptophan hydroxylase (*TPH*), cholecystokinin (*CCK*) and acetylcholine receptor alpha-subunit (*CHRNA7*) ([12-17]). The genes *DRD2* and *SLC6A3* were selected as the focus of this work because of the interest in *DRD2* in relation to alcoholism and addiction in general (see above) and the prominent role that *SLC6A3* plays in nicotine reuptake. These two genes also potentially influence each other's effect on smoking behaviors.

1.2.2 Twin Studies

The results of numerous twin studies conducted since the mid-twentieth century in various populations support a hypothesis that genotype is a major factor in smoking behavior.[3, 6] The reported heritability estimates have been as high as 84% (28 to 84%). Recent studies have investigated distinct phases of smoking behaviors, from initiation to dependence and persistence, and found support for genetic involvement across all phases. [3, 6, 18]. In a study of 2,163

female twins in Virginia, Kendler, et al., reported that the risk factors for initiation and dependence are related but not identical. Heritability rates for nicotine dependence were around 72%.[19] In a study of 3,356 members of a male veteran twin pair registry the heritability of nicotine dependence was 60.3% (95% confidence interval: 55.4 – 65.2%).[20] Although twin studies have been valuable in establishing a genetic link to smoking behavior, they are unable to provide insight into which specific genes influence behavior.

1.2.3 The DRD2 Gene

As explained previously, the dopamine pathway is a major reward pathway implicated in addiction to nicotine and other drugs. Among the various dopamine receptors, *DRD2* has received the most attention because of an early finding of an association between the *TaqIA* restriction fragment length polymorphism (RFLP) on the *DRD2* gene, located on chromosome 11q23, and alcoholism. This has led to *DRD2* research for various addictions and disorders, including nicotine addiction.

1.2.3.1 DRD2 Variants

Many variants in the *DRD2* gene have been found. We have selected *TaqIA* and *TaqIB* because of their importance in the literature with regard to smoking behaviors and *C957T* and *–141C Ins/Del* because they are functional changes that have not been thoroughly investigated in relation to smoking cessation.

Table 1 summarizes the frequencies of each variant in Caucasians, the nucleotide change associated with each variant, the reported effect of the change, and the location of each variant on chromosome 11. Table 2 summarizes a review of the literature for the allele frequencies in a variety of populations.

1.2.3.2 Associations of Selected DRD2 Variants with Smoking Behaviors

Of the selected *DRD2* variants *TaqIA* is clearly the most common variant studied in relation to smoking behaviors (table 3). Although early studies were promising, findings from more recent studies have not strongly supported the hypothesis of a direct link between *TaqIA*

and smoking cessation but have reported interesting findings in relation to gender differences and cessation treatment.

One of the earliest reports of a link between *DRD2* variants and cigarette smoking was from a study conducted in 312 non-Hispanic Caucasian case subjects who were part of a smoking cessation clinic that targeted individuals who had at least one unsuccessful attempt at cessation. Control subjects were a combination of 235 subjects obtained by the investigators and 479 subjects whose data were obtained by the investigators from abstracts presented in poster sessions. Control subjects were all screened to exclude problems with addiction other than tobacco. Two of the control subjects were smokers. The controls were in Hardy-Weinberg equilibrium, but the cases were significantly out of equilibrium. The frequency of the *A1* allele was significantly different between cases (0.264) and controls (0.147). Relationships between other smoking related variables, such as age at initiation and maximum quit duration, and the *A1* allele were also reported.[21]

A sample of 354 non-Hispanic Caucasian current smokers (n=57), former smokers (n=115) and nonsmokers (n=182) was genotyped for the *A1* allele. The prevalence of the variant was significantly different among the three groups (p=0.018), and a significant trend was reported with the frequency of the allele increasing from nonsmokers to former smokers to current smokers. When all smokers (former and current) were compared to nonsmokers the odds ratio was 1.85. When this comparison was limited to current smokers only, the odds ratio increased to 2.15. [22]

In a study of 104 smokers who smoked at least 5 cigarettes per day and 117 non-tobacco users who either never smoked or had not used any tobacco-containing product for at least 10 years no association was found between the *TaqIA* variant and smoking status. This study was limited to Caucasians from the North East of England. [23]

Researchers from the M.D. Anderson Cancer Center frequency matched a group of 126 Caucasian control subjects to 157 newly diagnosed lung cancer patients. The case and control subjects were classified as never smokers and ever smokers who smoked more than 100 cigarettes in their lifetime. Ever smokers who quit at least one year before the interview were further classified as former smokers. The association between having at least one *TaqIA* allele or at least one *TaqIB* allele was observed. The *TaqIB* variant was more common in ever smokers, but this was only statistically significant in controls. Relationships were also observed between

both the *TaqIA* and *TaqIB* variants and early smoking initiation and number of cigarettes smoked per day. It should be noted that ever smokers greatly outnumbered never smokers among both cases (142:15) and controls (113:13). [7]

In a follow-up study conducted in 208 African-Americans and 154 Mexican-Americans with and without lung cancer the same group of researchers from M.D. Anderson Cancer Center identified relationships between *DRD2* genotype and several smoking variables among control subjects only. Although few of these relationships reached statistical significance, they may be of interest for larger studies. Among the 111 Mexican-American controls the *A1A1* genotype appeared to be positively related to smoking (20% were never smokers and 60% were current smokers) and the *A2A2* genotype appeared to be negatively related to smoking (53.1% were never smokers and 28.1 % were current smokers). Similarly, among Mexican-American controls the *B1B1* genotype appeared to be positively related to smoking (21.4% were never smokers and 64.3% were current smokers) and the *B2B2* genotype appeared to be negatively related to smoking (53.7% were never smokers and 19.5 % were current smokers). While a $\chi 2$ test for trend was strongly significant for the *B1* frequency among never smokers, former smokers, and current smokers, the test for trend for the *A1* frequency did not reach statistical significance. Relationships between genotype and age at initiation and quantity smoked were also reported.[24]

These early reports have been questioned due to potential confounding that may have been introduced in the selection of the control populations.[5]

Lerman, et al., reported that the *SLC6A3*-9 allele was found less frequently among a group of 289 smokers (smoked at least five cigarettes per day for at least 1 year) when compared to 233 non-smokers (smoked less than 100 cigarettes in their lifetime). No differences were found between smokers and non-smokers in the prevalence of the *TaqIA* variant; however, the study suggested that *DRD2* might modify the effects of the dopamine transporter gene.[25]

In a study of 332 Japanese individuals, the association between smoking and the *TaqIA* variant and the –141C Insertion/Deletion (Ins/Del) variant was examined. Subjects were classified as never smokers who smoked less than 100 cigarettes in their lifetime (n=198), former smokers who quit at least one year prior to the interview (n=57), and current smokers who may have quit within one year of the interview (n=77). The ever smokers group was a combination of the current and former smokers (n=134). In contrast to previous reports, the *TaqIA* wild type

genotype was significantly more frequent in the ever smokers than in the never smokers. No relationship between *–141C Ins/Del* and smoking status was observed.[26]

A study of 101 male and 66 female Korean subjects with schizophrenia examined the relationship between the *TaqIA* allele and smoking behavior. Only smokers with a score greater than seven on the Fagerstrom test for nicotine dependence and non-smokers with no previous smoking experience were included in the study. The findings from this study are unique in that they present the hypothesis that the relationship between *TaqIA* and smoking shows gender-specific molecular heterosis. Significantly more heterozygous males were smokers than homozygous males of either genotype (wild type or variant). Fewer heterozygous females were smokers than homozygous females of either genotype, but this did not reach statistical significance.[27] The findings of this report were further supported in a study in 187 healthy Korean subjects. The follow-up study used the same definitions for smokers and non-smokers. In males, this study reported significantly higher *A1* allele frequency and prevalence in smokers compared to non-smokers. The data for females suggested an inverse relationship between smoking and allele frequency and prevalence, but these findings were not statistically significant. In females, significantly fewer heterozygotes were observed in the group of smokers.[28]

Johnstone, et al., investigated the impact of *DRD2 TaqIA* on smoking behaviors among participants in the OXCHECK study, a large population based cohort study that was conducted in the United Kingdom. Current smokers were classified into four groups: low consumption (1-9 cigarettes per day), mid consumption (10-19 cigarettes per day) and high consumption (20+ cigarettes per day). Three hundred participants were selected from each group and from the group of never smokers. A total of 975 participants were successfully genotyped. No associations were found between *DRD2 TaqIA* genotype and level of smoking consumption, smoking status (never smoker vs. current smoker) or age at smoking initiation.[29]

Three studies have investigated the influence of *DRD2 TaqIA* genotype on the use of bupropion for smoking cessation. In a clinical trial of bupropion treatment for smoking cessation conducted in the United States, 418 participants of European Caucasian descent were randomly assigned to receive either a placebo (n=191) or bupropion (n=227) for 10 weeks along with standard behavioral counseling. All participants were successfully genotyped for both *DRD2 TaqIA* and *SLC6A3*. Neither *DRD2 TaqIA* nor *SLC6A3* genotype had a significant effect on smoking behavior at the end of treatment nor at the 6-month follow-up; however, *SLC6A3* had a

significant effect on smoking cessation at the end of treatment for individuals with the *TaqIA A2* allele. In Logistic regression analyses the gene-gene interaction term was significant with the *SLC6A3*-9 allele being significantly associated with cessation at the end of treatment for individuals with the *TaqIA A2* allele. This effect was not observed at 6-months post-treatment, and a significant time**DRD2***SLC6A3* interaction effect was observed.[30] Similarly among 451 Caucasian participants in an open-label, randomized effectiveness study of bupropion and counseling for smoking cessation, Swan et al. found no significant difference in smoking cessation at one year and presence or absence of the *A1* allele. However, among female participants, those with an *A1* allele were significantly more likely to stop taking the bupropion than those without an *A1* allele. The number of copies of the *A1* allele that a woman carried was also associated with smoking cessation at one year. Women were significantly more likely to quit smoking if they carried more *A2* alleles.[31] In a smaller randomized trial comparing bupropion to placebo (n=29) withdrawal cravings were measured among participants who had been on bupropion for two weeks. Those participants with carrying the *A1* allele did not experience significant reductions in cravings while those with the *A2A2* genotype did. This report did not address differences in smoking status with regard to genotype.[32]

The strong biological rationale for the impact of *DRD2* on smoking behaviors and the paucity of data available on the topic provide the impetus for us to look at this potential association. While the previous studies described above provide us with interesting research questions, the inconsistency of their results suggest that more research is needed on this variant.

1.2.4 The SLC6A3 Gene

The *SLC6A3* gene is located on chromosome 5p15.3 and encodes the dopamine transporter (DAT) reuptake protein that removes dopamine from the synapse. The important role that DAT plays in removing dopamine from the synapse has led to research on *SLC6A3* and various dopamine related disorders. Variations in a 40-base pair repeat, occurring primarily as a 9- or 10-repeat, in the 3' untranslated region are related variations in the density of transporter molecules on the surface of neurons.[33]

1.2.4.1 SLC6A3 VNTR Variant

We selected the *SLC6A3* VNTR variant because it is the most studied of the DAT genetic variants and because of previously reported association between this variant and *DRD2*.[25, 30]

As previously stated, the *SLC6A3* VNTR occurs primarily as a 9- or 10-repeat allele. The 9-variant allele is considered the variant allele, and the 10-repeat is considered the wild type. Table 4 summarizes the frequencies of these alleles in Caucasians as well as the proposed effect of the allele. Table 5 summarizes a review of the literature for the allele frequencies in a variety of populations.

1.2.4.2 Associations of SLC6A3 VNTR Variants with Smoking Behaviors

We found five reports that investigated a link between *SLC6A3* and smoking behaviors. Four of these addressed the specific issue of smoking cessation. The results of this literature review are shown in table 6. Initial reports of a main effect relationship between the *SLC6A3*-9 allele and reduced smoking (either quitting or not smoking vs. smoking) were not replicated in subsequent studies. However, it is difficult to conclude whether or not this variant is significantly associated with smoking cessation because of the limited number of reports available.

In a study of 1,107 individuals recruited through several National Institutes of Health protocols not specific to smoking cessation (cancer risk-related behaviors, personality genetics, sexual behavior), Sabol, et al., found a significant association between *SLC6A3* genotype and smoking cessation among 593 nonsmokers (less than 100 cigarettes), 283 current smokers, and 231 former smokers. The *SLC6A3*-9 allele was more common among former smokers compared to those who were current smokers.[34]

In a previously described study, Lerman, et al., reported that the *SLC6A3*-9 allele was found less frequently among a group of 289 smokers when compared to 233 non-smokers. Although no differences were found between smokers and non-smokers in the prevalence of the *TaqIA* variant; however, the study suggested that *DRD2* might modify the effects of *SLC6A3*. Caucasian participants with *SLC6A3*-9 genotypes were significantly more likely to be non-smokers only among those participants with the *TaqIA A2A2* genotype. A similar, but nonsignificant trend was seen among African-American participants.[25] In a subsequent randomized trial of bupropion for smoking cessation described earlier in this dissertation *SLC6A3* was not significant as a main effect in the entire sample, but was significant among

participants with the *DRD2 TaqIA A2A2* genotype. In the multivariate model in the entire sample the *DRD2*SLC6A3* interaction term was significant.[30]

In contrast, Vandenberg, et al., reported no relationship between *SLC6A3* and smoking behavior among 595 randomly selected adult volunteers when the same group classifications were used that were used in the previously described reports by Lerman and Sabol (193 nonsmokers who smoked <100 cigarettes, 88 former smokers, 48 current smokers). However contrary to the previously reported associations, when the 135 never-smokers (0 lifetime cigarettes) and 59 non-smokers (<100 lifetime cigarettes) were analyzed as separate groups, *SLC6A3*-10 was more frequent among never smokers. [33]

Jorm, et al. found no association between the *SLC6A3* gene and either smoking initiation or cessation in a community sample of 861 Caucasian participants from Australia. [35]

1.3 OBJECTIVE

The primary research findings from this PhD study of the genetic basis of cigarette smoking cessation are presented in the form of three distinct manuscripts. Each manuscript describes research results from the same research population, current 50 to 79 year old cigarette smokers followed for one year after entry into a research-based lung cancer screening program (the Pittsburgh Lung Screening Study, PLuSS). The first manuscript describes the association between questionnaire-based risk factors and cigarette smoking cessation one year after entry into PLuSS. The second and third manuscripts describe risk associations between cigarette smoking cessation and *DRD2* (manuscript 2) and *SLC6A3* (manuscript 3).

Table 1: A summary of the selected DRD2 polymorphisms. Frequencies are reported for Caucasians

Allele	Frequency	Nucleotide Change	Effect	Chromosome Location	ID Number
TaqIA	19.3%[21]	3' C>T	Decreased receptor density [21, 28]	112776038[15]	Rs1800497
TaqIB	17.0%[29]	3' A>G	Unknown, but variant is closer to coding region than TaqIA [29]	112801496[15]	Rs1079597
C957T	57.0%[6]	C957T	Decreased stability of mRNA/Decreased protein synthesis [6]	112788669[24]	Rs6277
-141C Ins/Del	11.0%[2]	Deletion of -141 C	Decreased promoter activity [1, 2, 31]	112851462[15]	Rs1799732

Table 2: Selected DRD2 polymorphisms, frequencies of the variant allele reported in the literature

Author	Geographic Area	N	Frequency Of Variant Allele
TaqIA			
Lerman (1999)[21]	USA	1044 Total: 444 Caucasians 78 African Americans	 0.193 0.301
Erblich (2004)[7]	New York, East Harlem, USA	108 smokers, predominately African American	0.361
Cinciripini (2004)[4]	Texas, USA	134 Total	0.264
Spitz (1998)[29]	Texas, USA	283 Total: 157 lung cancer cases 126 controls	0.222 0.218 0.228
Wu (2000)[36]	Texas, USA (limited to African-Americans and Mexican-Americans)	357 Total: 139 lung cancer cases 218 controls	0.387 0.374 0.394
Comings (1996)[5]	California, USA (limited to non-Hispanic Caucasians)	424 Total: 312 smokers at least 1 unsuccessful quit attempt 235 controls (includes smokers)	0.270 0.264 0.277
Lerman (2004)[22]	USA (limited to smokers of European ancestry)	71 Total	0.260
Noble (1994)[25]	Nevada and California, USA (Limited to non-Hispanic Caucasians)	354 Total	0.196
Johnstone (2004)[12]	United Kingdom	975 Total: 470 smokers 145 never smokers	0.200 0.208 0.197

Table 2 continued

Author	Geographic Area	N	Frequency Of Variant Allele
TaqIA			
Gorwood (2000)[9]	France (limited to males)	162 Total: 113 alcohol dependent 49 controls	0.395 0.389 0.408
Xu (2004)[37]	Germany	418 Total: 227 heroin-dependent cases 191 controls	0.198 0.194 0.202
Xu (2004)[37]	China	799 Total: 486 heroin-dependent cases 313 controls	0.392 0.401 0.379
Lee (2003)[18]	Korea	187 Total: 94 Smokers 93 Non-smokers	0.41 0.42 0.40
Lee (2002)[19]	Korea (limited to schizophrenics)	167 Total: 96 smokers 71 never smokers	0.47 0.48 0.47
Suzuki (2000)[32]	Japan (limited to schizophrenics)	25 Total	0.34
TaqIB			
Spitz (1998)[29]	Texas, USA	283 Total: 157 lung cancer cases 126 controls	0.160 0.166 0.152
Wu (2000)[36]	Texas, USA (limited to African-Americans and Mexican-Americans)	308 Total: 117 lung cancer cases 191 controls	0.260 0.231 0.277
Xu (2004)[37]	China	772 Total: 466 heroin-dependent cases 306 controls	0.538 0.480 0.587
Xu (2004)[37]	Germany	372 Total: 266 heroin-dependent cases 112 controls	0.717 0.720 0.710
Yoshida (2001)[38]	Japan	332	0.357
C957T			
Duan (2003) [6]	USA	146 Total: 94 European-American 51 African-American	0.40 0.57 0.09
Lawford (2005) [17]	Australia (limited to Caucasians)	301 Total: 153 schizophrenics 148 controls	0.521 0.445 0.581

Table 2 continued

Author	Geographic Area	N	Frequency Of Variant Allele
-141C Ins/Del			
Xu (2004)[37]	Germany	662 Total: 471 heroin-dependent cases 191 controls	0.105 0.104 0.107
Xu (2004)[37]	China	784 Total: 475 heroin-dependent cases 309 controls	0.121 0.118 0.083
Hori (2001) [11]	Japan	442 Total: 241 cases with schizophrenia 201 controls	0.17 0.18 0.16
Katsuragi (2001)[14]	Japan	105 Total	0.252
Yoshida (2001)[38]	Japan	332 Total	0.161

Table 3: Reports investigating a link between DRD2 polymorphisms and smoking behaviors. Odds ratios were calculated as the odds of the reduced smoking behavior in the presence of the variant allele

Author	Geographic Area	Variant	Comparison Groups	OR (95% CI)	Interactions
Comings (1996)[5]	California, USA (limited to non-Hispanic Caucasians)	*TaqIA*	Smokers = 312 Controls = 714	0.37 (0.28-0.49)	
Noble (1994)[25]	Nevada and California, USA (Limited to non-Hispanic Caucasians)	*TaqIA*	Current Smokers = 57 Nonsmokers = 182	0.464 (0.251-0.857)	
			Current Smokers = 57 Past Smokers = 115	0.795 (0.419-1.509)	
Singleton (1998)[28]	England	*TaqIA*	Smokers = 104 Nonsmokers = 117	1.679 (0.964-2.924)	
Spitz (1998)[29]	Texas, USA	*TaqIA*	Ever Smokers = 173 Never Smokers = 107	0.744 (0.324-1.711)	
		TaqIB	Ever Smokers = 193 Never Smokers = 76	0.182 (0.042-0.787)	
Lerman (1999)[21]	USA	*Taq1A*	Smokers = 289 Nonsmokers = 233	0.830 (0.580-1.188)	Interaction with SLC6A3 genotype
Wu (2000) [36]	Texas, USA (limited to African-Americans and Mexican-Americans)	*TaqIA*	Current Smokers = 72 Former Smokers = 59	0.988 (0.473-2.066)	
		TaqIA	Current Smokers = 72 Never Smokers = 87	0.807 (0.417-1.561)	
		TaqIB	Current Smokers = 60 Former Smokers = 51	0.565 (0.265-1.204)	
		TaqIB	Current Smokers = 60 Never Smokers = 80	0.716 (0.366-1.402)	
Yoshida (2001)[38]	Japan	*TaqIA*	Current Smokers = 77 Former Smokers = 57	0.645 (0.322-1.293)	
		TaqIA	Current Smokers = 77 Never Smokers = 198	2.25 (1.314-3.857)	
		-141C Ins/Del	Current Smokers = 76 Former Smokers = 57	1.227 (0.585-2.575)	
		-141C Ins/Del	Current Smokers = 76 Never Smokers = 194	0.947 (0.527-1.702)	
Lee (2002)[19]	Korea Limited to individuals with schizophrenia	*TaqIA*	Smokers = 96 Nonsmokers = 71	0.850 (0.425-1.700)	Gender specific heterosis: Male heterozygotes more likely to smoke (sig), female heterozygotes less likely (ns)
Lee (2003)[18]	Korea	*Taq1A*	Smoker = 94 Nonsmoker = 93	0.937 (0.506-1.736)	Gender specific heterosis was reported
Cinciripini (2004)[4]	Texas, USA	*Taq1A*	134 Smokers OR is for abstinence over several time points within 1 year follow-up	0.649 (0.424-0.990)	
Johnstone (2004)[12]	United Kingdom	*TaqIA*	Current Smokers = 732 Never Smokers = 243	1.212 (0.900-1.633)	
Swan (2005) [33]	Washington, USA Limited to Caucasians	*Taq1A*	Continued Smokers = 276 Quitters at 1 year = 140	0.777 (0.500-1.206)	

Table 4: A summary of the frequencies of the SLC6A3 polymorphisms in Caucasians and the effect of the polymorphism

Allele	Frequency	Nucleotide Change	Effect
SLC6A3-10 (WT)	73%	10-copy repeat	None [26]
SLC6A3-9	27%	9-copy repeat	Reduced concentration of transporter molecules on the surface of neurons [26, 35]

Table 5: Frequencies of the 9-repeat SLC6A3 allele reported in the literature

Author	Geographic Area	N	Frequency of 9-repeat allele
Sabol (1999)[26]	USA	1107 Total	0.267
Lerman (1999) [21]	USA	522 Total: 444 Caucasians 78 African Americans	0.208 0.193 0.301
Vandenbergh (2002)[35]	Continental USA	579 Total: 515 Whites 31 Blacks	0.247 0.255 0.194
Erblich (2004)[7]	New York, East Harlem, USA	108 smokers – Predominately African American	0.306
Lerman (2003)[23]	Washington, DC and New York, USA Limited to European Caucasian ancestry	418 smokers	0.285
Stein (2005)[30]	Illinois, USA	47 children with attention deficit hyperactivity disorder (ADHD)	0.362
Van Dyck (2005)[34]	Connecticut, USA Limited to European Americans	96 Total	0.24
Jorm (2000)[13]	Australia	861 Caucasians	0.277
Köhnke (2005) [16]	Germany	318 Total: 216 alcoholics 102 controls	0.42 0.48 0.32
Galili-Weisstub (2005)[8]	Israel	68 Total	0.46
Simsek (2005) [27]	Oman	110 Total	0.332
Simsek (2005) [27]	Oman	202 Total: 92 children with ADHD 110 healthy subjects	0.327 0.321 0.332
Cheon (2005) [3]	Korea	11 children with ADHD	0.091
Hong (2003) [10]	Taiwan	210 Total: 98 methamphetamine dependent cases 112 controls	0.083 0.089 0.078
Lin (2003) [10]	Taiwan	447 Total: 193 cases with Parkinson's Disease 254 controls	0.069 0.083 0.059

Table 6: Reports investigating a link between SLC6A3 polymorphisms and smoking behaviors. Odds ratios were calculated as the odds of the reduced smoking behavior in the presence of the variant (9-repeat) allele

Author	Geographic Area	Comparison Groups	OR (95% CI)	Interactions
Sabol (1999)[26]	USA	593 Nonsmokers 514 Current and Former Smokers	0.89 (0.70-1.12)	Non-significant trend for interaction with *DRD2 TaqIA*
		231 Former Smokers 283 Current Smokers	1.49 (1.05-2.11)	
Lerman (1999)[21]	USA	233 Smokers 289 Controls (<100 lifetime cigarettes)	1.44 (1.02-2.04)	Interaction with *DRD2 TaqIA*
		Caucasians only: 237 Smokers 207 Controls (<100 lifetime cigarettes)	1.40 (0.96-2.03)	
		African-Americans only: 52 Smokers 26 Controls (<100 lifetime cigarettes)	1.19 (0.44-3.24)	
Jorm (2000)[13]	Australia	452 Nonsmokers 198 Current Smokers	1.07 (0.77-1.50)	
		211 Former Smokers 198 Current Smokers	1.16 (0.79-1.72)	
Vandenbergh (2002)[35]	Continental USA (random digit dialing used)	153 Former Smokers 98 Current Smokers	0.77 (0.46-1.28)	
		214 Never Smokers 98 Current Smokers	0.61 (0.38-0.99)	
Lerman (2003)[20]	Washington, DC and New York, USA Limited to European Caucasian ancestry	At the end of bupropion treatment: 201 Abstinent 217 Smoking	1.16 (0.79-1.70)	*DRD2 TaqIA*SLC6A3* at end of treatment and time**SLC6A3 *TaqIA*
		Six months following bupropion treatment: 115 Abstinent 303 Smoking	1.15 (0.75-1.77)	

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2.0 PREDICTORS OF SMOKING CESSATION ONE YEAR AFTER ENROLLMENT IN A LUNG SCREENING STUDY

2.1 ABSTRACT

Introduction

Despite smoking prevention and cessation efforts, many people continue to smoke. Low-dose computed tomography (CT) screening programs for the early detection of lung cancer may provide a "teachable moment" for clinicians to discuss lung health and the benefits of smoking cessation with a large number of patients.

Objective

This cohort study attempts to identify differences between smokers who successfully quit smoking one year after entering a lung screening program and those who do not.

Participants

Participants are part of the Pittsburgh Lung Screening Study (PLuSS), a research-based low-dose CT screening program in which current and former cigarette smokers, ages 50 to 79 years, were recruited from the general population in the Pittsburgh area. Eligible members of the cohort must have smoked at least one-half pack per day for at least 25 years.

Methods

Questionnaire data from all baseline smokers who completed baseline and follow-up surveys were evaluated. Subjects (n=2046) were classified according to one-year quit status (Nonquitters, Unsuccessful Quitters, Successful Quitters) and to whether the CT necessitated a referral.

Relative risks were calculated, and multinomial logistic regression was used to identify the independent effect of the baseline CT result.

Results

The relative risk of being a Successful Quitter at one year after receiving a CT referral was 1.39 (95% Confidence Interval (CI): 1.14-1.70). Even after controlling for marital status, gender, symptoms of lung disease, and pulmonary conditions, a CT referral was significantly related to quitting behavior ($p<0.001$). Relative to participants with CT results not resulting in a referral, participants who had CT results necessitating a referral were more often in the Unsuccessful Quitter group (OR: 1.43, 95% CI: 1.17-1.74, compared to Nonquitter) and to be in the Successful Quitter group (OR: 1.24, 95% CI: 0.96-1.61, compared to Unsuccessful Quitter).

Conclusion

This association supports the notion that CT screening can influence smoking behaviors, thereby creating a "teachable moment" for smoking interventions..

2.2 INTRODUCTION

Despite ongoing smoking prevention and cessation efforts, many people continue to smoke. Almost half of all adults have smoked in their lifetime and, of those adults who have smoked, over half continue to smoke.[1] Although the prevalence of cigarette smoking has decreased over the past few decades in reaction to public health campaigns,[2] many smokers are still unable to quit. According to the National Health Interview Survey, 70% of smokers indicated that they wanted to quit; however, only 4.7% could abstain from smoking for at least three months.[1]

Public health campaigns have improved awareness among past and current smokers that smoking increases the risk of lung cancer. We presume that personal knowledge of lung cancer risk attracts many smokers to screening programs. Although early attempts at lung cancer screening programs were discouraging, technological advances in screening modalities, specifically low-dose spiral computed tomography (CT) which has reduced both the level of radiation exposure and the amount of time required to complete the screening, have renewed

interest in lung cancer screening.[3, 4] Low-dose CT screening programs have been initiated worldwide.[5-9] In addition to the potential for early detection of disease, a possible benefit of mass screening with low-dose CT screening is a "teachable moment" for clinicians to discuss lung health and the benefits of smoking cessation with a large number of patients.[7, 10] Identifying characteristics of smokers who enter lung screening programs and differences between those smokers who do and do not quit after the screening will enable clinicians to take advantage of this "teachable moment" and apply interventions more effectively at screening visits.

2.3 METHODS

2.3.1 Population

The current analysis focuses on participants in the Pittsburgh Lung Screening Study (PLuSS), a research-based low-dose CT screening program conducted as part of the University of Pittsburgh Lung Cancer SPORE program. PLuSS subjects were recruited through mass media (paid newspaper advertising and public service announcements), physician referral, and mass mailings. The cohort comprises current and former cigarette smokers between the ages of 50 and 79 years who were recruited between January, 2002 and April, 2005. Eligible members of the cohort must smoke or have smoked at least one-half pack per day for at least 25 years. Former smokers must have quit no more than 10 years prior to enrollment.

2.3.2 Procedures

As part of the PLuSS study protocol, eligible subjects were asked to complete a standardized, self-administered baseline survey. The survey was based on the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Study baseline questionnaire and contained both multiple choice and open-ended questions regarding demographic characteristics, past and current smoking behaviors, family history of cancer, personal history of cancer, and personal history of other

smoking-related conditions and symptoms. The survey responses were visually edited and double entered.

Clinical assessments included a baseline forced expiratory spirometry conducted and analyzed according to the American Thoracic Society and Global Initiative for Chronic Obstructive Lung Disease (GOLD) Standards[11] and a low-dose spiral CT scan. Detailed results from the assessments were mailed to participants. Participants who received reports of suspicious nodules from their CT scan were invited to discuss the findings with the PLuSS physician.

A brief telephone interview was to be conducted approximately one year after the baseline CT scan to determine if any changes in vital status, cancer status, or smoking status had occurred. For the current analysis, questionnaire data from all baseline smokers who completed both the baseline and the one-year follow-up survey were evaluated.

Participants were classified as continuing smokers versus abstinent at one year based on their response to the annual study update question, "Are you currently smoking cigarettes?" For these analyses, participants who were abstinent at one year were restricted to those participants who remained abstinent for more than 30 days prior to the follow-up interview (Successful Quitters). A small number of participants (n=47) who were not smoking at the one year follow-up but had quit for 30 days or fewer were excluded from the analyses. Continuing smokers were further divided into those who had not made any quit attempt in the follow-up interval (Nonquitters) and those who had made an attempt but were smoking at the time of the follow-up interview (Unsuccessful Quitters). One participant was excluded because quit status could not be determined from the information provided during the follow-up interview.

2.3.3 Data Analysis

Predictor variables included the following demographic variables: Gender (Male vs. Female), Age (by decade of life at baseline), Race/Ethnicity (White Non-Hispanic vs. other), Education (High School or less vs. other), and Marital Status (Married/living as Married vs. Divorced/Separated/Widowed vs. Never Married). Predictor variables also included Family History of Any Cancer (Yes vs. No), Family History of Lung Cancer (Yes vs. No) and Personal History Any Cancer Except Lung (Yes vs. No). Participants were asked if they had recently

experienced any of the following symptoms: Hemoptysis, Phlegm, Cough, Wheeze, Dyspnea, Edema, Weight Loss. In addition to considering these variables individually, two variables, Number of Symptoms (0 vs. 1 vs. 2 or more) and Any Symptoms (Yes vs. No), were derived from the symptom variables. Participants were asked if a doctor had told them that they had any of the following conditions: Bronchitis, Emphysema, Asthma, Heart Attack, Stroke. They were also asked if they had undergone a coronary artery bypass grafting (CABG) procedure. In addition to considering the condition and CABG variables individually, two variables, Number of Conditions (0 vs. 1 vs. 2 or more) and Any Conditions (Yes vs. No), were derived from individual items. Information from the baseline visit was used to construct the following variables: Referral required due to abnormal CT finding (Yes vs. No), Coronary Calcifications found at CT, emphysema found at CT (Yes vs. No), GOLD Stage (GOLD 0: At Risk vs. GOLD I/II : Mild/Moderate vs. GOLD III/IV : Moderate/Severe), Clean Bill of Health defined as no CT referral or and GOLD 0) . Time since the subject's last chest x-ray or CT scan was also evaluated (Less than 1 year ago vs. 1 to 2 years ago vs. 2 to 3 years ago vs. More than 3 years ago vs. Never). Predictor variables related to smoking history included Start Age (less than 18 vs. 18 and older), Start Age (Continuous), Baseline Cigarettes Per Day (Less than 20 vs. 20 to 39 vs. 40 or more), and Smoking Duration (Continuous).

Differences involving the predictor variables were evaluated individually according to smoking behavior. We used chi-squared tests and analysis of variance to evaluate the association between each risk factor and smoking behavior one year later. The focus of subsequent multivariate analyses was to develop a parsimonious model that described the independent contribution of CT referral on subsequent smoking behavior while controlling for factors that had a material effect on the relationship between CT referral and smoking behavior. Using multinomial logistic regression,[12] we evaluated the independent contribution of all selected predictors of behavior. The advantage of using this method is that the impact of the predictors on all three outcomes can be evaluated simultaneously. [12] The log-likelihood ratio test was used to evaluate the statistical significance of main effects and two-way interactions. If a variable had no main effect or material effect on the association between CT referral and smoking it was dropped from the model. Using all variables except CT referral we tried to obtain the best-fit model. CT referral was then added to the model.

2.4 RESULTS

Of the 3755 PLuSS participants, 3411 completed the one-year follow-up prior to this analysis. Approximately half of the participants who completed the follow-up were male (50.9%) and most were Caucasian (92.9%). The average age at baseline was 59.7 years. Participants had been smoking for an average of 41.2 years. CT referral was recommended for 1609 (42.8%) participants. Primarily the referrals were requested due to the presence of small non-calcified lung nodules that created suspicion for some level of lung cancer; however, other abnormalities involving the airway or heart were also cause for referral. Among the 2275 (60.6%) smokers at baseline, over half (57.7%) smoked between one and two packs of cigarettes per day, and few (6.6%) smoked more than two packs per day. The one-year assessment was completed at a median of 349 days (interquartile range = 24 days).

Expired air carbon monoxide measures were used to validate self-reported smoking status in a convenience sample of 228 PLuSS participants who returned for a follow-up visit between August, 2005 and January, 2006. One participant took the test on two separate occasions approximately one month apart; thus, a total of 229 readings are reported. Participants were asked their smoking status prior to taking the test. A total of 108 participants indicated that they were not smoking, and 121 indicated that they were smoking. Of the 108 participants who reported not smoking, 13 (12%) tested positive for smoking. Of the 121 participants who reported that they were smoking, 9 (7%) tested negative for smoking.

Figure 1 classifies participants (N=3755) in the source population (PLuSS) according to the factors (smoking status at baseline and one year later) used to construct the comparison groups of interest (Successful Quitters, Unsuccessful Quitters, Nonquitters). Among the 2046 baseline smokers for whom one year quit status is available, 59.5% [(370+847)x100/2046] had made a quit attempt and 15.7% [321x100/2046] were abstinent for more than 30 days prior to and at the time of the follow-up interview. The final sample for this analysis was comprised of 1997 participants: 829 in the Nonquitter group, 847 in the Unsuccessful Quitter group, and 321 in the Successful Quitter group. Baseline variables of baseline smokers who completed the one-year follow-up (N=2046) were compared to those who did not complete follow-up (N=229). Of the 229 who did not complete the follow-up telephone questionnaire, 77 (33.6%) were not eligible because they had not completed the baseline CT scan, 71 (31.0%) were not eligible

because their baseline CT scan was less than one year prior to this analysis, 12 (5.2%) were deceased, 2 (0.9%) refused to complete the follow-up, and 67 (29.3%) could not be reached. Baseline smokers who completed the follow-up were more likely to be older ($p=0.03$), white non-Hispanic ($p=0.02$), educated beyond high school ($p<0.01$), and married ($p<0.01$).

The primary study results appear in table 7. The Successful Quitter group was comprised of 18.9% of the participants receiving a CT referral and 13.5 % of the participants not receiving a CT referral (Relative Risk: 1.39, 95% Confidence Interval (CI): 1.14 – 1.70). See table 7. We combined the Successful Quitter and Unsuccessful Quitter groups to calculate the relative risk of making a quit attempt in the one-year follow-up interval given a CT referral. When the Successful Quitter and Unsuccessful Quitter groups were combined, 64.0 % (18.9% + 45.1%) of participants receiving a CT referral and 54.3% (13.5% + 40.8%) of participants not receiving a CT referral reported making a quit attempt in the one-year follow-up interval (Relative Risk: 1.18, 95% CI: 1.10 – 1.27). Table 7 also shows other factors associated with the outcomes of interest (gender, race, marital status, smoking intensity, number of symptoms and number of conditions).

The list of candidate variables for use in the logistic regression analysis was restricted by identifying those variables that best represented each conceptual domain of interest (eg. symptoms) and by giving preference to variables with the best distributional properties and the strongest association with the smoking outcome. For example, Number of Symptoms was selected because it was more informative and showed a stronger association with smoking behavior than the dichotomous variable Any Symptoms. Number of Symptoms also served as a summary variable for the individual symptom variables, which varied in their distributional properties and strength of association. CT screening referral showed a significant association with smoking behavior at one year ($p<0.001$) with a higher proportion of participants referred because of an abnormal CT scan in both the Successful Quitter and Unsuccessful Quitter groups than those not referred. The lightest smokers appeared to have more success in quitting than heavier smokers ($p=0.050$).

For the final model, we considered the impact of gender, marital group, number of symptoms, number of conditions, CT referral, and baseline cigarettes per day. Marital status was missing in three participants; therefore, these participants were excluded from the regression analysis. The variables decade of age and baseline cigarettes per day were not significant as main

effects and did not impact the relationship between CT referral and quit attempt status; hence, they were excluded from the final model. The high proportion of white non-Hispanic participants makes comparisons by race difficult. Therefore, once a final model was established it was rerun in only white non-Hispanic participants to determine if race had an impact on the findings. Significant interaction terms included marital group by number of conditions and gender by number of symptoms. Thus, the final model included gender, marital status, number of symptoms, number of conditions, CT referral and the interaction terms marital group by number of conditions and gender by number of symptoms.

Overall, controlling for gender, marital status, number of symptoms and conditions, and interaction terms, CT referral is significantly related to quitting behaviors ($p<0.001$). In comparing individuals who receive a referral after their CT scans versus those who do not, the odds ratio for having being in the Unsuccessful Quitter group versus Nonquitter group at the one year of follow-up is 1.43 (95% Confidence Interval (CI): 1.18-1.79), and the odds ratio for being in the Successful Quitter group versus being in the Unsuccessful Quitter group is 1.24 (95% CI: 0.96-1.61). CT referral remains highly significant when the model is restricted to white non-Hispanic participants. Restricting the Unsuccessful Quitter group to only those participants who had made a serious attempt to quit (lasting more than seven days), we repeated the analysis and found associations between quit attempt status and CT referral that were similar to those in the entire group ($p<0.01$, Unsuccessful Quitter versus Nonquitter OR: 1.50, 95% CI: 1.20-1.88; Successful Quitter versus Unsuccessful Quitter OR: 1.18, 95% CI: 0.89-1.56). We then conducted binary logistic regression to determine if similar relationships were apparent when comparing the NonQuitter group to those who did attempt to quit (combining the Successful Quitter and Unsuccessful Quitter groups) and found that participants who had a CT referral were significantly more likely quit (Successfully or unsuccessfully) than those who did not have a CT referral (OR: 1.52, 95% CI: 1.26-1.82). However, when participants in the Unsuccessful Quitter and the Successful groups were compared, CT referral was no longer significant (OR: 1.22, 95% CI: 0.94-1.59).

Additional analyses were conducted to further investigate the contribution of interaction terms to the model. The results are displayed in figures 2 and 3. In examining the role of marital status and conditions on quitting behaviors, number of conditions only had a significant impact on being in the Unsuccessful Quitter group versus the Nonquitter group in participants who were

formerly married (divorced/separated/widowed). Participants who were formerly married were more likely to be in the Unsuccessful Quitter group if they had at least one condition versus none. In general, the trend among all three marital groups appeared to be that having more symptoms increased the odds of being in the Unsuccessful Quitter group. Number of conditions only had a significant impact on having a being in the Successful Quitter group versus being in the Unsuccessful Quitter group in participants who were married. Married participants were less likely to be in the Successful Quitter group if they had two or more conditions versus no conditions. The gender by symptoms interaction term followed a similar pattern. Men were significantly more likely to be in the Unsuccessful Quitter group when they had symptoms. Both men and women seemed to be less likely to be in the Successful Quitter group when they had two or more symptoms; however, these odds ratios were not significant.

2.5 DISCUSSION

The present study supports the hypothesis that lung screening provides practitioners with a "teachable moment" to discuss smoking cessation with their patients.

Previous reports on the impact of lung screening on smoking behavior have been inconsistent. Ostroff et al. surveyed participants in the Early Lung Cancer Action Program (ELCAP) who were baseline smokers to determine if participation in the program influenced their smoking behaviors or their thoughts about smoking. A large number of participants reported quitting (23%) or cutting back (27%). Few factors appeared to differentiate those who quit or reduced smoking from those who did not change or increased their smoking. Older subjects were less likely to quit and subjects with an abnormal CT finding, particularly women, were more likely to quit.[6] Cox et al. conducted a similar investigation of baseline smokers and former smokers who enrolled in a low-dose CT lung cancer screening study at the Mayo Clinic. This study also found abstinence rates that were higher than expected (17.9% observed versus 5 to 7% expected in the general population). Self-reported abstinence was biochemically validated, and 98% of the reports were accurate. Subjects with abnormal pulmonary function test findings were more likely to quit at one year. However, CT findings did not appear to influence quitting

at one year. Also contrary to the Ostroff findings, in a univariate analysis of baseline smokers, older age positively predicted abstinence. This was not significant in a multivariate analysis.[7]

The current study has a quit rate similar to that found in the Mayo Clinic study even when the participants who quit for less than 30 days are excluded (15.7%). Thus, all three studies reported higher than expected cessation rates.[13] The current study shows that individuals who received CT referral were more likely to try to quit than those who did not. Although we cannot disregard the possibility that this difference is due to a reduction in cessation among those who did not receive a referral, the higher than expected cessation rates overall lead us to believe that the CT referrals promoted smoking cessation. However, these differences highlight the need for clinicians to not only take advantage of the teachable moment among patients with abnormal results but also to consider how to create a teachable moment when conveying normal test results. Because PLuSS participants were often counseled in smoking cessation after receiving an abnormal result, we cannot differentiate between the consequences of receiving an abnormal result from the consequences of the counseling that occurred from the referral. Differences in how abnormal CT results are handled may explain the differences in the results across the three studies. The Mayo Clinic study that showed no association between abnormal CT results and smoking cessation did find an association with abnormal pulmonary function test findings. Thus, in all three studies abnormal screening results were associated with cessation rates. This highlights the potential of various modes of lung screening to provide teachable moments for smoking cessation.

This study found interactions between marital status and number of conditions and between gender and number of symptoms. The significance of these interactions is unclear. They may be spurious findings due to multiple testing during the model building procedure. They may relate to selection bias. For example, individuals with multiple symptoms or conditions may have been pressured to quit smoking more than those without symptoms or conditions. Because this study was limited to baseline smokers, only those participants with multiple conditions or symptoms who continued to smoke despite this pressure would have been included in this analysis. The added advice provided by PLuSS may have had minimal impact on these participants. Conversely, for a participant who was not under much pressure to quit because of otherwise good health, the advice provided by PLuSS may have had a greater impact.

The baseline predictor variables appeared to have a stronger effect on making a quit attempt than on being abstinent at one year. This, along with the high number of participants who made quit attempts but were smoking again by the one year follow-up, highlights the lack of effective quit strategies. Teachable moments may help clinicians provide motivation for participants to quit smoking but they may not ultimately help smokers quit smoking. This finding also may suggest that different factors, such as genetics, may play a role in successfully quitting.

Age, smoking duration, and smoking intensity were all eligibility criteria for entry into PLuSS; thus, the amount of variation in these criteria was limited. If greater variation in these factors was present in the study population, we may have found significant associations with smoking behavior.

Self-selection bias could have impacted the one-year follow-up results if a significant number of participants chose not to complete the one year follow-up questionnaire. Of the 229 who did not complete the follow-up 160 (70%) could not complete the follow-up because they were not eligible or they were deceased. Thus, of the 2115 who could have completed the follow-up, 97% did complete it. Differences in a few sociodemographic variables existed between participants who completed the one-year follow-up and those who did not; however, no differences existed in CT referral rates between the two groups ($p=0.758$). Therefore, self-selection bias would seem to have very little effect on the results of this study.

Because the study sample was predominantly Caucasian, generalizations regarding differences in behavior based on race could not be made. However, these data do show an interesting trend. As shown in table 7, Non-Caucasians were more likely to have an unsuccessful quit attempt, but Caucasians were more likely to succeed in quitting. Non-Caucasians in this sample were predominately African-American (91%), and previous reports have highlighted disparities in smoking cessation between Caucasians and African-Americans.[14] If verified in future studies, this finding could indicate that current smoking cessation treatment strategies need to be modified to suit the needs of Non-Caucasians.

Although we estimated the validity of self-reported smoking status for a subset of the PLuSS population, we were unable to measure the validity of self-reported smoking status in the entire sample at their one-year follow-up visit. The available data indicate that 12% of smokers may have misreported their smoking status. The rates of reporting error do not differ

significantly by CT referral group. Thus, misclassification may have influenced the intensity, but not the direction of the effect of an abnormal CT finding on smoking status.

Due to the inconsistencies of currently available reports of the impact of lung screening on smoking behavior, additional studies are needed. If future studies support the hypothesis that lung screening promotes smoking cessation then cost effectiveness analyses should consider not only the early detection of lung cancers with screening, but also the prevention of lung disease with increased smoking cessation.

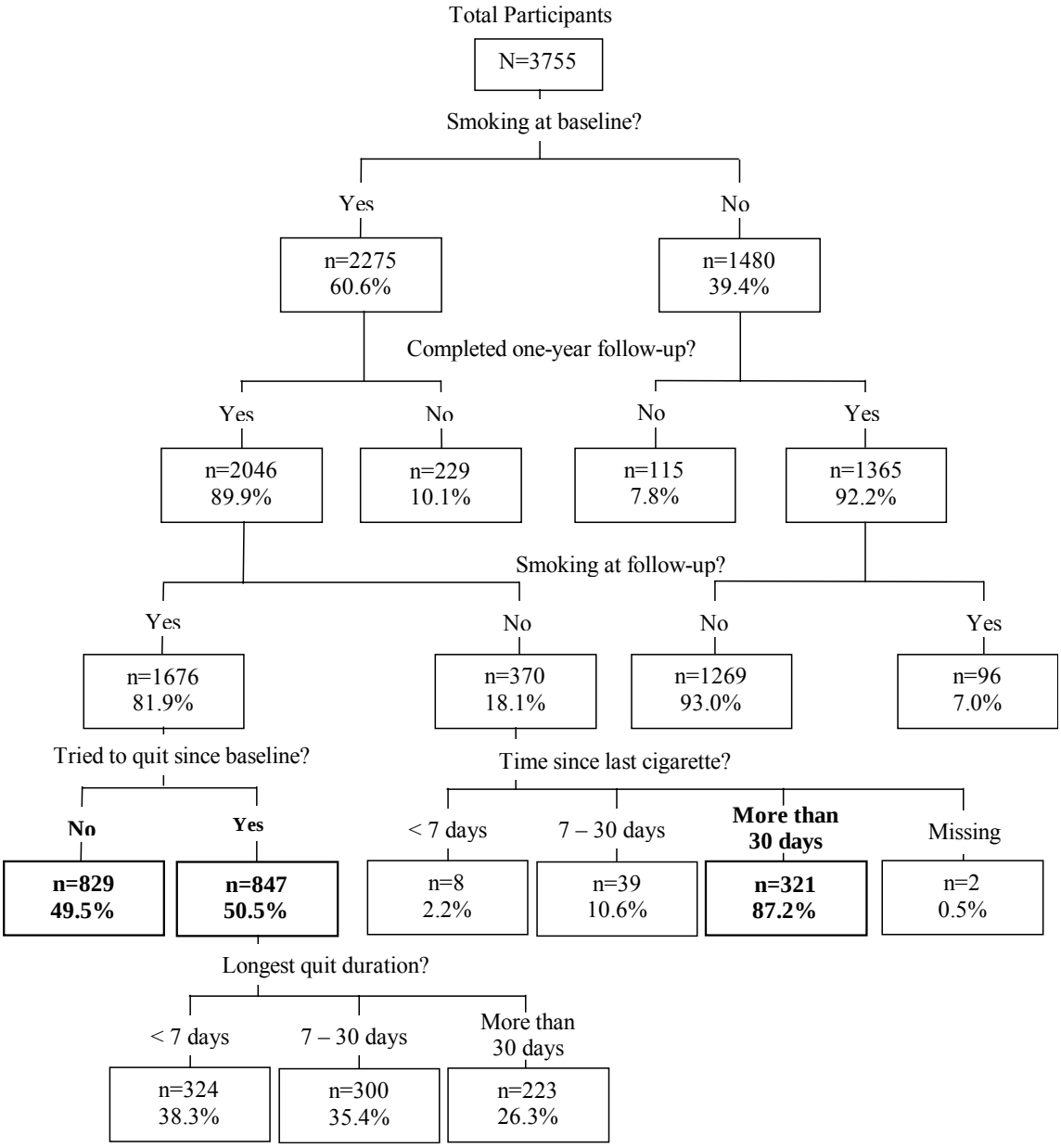

Figure 1: Smoking status of participants at baseline and follow-up

Table 7: Subjects, number (N) and percentage (%), according to baseline personal characteristic, and cigarette smoking behavior on follow-up, stratified according to baseline personal characteristic

			Smoking behavior on Follow-up (%)			
Characteristic	N	%	Nonquitter (N=788)	Unsuccessful Quitter (N=817)	Successful Quitter (N=305)	p-value
Overall	1,910	100.0	41.3	42.8	16.0	
Demographic						
Gender						0.006
men	931	48.7	44.9	39.6	15.5	
women	979	51.3	37.8	45.8	16.4	
Age (years)						0.188
50-59	1,178	61.7	43.0	42.0	14.9	
60-69	565	29.6	39.1	44.1	16.8	
70-79	167	8.7	35.9	43.7	20.4	
Race						
white	1,752	91.7	42.4	41.3	16.4	0.000
non-white	137	7.2	28.5	60.6	10.9	
Education						
post high school	1,446	75.7	41.5	42.5	16.0	0.921
no greater than high school	464	24.3	40.5	43.5	15.9	
Marital Status						
Married	1,200	62.8	43.7	40.0	16.3	0.010
Divorced/Separated/Widowed	568	29.7	35.7	48.4	15.8	
Never Married	139	7.3	42.4	44.6	12.9	
Baseline smoking behavior						
Duration (years)						
<46	1,352	70.8	42.2	42.5	15.2	0.261
46+	558	29.2	38.9	43.4	17.7	
Intensity (cigarettes per day)						
<20	663	34.7	38.0	42.7	19.3	0.042
20-39	1,124	58.8	43.1	42.6	14.3	
40+	123	6.4	42.3	44.7	13.0	
Medical factors						
Number of symptoms						
0	292	15.3	44.2	36.6	19.2	0.001
1	414	21.7	39.9	39.4	20.8	
2+	1,204	63.0	41.0	45.4	13.5	
Number of conditions						
0	1,283	67.2	42.6	40.8	16.6	0.013
1	361	18.9	41.3	42.4	16.3	
2+	266	13.9	35.0	52.6	12.4	
CT screening referral						
yes	873	45.7	36.0	45.1	18.9	0.000
no	1,037	54.3	45.7	40.8	13.5	

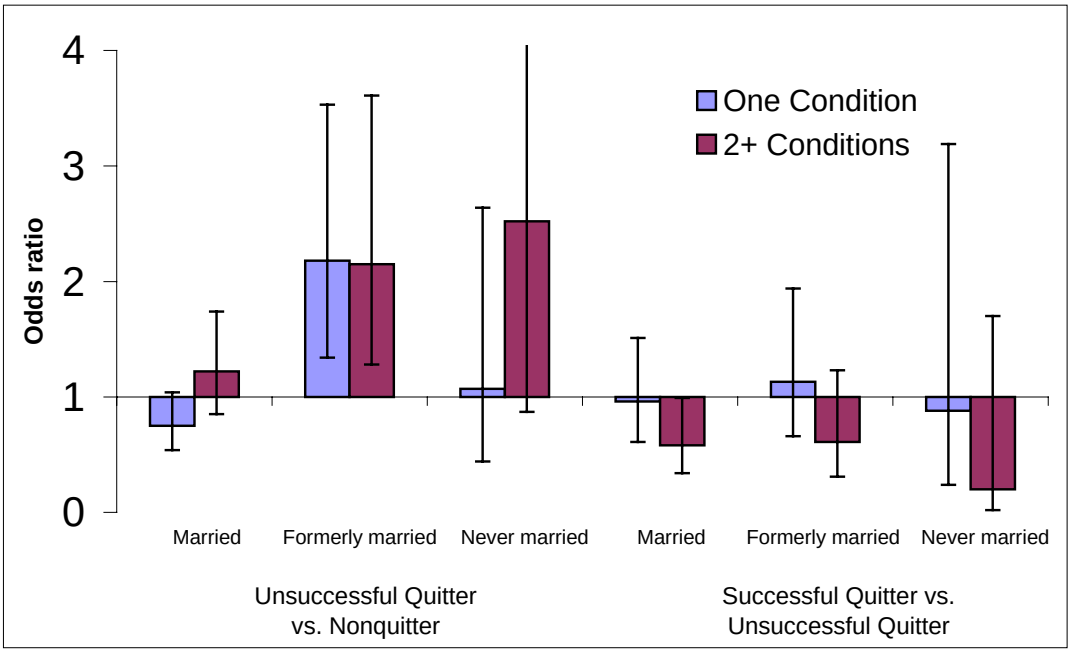

Figure 2: Odds of advancing on the cessation continuum given marital status and number of conditions (no conditions serves as the comparison group)

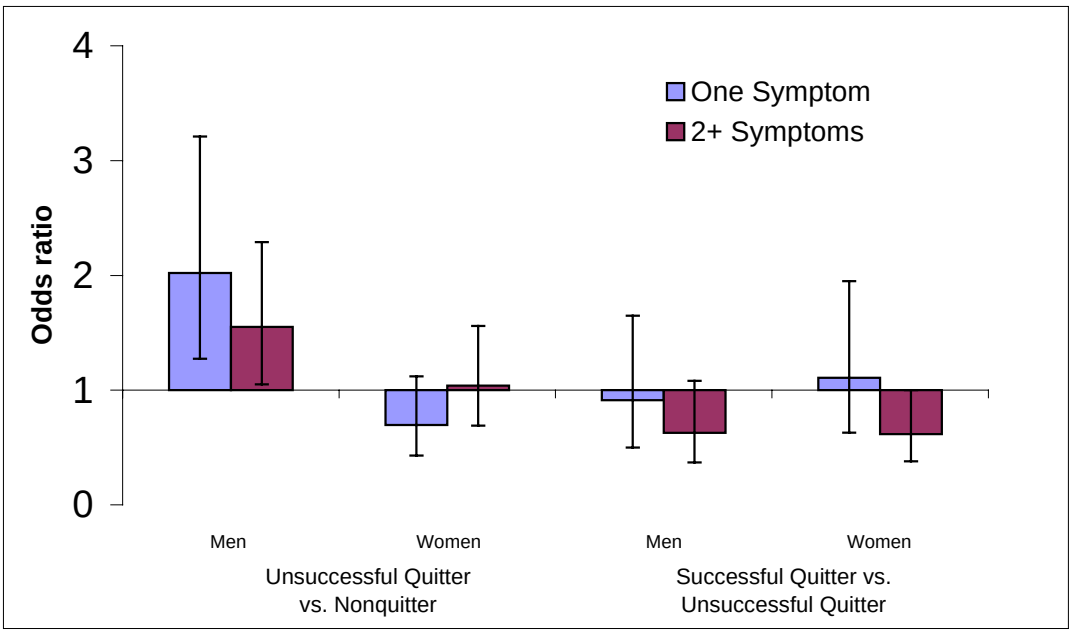

Figure 3: Odds of advancing on the cessation continuum given gender and number of symptoms (no symptoms serves as the comparison group)

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3.0 SMOKING AND THE DOPAMINE PATHWAY: VARIANTS OF THE DOPAMINE RECEPTOR 2 (DRD2) GENE

3.1 ABSTRACT

Introduction

Smoking cessation strategies continue to have disappointing results. By determining the interindividual genetic differences that influence smoking behaviors, we may be able to develop tailored strategies that increase the likelihood of successful cessation.

Objective

This study attempts to determine genetic influences on the relationship between the dopamine pathway and smoking cessation by examining associations with the *DRD2* variants *TaqIA (A2* vs. *A1)*, *TaqIB (B2* vs. *B1)*, *C957T (C* vs. *T)*, and *–141C Ins/Del (C* vs. *Del)*.

Participants

Participants are part of the Pittsburgh Lung Screening Study (PLuSS), a research based low-dose CT screening program containing current and former cigarette smokers, ages 50 to 79 years, recruited from the general population in the Pittsburgh area. Eligible members of the cohort must have smoked at least one-half pack per day for at least 25 years.

Methods

Questionnaire data from all baseline smokers who completed baseline and follow-up surveys were evaluated. Subjects were classified according to one-year smoking status (Not Abstinent at one year, Abstinent at one year). All individuals in the Abstinent group were included in the genotype analysis. Individuals in the Not Abstinent group were frequency matched by gender,

decade of age, and time of enrollment (three month intervals) in a three to one ratio to the Abstinent group. Logistic regression was used to identify the effect of individual genotypes on abstinence at one year.

Results

After controlling for the matching variables and other genotypes, the *DRD2 TaqIA* polymorphism was significantly associated with being abstinent at one year (p=0.01). Compared to participants who had the homozygous wild type *TaqIA* genotype (*A2A2*), participants who carried at least one variant allele (*A1*) were less likely to quit (Odds Ratio: 0.47, 95% CI: 0.24-0.94).

Conclusion

This association supports the hypothesis that genetic variation in the dopamine pathway influences smoking cessation.

3.2 INTRODUCTION

The dopamine pathway is a major reward pathway implicated in addiction to nicotine and other addictive drugs. The effectiveness of bupropion, an antidepressant that acts on the dopamine pathway and that helps smokers quit, supports the hypothesis that genetic variation along the dopamine pathway may cause differences in smoking behaviors including cessation. Among the five dopamine receptors, *DRD2* received the most attention initially because of the proposed association between the *TaqIA* restriction fragment length polymorphism (RFLP) on the *DRD2* gene, located on chromosome 11q23, and alcoholism. This has led to *DRD2* research for various addictions and disorders, including nicotine addiction.

The *TaqIA* restriction fragment polymorphism (RFLP) occurs 10 kilobases (kb) into the 3' untranslated region of the *DRD2* gene and results from a cytosine (C) to thymine (T) single nucleotide polymorphism (SNP). In addition to the initial association studies linking this variant to alcoholism, a link between this variant and reduced receptor density has been reported. This combination of findings has contributed to this variant being the most studied *DRD2* allele. *A2* is

the wild type allele, and *A1* is the variant. Reported frequencies of the *A1* allele in Caucasians range from 16% to 41% with most reports ranging between 19% and 28%. Studies investigating the relationship between the *TaqIA* variant and smoking behaviors have had mixed results. Most studies have found increased odds of smoking with the *A1* allele; however, few of these studies have reached statistical significance.[1-3] Many studies have investigated the relationship between the presence of any variant allele and smoking behaviors; however, two studies from the same investigators in Korea found evidence of gender-specific heterosis when *TaqIA* was evaluated as a three level variable.[4, 5]

The *TaqIB* RFLP is an adenine (A) to guanine (G) SNP. Unlike *TaqIA*, no association between receptor density and *TaqIB* has been reported; however, the *TaqIB* RFLP has been the subject of increased study because it is closer to the coding regions of the gene than *TaqIA*. Among Caucasians, the frequency of this allele has been reported as 16%. [6] In two reports that investigated the relationship between this variant and smoking, the *TaqIB* variant allele (*B1*) appeared to be positively related to smoking; however, this only reached statistical significance in specific subject groups. In one report, control participants and cancer cases were evaluated separately, and statistical significance was reached only in controls. Similarly, in the other report, Mexican-American and African-American participants were evaluated separately, and statistical significance was reached only in the Mexican-American participants. [3, 6]

C957T is a SNP which results in a synonymous amino acid change in the *DRD2* enzyme. In a study evaluating the impact on mRNA stability, the presence of the *T* allele (variant) resulted in a decreased half-life of mRNA. The frequency of the *T* allele in a European-American population of 94 subjects was 57%.[7] The National Center for Biotechnology Information's dbSNP database cites a frequency of the T allele among Caucasians as 48%.[8] Although most of the research on this variant has focused on functionality of the variant rather than potential associations with disease, in a recent report Lerman et al. investigated potential associations between this SNP and smoking cessation pharmacotherapy in two clinical trials. The first trial compared bupropion therapy to placebo in 414 participants. No interaction with treatment group was observed, but in multivariate analyses, participants with the *CC* or *CT* genotypes were less likely than those with the *TT* genotype to be abstinent at six months post-treatment with bupropion or placebo (p=0.05). No statistically significant association was observed in the univariate analysis. The second trial compared two forms of nicotine replacement therapy,

transdermal nicotine and nicotine spray, in 368 participants. Participants with the *CC* or *CT* genotypes were also less likely than those with the *TT* genotype to be abstinent at the end of treatment (p=0.03), but no interaction was found with nicotine therapy type.[9]

The *–141C Insertion/Deletion* (*–141C Ins/Del*) variant is a functional polymorphism that results in the deletion of a single cytosine from a pair of cytosines in the promoter region of *DRD2*.[10, 11] The presence of this variant has been shown to reduce promoter activity by more than half.[11] In German Caucasians, the frequency of deletion was reported to be 9.5%.[12] Among a group of alcohol and drug dependent Caucasians and their parents, the allele frequencies for a deletion ranged from 11% to 16%.[11] We could find only two reports that investigated the relationship between this variant and smoking behaviors. In one, no relationship between this variant and smoking status was observed.[13] In the second, Lerman et al. examined potential associations between this SNP and pharmacotherapy (bupropion therapy and nicotine replacement therapy) as described above for *C957T*. An interaction between treatment group and *–141C Ins/Del* genotype was observed in both trials. In the placebo group of the bupropion trial participants with the *CC* genotype were less likely to quit, but in the bupropion group participants with the any deletion were less likely to quit (p=0.01 at the end of treatment, p=0.08 at 6 months). Overall, at the end of treatment participants with any deletion were more likely to be abstinent. No interaction between genotype and treatment group were observed.[9]

Figure 4 shows the location and dbSNP identification numbers of the four selected variants along the *DRD2* gene. The purpose of this investigation was to determine if these variants, alone or in combination, are associated with smoking cessation in participants in a lung screening study.

3.3 METHODS

3.3.1 Population

The current study focuses on participants in the Pittsburgh Lung Screening Study (PLuSS), a research-based low-dose computed tomography (CT) screening program conducted as part of the University of Pittsburgh Lung Cancer SPORE program. PLuSS participants, recruited between

January, 2002 and April, 2005, are current and former cigarette smokers between the ages of 50 and 79 years who smoke or smoked at least one-half pack per day for at least 25 years. At baseline, participants completed a questionnaire and assessment that included a CT scan and pulmonary function test. At approximately one year after their baseline CT scan, participants completed the annual study update that included a repeat CT scan and a brief telephone questionnaire to determine if any changes in vital status, cancer status, or smoking status had occurred.

Expired air carbon monoxide measures were used to validate self-reported smoking abstinence in a subset of 228 PLuSS participants who returned for a follow-up visit between August, 2005 and January, 2006. A total of 229 readings were compared to self-reported smoking abstinence. Participants were asked their smoking status prior to taking the test. Using the recommended cutoff of 8 parts per million or higher as a positive test for smoking[14], we found that 13 (12%) of the 108 participants who reported not smoking tested positive for smoking and 9 (7%) of the 121 participants who reported that they were smoking tested negative.

Genotyping analyses were conducted in a subset of PLuSS participants who were smoking at baseline and had provided information regarding their smoking status during the annual study update at the time of subset selection. Participants who had lung cancer diagnosed in the one-year follow-up interval were excluded. Because the PLuSS population was over 90% Caucasian, the genotyping subset was limited to Caucasians.

Participants were classified as Abstinent versus Not Abstinent at one year based on their response to the annual study update question, "Are you currently smoking cigarettes?" The Abstinent at One Year group was further restricted to those participants who remained abstinent for more than 30 days prior to the follow-up telephone questionnaire. For the primary case-control analysis, participants who were in the Not Abstinent group served as the control group and the Abstinent group served as the case group. Subset selection occurred at two time points based on the status of the PLuSS database: October, 2004 and July, 2005. All individuals in the Abstinent group were included in the subset. Those in the Not Abstinent group were frequency matched to those in the Abstinent at group by gender, decade of age, and time of enrollment (in three month intervals). Within each stratum (cross of gender, decade of age, and time of enrollment), three individuals who were not abstinent at one year were selected for each individual in the Abstinent group. In cases where a stratum did not contain enough Not Abstinent

participants, Not Abstinent participants were selected from an adjacent time of enrollment or decade of age stratum. This modification of the matching strategy was only necessary for the October, 2004, subset. Of the 900 specimens that were requested (225 Abstinent at one year, 675 Not Abstinent at one year), 881 had blood specimens available and were successfully genotyped for *TaqIA* and *TaqIB* (219 Abstinent at One Year, 662 smokers). *C957T* results could not be obtained for 13 additional participants, and *–141C Ins/Del* results could not be obtained for one additional participant. In the final data set, no significant case-control group differences in gender, decade of age, or time of enrollment between individuals who were abstinent at one year and smokers was observed.

3.3.2 Genotyping Procedures

The *DRD2* (*TaqIA, TaqIB, C957T, -141C Ins/Del*) polymorphisms were genotyped by TaqMan allele discrimination assays using the Applied Biosystems 7700 system (Applied Biosystems, Foster City, CA). These assays were designed using the Applied Biosystems Primer express software, version 1.5. All reactions were performed using 1X Universal Master mix, 200nM VIC or FAM labeled probes and 20ng of genomic DNA. For *DRD2 TaqIA* and *TaqIB* site screening, 600 nM PCR primer concentrations were used. For the *C957T* and *-141C Ins/Del* polymorphisms, 900 nM PCR primer concentrations were used. Thermal cycling was initiated with a pre-PCR step, 2 min incubation at 50° C, followed by 10 min at 95° C, and then by 49 cycles of 15 sec at 95° C and 1 min at 67° C (*TaqIA*), 59° C (*TaqIB*), 64° C (*C957T*) or 55° C (*-141C Ins/Del*). Positive and negative PCR controls were included with each amplification reaction. An additional 10% of samples were repeated to verify the reproducibility of the assay. All results were interpreted independently by two laboratory personnel who were blinded to the case-control status of participants. In the event of a discrepancy, the genotyping assay was repeated until concordance was reached.

3.3.3 Data Analysis

Matching variables included gender (male vs. female), age (by decade of life at baseline), and enrollment date (by quarter). Other potential risk factors included sociodemographic variables, health-related variables, and smoking history variables. Sociodemographic variables were Education (high school or less vs. other), and Marital Status (married/living as married vs. divorced/separated/widowed vs. never married). Health-related variables were Family History of Any Cancer (yes vs. no), Family History of Lung Cancer (yes vs. no), Personal History of Any Cancer Except Lung (yes vs. no), Number of Symptoms of lung disease (0 vs. 1 vs. 2 or more), Number of Health Conditions (0 vs. 1 vs. 2 or more), and Referral required due to abnormal CT finding (yes vs. no). Participants were asked if they had recently experienced any of the following symptoms: Hemoptysis, Phlegm, Cough, Wheeze, Dyspnea, Edema, Weight Loss. The variable Number of Symptoms was derived from the individual symptom variables. Participants were asked if a doctor had told them that they had any of the following conditions: Bronchitis, Emphysema, Asthma, Heart Attack, Stroke. They were also asked if they had undergone a coronary artery bypass grafting (CABG) procedure. The Number of Conditions variable was derived from the individual items. Smoking history variables included Start Age (continuous), Baseline Cigarettes Per Day (less than 20 vs. 20 to 39 vs. 40 or more), Smoking Duration (continuous) and Smoking Duration (less than 46 years or 46 years or more). The genetic variables were examined by genotype (*TaqIA*: *A1A1* vs. *A1A2* vs. *A2A2*; *TaqIB*: *B1B1* vs. *B1B2* vs. *B2B2*; *C957T*: *CC* vs. *CT* vs. *TT*; *-141C Ins/Del*: *CC* vs. *C-* vs. --) and by presence or absence of the variant allele.

Hardy-Weinberg equilibrium was assessed in the control population for each variant. The relationships between risk factors, genetic variables and one-year smoking abstinence were initially assessed using univariate analysis techniques (chi-squared tests, t-tests, and Fisher's exact tests as appropriate). The focus of the analysis was to determine the impact of genotype on smoking cessation, so univariate tests also included an evaluation of relationships between genetic variables and other risk factors in the control population (continuing smokers). Crude odds ratios were calculated to initially evaluate the contribution of the genetic variables to smoking cessation. We used logistic regression to calculate adjusted odds ratios for each genetic

variable by controlling for matching variables. Logistic regression was used to evaluate a full model that included all of the genetic variables.

A second model was constructed that included non-genetic variables. Variables that were statistically significantly associated with smoking abstinence in the univariate analysis were included in the model building analysis as were variables that showed associations with smoking abstinence in a previous evaluation of the PLuSS population. The log-likelihood ratio test was used to evaluate the statistical significance of the main effects and two-way interactions. Once the best fitting base model was established without the genetic variables, the genetic variables were added.

Exploratory analysis of *DRD2* haplotypes was conducted by first constructing haplotypes using the PHASE program (Version 2.1). The case-control permutation test in PHASE was used to determine if significant differences existed in the haplotype frequencies between the Abstinent at One Year group and the continuing smokers. Chi-square tests were then used to evaluate the association of each of the five most frequent haplotypes with quitting. The odds of quitting given the presence of each of the five most frequent haplotypes were also calculated.

3.4 RESULTS

The allele frequencies for each gene are displayed in table 8 and are consistent with frequencies reported in the literature for Caucasians. *TaqIA* and *C957T* were in Hardy-Weinberg equilibrium while the homozygous variant genotype appeared to be underrepresented in the control population for *TaqIB* and *–141C Ins/Del*. Results of the univariate analysis for selected variables are displayed in table 9. Number of symptoms at baseline (p=0.006), *TaqIA* genotype (p=0.001) and presence of *TaqIA* variant allele (p=0.029) were the only predictor variables that were significantly associated with smoking behavior at one year. The direction of the *TaqIA* and *TaqIB* genotypes in relation to smoking appears to follow a pattern of heterosis, with heterozygous being less likely to quit smoking; however, the number of individuals with the homozygous variant genotypes were very small.

Few variables were associated with genotype as shown in tables 10 through 13. Gender differences were significant for *TaqIB* and showed a trend toward significance for *C957T*, with

males being over represented in the group with a variant allele for *TaqIB* and in the homozygous wild type group for *–141C Ins/Del*. Due to small cell sizes, time of enrollment was grouped in six month rather than three month intervals. A significant association was found between *TaqIB* genotypes and CT screening referral (p=0.043). Participants with the *B1* allele were more likely to have a CT scan that necessitated a referral.

Table 14 shows the odds ratios for each variant. *TaqIA* had a statistically significant relationship with smoking behavior even after adjusting for matching variables and other genetic variables (p=0.03). Compared to participants with the *A2A2* genotype (homozygous wild type) participants carrying a variant allele were less likely to be abstinent at one year. No other SNPs were significant when either the crude or adjusted odds ratios were calculated. Similarly, based on analyses described below, number of symptoms was added to the model to determine if it affected the relationship between *TaqIA* and smoking abstinence. The association between *TaqIA* and smoking abstinence did not change when number of symptoms was added to the model (data not shown).

For the regression analysis that included non-genetic variables, the matching variables (gender, decade of age, and time of enrollment) were initially forced into the model. No two-way interactions of the matching variables were significant. Number of symptoms, baseline packs per day, and CT referral were then entered into the model and two-way interactions were assessed. Number of symptoms was significant; baseline packs per day, CT referral and interaction terms were not significant. Thus, the base model contained the matching variables and number of symptoms. *TaqIA* and related interaction terms were entered into the model. *TaqIA* was significant as a main effect. No two-way interactions were significant. *TaqIB* and related interaction terms were then added to the base model. *TaqIB* was not significant as a main effect or as part of two-way interactions. *C957T* and interaction terms were entered into the base model limited to the 653 participants with complete data for this variant. The *C957T* by age interaction term was significant. This remained true when *TaqIA* was added to the model. The *–141C Ins/Del* genotype was assessed in the 880 participants who had complete data for this variant. The *–141C Ins/Del* genotype and interaction terms were not significant when they were added to the base model. (Data not shown.)

We repeated the model building exercise limiting the comparison to the 323 control participants who reported trying to quit during the one year follow-up versus the 214 cases for

whom the complete genotype data were available. Number of symptoms, *C957T*, and *C957T* by age remained statistically significant ($p<0.05$ for all). We then compared the participants who tried to quit (n=537) to the control participants who reported that they did not try to quit (n=330). The best fitting model comparing these two groups did not contain any genetic variables but included only the CT by age interaction term. (Data not shown.)

To further examine the *C957T* by age interaction term, the contribution of *C957T* to smoking cessation was examined separately for each decade of age (table 15). The chi-square test was significant at $p<0.05$ only for participants age 50 to 59. The adjusted odds ratio for this group was also statistically significant. In this age group participants carrying a variant allele were more likely to be abstinent at one year (OR: 2.13, 95% CI: 1.18-3.83). Although age is significantly associated with smoking duration, smoking duration is not associated with cessation as either a categorical variable or as a continuous variable (data not shown).

No significant differences were found in the haplotype frequencies between cases and controls ($p=0.24$). Of the 16 potential haplotypes, 12 were included in the best pairs summary for the 867 participants with complete genotyping data. The level of certainty for the best pairs was greater than 75% for 830 (95.7%) of participants. Of the 12 haplotypes, 5 were present with a frequency of at least 5%. These haplotypes are listed in table 16. The remaining haplotypes had a combined frequency of 2%. All five haplotypes were evaluated as dichotomous variables (presence vs. absence of the haplotype). Haplotype number 4 was further evaluated as a three level variable based on the number of copies of the haplotype that were present (0 vs. 1 vs. 2). Chi square analyses were significant only for haplotype 4 coded as a three level variable. The odds ratios for this variable in relation to smoking cessation are shown in table 17. Having two copies of the haplotype significantly increases the odds of quitting.

3.5 DISCUSSION

A variety of evidence suggests that the dopamine pathway is involved in smoking behaviors and specifically in smoking cessation. The findings from this study further support that hypothesis by linking variants in the *DRD2* gene to quitting.

TaqIA showed the strongest association with quitting both in univariate and multivariate analyses. Similar to previous reports [1, 2], we found that participants carrying a variant allele were less likely to be abstinent at one year versus those who were homozygous for the wild type allele. This association was apparent regardless of whether crude odds ratios were calculated or various other genetic and non-genetic variables were included in the model.

To the best of our knowledge, an interaction between *C957T* and age has not been previously reported. This finding is difficult to explain and is not clarified when the analyses are stratified by age. A potential confounder is smoking cessation strategy. For example, if *C957T* genotype interacts with a cessation strategy such as bupropion, and bupropion use varies with age, this could explain the observed interaction between *C957T* and age. Because PLuSS was not designed as a smoking cessation study data regarding cessation strategies were not collected.

We know of only two other studies that investigated the relationship between *–141C Ins/Del* and smoking behavior. One study, conducted in Japan, found no association between *–141C Ins/Del* and current, former, and never smokers.[13] Our findings are consistent with that report. As with *C957T*, we may have found differences related to smoking cessation strategy if these data were available. Lerman et al. have reported interaction between *–141C Ins/Del* variants and smoking cessation strategies. [9]

In the haplotype analysis we found that individuals with the *A2 B2* T C haplotype were more likely to quit smoking than those with other haplotypes, and that this relationship increased with the number of copies of the haplotype. When individual allele frequencies are examined in relationship to smoking abstinence, only slight differences are seen in allele frequencies between continuing smokers and those who were abstinent at one year (data not shown), but the combination of alleles (haplotypes) results in a strong association. This suggests that multiple variations along the dopamine pathway may influence smoking cessation.

Our finding that genetics do not appear to be involved in trying to quit but only in succeeding in quitting underscores the need for quit strategies that are tailored to interindividual differences in smokers. Educational strategies and public awareness of the dangers of smoking may increase a person's motivation to quit, but genetics may determine whether the person ultimately succeeds in quitting.

Figure 4: Locations of selected variants on the DRD2 gene on Chromosome 11.

Table 8: Allele frequencies for DRD2 variants

Allele	Frequency	Percent
TaqIA		
A2	1427	81.0
A1	335	19.0
TaqIB		
B2	1521	86.3
B1	241	13.7
C957T		
C	752	43.3
T	984	56.7
-141C ins/del		
C	1548	88.0
-	212	12.0

Table 9: Subjects (all subjects, Not Abstinent at one year, and Abstinent at one year) distributed according to selected baseline characteristics

			Smoking behavior on Follow-up (%)		
Characteristic	N	%	Not Abstinent (N=662)	Abstinent (N=219)	p-value
Matching Variables					
Gender					0.858
men	423	48.0	48.2	47.5	
women	458	52.0	51.8	52.5	
Age (years)					0.981
50-59	530	60.2	60.3	59.8	
60-69	253	28.7	28.5	29.2	
70-79	98	11.1	11.2	11.0	
Consent Quarter					0.751
01/01/2002 - 03/31/2002	22	2.5	2.0	4.1	
04/01/2002 - 06/30/2002	108	12.3	12.7	11.0	
07/01/2002 - 09/30/2002	108	12.3	13.0	10.0	
10/01/2002 - 12/31/2002	82	9.3	8.8	11.0	
01/01/2003 - 03/31/2003	88	10.0	9.7	11.0	
04/01/2003 - 06/30/2003	128	14.5	14.8	13.7	
07/01/2003 - 09/30/2003	106	12.0	12.1	11.9	
10/01/2003 - 12/31/2003	116	13.2	13.1	13.2	
01/01/2004 - 03/31/2004	80	9.1	9.1	9.1	
04/01/2004 - 06/30/2004	43	4.9	4.8	5.0	
Baseline smoking behavior					
Intensity (cigarettes per day)					0.060
<20	285	32.3	30.2	38.8	
20-39	535	60.7	62.5	55.3	
40+	61	6.9	7.3	5.9	
Medical factors					
Number of symptoms					0.006
0	139	15.8	15.0	18.3	
1	193	21.9	19.8	28.3	
2+	549	62.3	65.3	53.4	
Number of conditions					0.177
0	605	68.7	67.2	73.1	
1	153	17.4	17.7	16.4	
2+	123	14.0	15.1	10.5	
CT screening referral					0.124
yes	427	48.5	47.0	53.0	
no	454	51.5	53.0	47.0	
Genotypes					
Taq1A					0.001
A2/A2	595	67.5	65.6	73.5	
A2/A1	237	26.9	29.8	18.3	
A1/A1	49	5.6	4.7	8.2	
Taq1B					0.060
B2/B2	650	73.8	72.8	76.7	
B2/B1	221	25.1	26.4	21.0	
B1/B1	10	1.1	0.8	2.3	
C957T*					0.200
C/C	175	20.2	21.1	17.2	
C/T	402	46.3	46.9	44.7	
T/T	291	33.5	32.0	38.1	
-141C Ins/Del**					0.870
C/C	692	78.6	78.2	79.8	
C/ -	164	18.6	19.0	17.4	
- / -	24	2.7	2.7	2.8	
Taq1A					0.029
A2/A2	595	67.5	65.6	73.5	
Any A1	286	32.5	34.4	26.5	
Taq1B					0.255
B2/B2	650	73.8	72.8	76.7	
Any B1	231	26.2	27.2	23.3	
C957T*					0.214
C/C	175	20.2	21.1	17.2	
Any T	693	79.8	78.9	82.8	
-141C Ins/Del**					0.624

Table 10: Control subjects (Not Abstinent at one year by TaqIA genotype) distributed according to selected baseline characteristics

Characteristic	N	%	TaqIA Genotype (N=662) A2A2 (N=434)	TaqIA Genotype (N=662) Any A1 (N=228)	p-value
Matching Variables					
Gender					0.183
men	319	48.2	46.3	51.8	
women	343	51.8	53.7	48.2	
Age (years)					0.102
50-59	399	60.3	60.4	60.1	
60-69	189	28.5	30.2	25.4	
70-79	74	11.2	9.4	14.5	
Consent Time					0.467
01/01/2002 - 06/30/2002	97	14.7	13.6	16.7	
07/01/2002 - 12/31/2002	144	21.8	21.9	21.5	
01/01/2003 - 06/30/2003	162	24.5	26.3	21.1	
07/01/2003 - 12/31/2003	167	25.2	24.0	27.6	
01/01/2004 - 06/30/2004	92	13.9	14.3	13.2	
Baseline smoking behavior					
Intensity (cigarettes per day)					0.505
<20	200	30.2	29.5	31.6	
20-39	414	62.5	62.4	62.7	
40+	48	7.3	8.1	5.7	
Medical factors					
Number of symptoms					0.305
0	99	15.0	13.8	17.1	
1	131	19.8	18.9	21.5	
2+	432	65.3	67.3	61.4	
Number of conditions					0.959
0	445	67.2	67.1	67.5	
1	117	17.7	18.0	17.1	
2+	100	15.1	15.0	15.4	
CT screening referral					0.184
yes	351	53.0	51.2	56.6	
no	311	47.0	48.8	43.4	

Table 11: Control subjects (Not Abstinent at one year by TaqIB genotype) distributed according to selected baseline characteristics

Characteristic	N	%	TaqIB Genotype (N=662) B2B2 (N=482)	TaqIB Genotype (N=662) Any B1 (N=180)	p-value
Matching Variables					
Gender					0.049
men	319	48.2	45.9	54.4	
women	343	51.8	54.1	45.6	
Age (years)					0.096
50-59	399	60.3	60.2	60.6	
60-69	189	28.5	30.1	24.4	
70-79	74	11.2	9.8	15.0	
Consent Time					0.644
01/01/2002 - 06/30/2002	97	14.7	13.7	17.2	
07/01/2002 - 12/31/2002	144	21.8	22.0	21.1	
01/01/2003 - 06/30/2003	162	24.5	25.7	21.1	
07/01/2003 - 12/31/2003	167	25.2	24.7	26.7	
01/01/2004 - 06/30/2004	92	13.9	13.9	13.9	
Baseline smoking behavior					
Intensity (cigarettes per day)					0.588
<20	200	30.2	30.1	30.6	
20-39	414	62.5	62.0	63.9	
40+	48	7.3	7.9	5.6	
Medical factors					
Number of symptoms					0.379
0	99	15.0	14.1	17.2	
1	131	19.8	19.1	21.7	
2+	432	65.3	66.8	61.1	
Number of conditions					0.830
0	445	67.2	66.6	68.9	
1	117	17.7	17.8	17.2	
2+	100	15.1	15.6	13.9	
CT screening referral					0.043
yes	351	53.0	50.6	59.4	
no	311	47.0	49.4	40.6	

Table 12: Control subjects (Not Abstinent at one year by C957T genotype) distributed according to selected baseline characteristics

Characteristic	N	%	C957T Genotype (N=653) CC (N=138)	CT (N=515)	p-value
Matching Variables					
Gender					0.285
men	315	48.2	44.2	49.3	
women	338	51.8	55.8	50.7	
Age (years)					0.073
50-59	394	60.3	62.3	59.8	
60-69	187	28.6	22.5	30.3	
70-79	72	11.0	15.2	9.9	
Consent Time					0.121
01/01/2002 - 06/30/2002	97	14.9	17.4	14.2	
07/01/2002 - 12/31/2002	144	22.1	18.1	23.1	
01/01/2003 - 06/30/2003	162	24.8	21.0	25.8	
07/01/2003 - 12/31/2003	164	25.1	24.6	25.2	
01/01/2004 - 06/30/2004	86	13.2	18.8	11.7	
Baseline smoking behavior					
Intensity (cigarettes per day)					0.624
<20	197	30.2	32.6	29.5	
20-39	408	62.5	61.6	62.7	
40+	48	7.4	5.8	7.8	
Medical factors					
Number of symptoms					0.790
0	99	15.2	13.8	15.5	
1	131	20.1	21.7	19.6	
2+	423	64.8	64.5	64.9	
Number of conditions					0.381
0	441	67.5	63.8	68.5	
1	116	17.8	21.7	16.7	
2+	96	14.7	14.5	14.8	
CT screening referral					0.684
yes	346	53.0	51.4	53.4	
no	307	47.0	48.6	46.6	

Table 13: Control subjects (Not Abstinent at one year by -141C Ins/Del genotype) distributed according to selected baseline characteristics

Characteristic	N	%	-141C Ins/Del Genotype (N=661) CC (N=518)	C - (N=144)	p-value
Matching Variables					
Gender					0.050
men	319	48.2	50.2	41.0	
women	343	51.8	49.8	59.0	
Age (years)					0.538
50-59	399	60.3	60.4	59.7	
60-69	189	28.5	27.8	31.3	
70-79	74	11.2	11.8	9.0	
Consent Time					0.584
01/01/2002 - 06/30/2002	97	14.7	14.5	15.3	
07/01/2002 - 12/31/2002	144	21.8	22.6	18.8	
01/01/2003 - 06/30/2003	162	24.5	25.3	21.5	
07/01/2003 - 12/31/2003	167	25.2	24.1	29.2	
01/01/2004 - 06/30/2004	92	13.9	13.5	15.3	
Baseline smoking behavior					
Intensity (cigarettes per day)					0.739
<20	200	30.2	30.9	27.8	
20-39	414	62.5	61.8	65.3	
40+	48	7.3	7.3	6.9	
Medical factors					
Number of symptoms					0.173
0	99	15.0	16.2	10.4	
1	131	19.8	20.1	18.8	
2+	432	65.3	63.7	70.8	
Number of conditions					0.093
0	445	67.2	69.3	59.7	
1	117	17.7	16.4	22.2	
2+	100	15.1	14.3	18.1	
CT screening referral					0.165
yes	351	53.0	54.4	47.9	
no	311	47.0	45.6	52.1	

Table 14: Crude and adjusted odds ratios for the effect of genotype on one-year quit status.

	Abstinent	Not Abstinent	Crude		Model set 1*		Model 2**	
Genotype			OR 95% CI	p-value^	OR 95% CI	p-value^^	OR 95% CI	p-value^^
TaqIA				0.030		0.027		0.033
A2/A2	161	434	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Any A1	58	228	0.69 (0.49-0.96)		0.68 (0.48-0.96)		0.47 (0.24-0.94)	
TaqIB				0.256		0.239		0.184
B2/B2	168	482	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Any B1	51	180	0.81 (0.57-1.16)		0.80 (0.56-1.16)		1.66 (0.79-3.51)	
C957T				0.214		0.153		0.261
CC	82	209	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Any T	133	444	1.29 (0.86-1.93)		1.35 (0.90-2.03)		1.29 (0.83-2.02)	
-141C Ins/Del								
C/C	174	518	1.00 (reference)	0.624	1.00 (reference)	0.561	1.00 (reference)	0.900
Any Del	44	144	0.91 (0.62-1.33)		0.892 (0.61-1.31)		0.98 (0.65-1.45)	

*Model Set 1 uses logistic regression to adjust the genotype-smoking outcome associations (expressed as odds ratios) for factors (gender, decade of age, and quarter of enrollment) used to match the case and controls groups. N=881 for the TaqIA and TaqIB models. N=868 fo the C957T model. N=880 for the -141C Ins/Del model.

**Model 2 uses logistic regression to adjust the genotype-smoking outcome associations (expressed as odds ratios) for matching factors and for each of the other genetic risk factors. N=867

^Chi-square test

^^Wald Test

Table 15: An evaluation of the interaction term C957T X decade of age in relation to smoking status at one year

		DRD2-957 Genotype Distributions (%)		
Decade of Age	Abstinent	C/C	Any T	p-value
50s				0.02
	Yes	16 (12.5)	112 (87.5)	
	No	86 (21.8)	308 (78.2)	
OR (95% CI)*		1.00	2.13 (1.18-3.83)	
60s				0.07
	Yes	17 (27.0)	46 (73.0)	
	No	31 (16.6)	156 (83.4)	
OR (95% CI)*		1.00	0.52 (0.26-1.05)	
70s				0.23
	Yes	4 (16.7)	20 (83.3)	
	No	21 (29.2)	51 (70.8)	
OR (95% CI)*		1.00	1.93 (0.54-6.87)	

*Adjusted for gender and time of enrollment (3 month intervals)

Table 16: Most frequent haplotypes for all 881 participants

Haplotype Number	Alleles Included in Each Haplotype				Frequency
	Taq1A	*Taq1B*	*C957T*	*-141C Insdel*	
1	A2	B2	C	C	0.20
2	A2	B2	C	Del	0.08
4	A2	B2	T	C	0.50
8	A1	B1	C	C	0.12
10	A1	B2	T	C	0.08

Table 17: Odds of being abstinent at one year based on the number of copies of haplotype 4, limited to participants with haplotype pairs that were determined with a certainty of more than 75% (N=830)

Number of copies of haplotype 4	OR	95% CI
1 versus 0	1.03	(0.68, 1.54)
2 versus 0	1.57	(1.02, 2.41)
2 versus 1	1.55	(1.07, 2.23)

Adjusted for adjusted for decade of age, gender, and time of enrollment (3 month intervals)

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4.0 SMOKING AND THE DOPAMINE PATHWAY: VARIANTS OF THE DOPAMINE TRANSPORTER GENE

4.1 ABSTRACT

Introduction

Dopamine availability may explain differences in responses to smoking and smoking cessation. Dopamine transporter (DAT) is a reuptake protein coded by the *SLC6A3* gene that removes dopamine from the synapse. Variations in *SLC6A3* may impact the number of transporter molecules on the surface of neurons and the functionality of DAT.

Objective

This study attempts to determine genetic influences on the relationship between the dopamine pathway and smoking cessation by examining a variable number tandem repeat in the *SLC6A3*.

Participants

Participants are part of the Pittsburgh Lung Screening Study (PLuSS), a research based low-dose CT screening program containing current and former cigarette smokers, ages of 50 to 79 years, recruited from the general population in the Pittsburgh area. Eligible members of the cohort must have smoked at least one-half pack per day for at least 25 years.

Methods

Questionnaire data from all baseline smokers who provided information on smoking status one year later were evaluated. Subjects were classified as Not Abstinent at one year or Abstinent at one year according to smoking status information that they provided during the one year telephone follow-up. All eligible participants who were Abstinent at one year (n=225) were

included in the genotype analysis. Those in the Not Abstinent group were frequency matched to the Abstinent at one year group by gender, decade of age, and time of enrollment (three month intervals) in a three to one ratio (n=675). *SLC6A3* data (genotype with respect to the number of 40 base-pair repeats in the 3' untranslated region of the *SLC6A3*) were available for 219 in the Abstinent at one year group and 662 in the Not Abstinent at one year group. Logistic regression was used to identify the effect of genotype on abstinence at one year.

Results/Conclusion

SLC6A3 genotype was not associated with smoking status at one-year (p=0.757). After adjusting for matching variables, compared to participants with the 10-repeat/10-repeat genotype the odds ratio for being Abstinent at one year in participants with the 10-repeat/9-repeat genotype was 1.10 (95% CI: 0.79-1.54) and with the 9-repeat/9-repeat genotype was 0.90 (95% CI: 0.50-1.62). Contrary to previous reports no significant gene-gene interaction with the *DRD2 TaqIA* variant was observed.

4.2 INTRODUCTION

The dopamine pathway is a major reward pathway implicated in addiction to nicotine and other drugs. Dopamine receptors, which are stimulated by dopamine, have been the focus of a majority of research related to potential associations between dopamine and addiction. It has been hypothesized that differences in the density of receptors may explain the interindividual differences in response to the dopamine release that occurs in smokers. However, other factors, such as the amount of dopamine available, may also explain these differences. Dopamine transporter (DAT) is a reuptake protein that removes dopamine from the synapse. This protein is coded by the *SLC6A3* gene.

The *SLC6A3* gene is located on chromosome 5p15.3.[1] DAT's role in removing dopamine from the synapse has led to research on *SLC6A3* and various dopamine-related disorders. Variations in a 40-base pair repeat in the 3' untranslated region have been linked to variations in the density of transporter molecules on the surface of neurons.[1] The 10-repeat allele (wild type) and the 9-repeat allele are the most frequently occurring variants, although a

wide range of repeats have been found. Laboratory studies suggest that variations in the number of repeats influence variations in gene transcription and expression.[2-5]

Two studies have found an association between reduced smoking behaviors and presence of the 9-repeat allele of *SLC6A3*. Sabol et al., found a significant association between *SLC6A3* genotype and smoking cessation among 593 nonsmokers (less than 100 cigarettes), 283 current smokers, and 231 former smokers. The 9-REPEAT ALLELE allele was more common among former smokers compared to those who were current smokers.[6] Similarly, Lerman et al., reported that the 9-repeat allele was found less frequently among a group of 289 smokers when compared to 233 non-smokers (less than 100 cigarettes). The Lerman report also compared *DRD2* variant allele frequencies. No differences were found between smokers and non-smokers in the prevalence of the *TaqIA* variant; however, the study suggested that *DRD2* may modify the effects of *SLC6A3*.[7] In a subsequent randomized trial of bupropion for smoking cessation, *SLC6A3* was not significant as a main effect in the entire sample, but was significant among participants with the *DRD2 TaqIA A2A2* genotype. In the multivariate model in the entire sample, the *DRD2 SLC6A3* interaction term was significant.[8]

In contrast, Vandenberg, et al., reported no relationship between *SLC6A3* and smoking behavior among 595 randomly selected adult volunteers when the same group classifications were used that were used in the previously described reports (nonsmokers who smoked <100 cigarettes, former smokers, current smokers). However contrary to the previously reported associations, when never-smokers (0 lifetime cigarettes) and non-smokers (<100 lifetime cigarettes) were analyzed as separate groups, the 10-repeat allele was more frequent among never smokers. [1] Jorm et al. found no association between the *SLC6A3* gene and either smoking initiation or cessation.[9]

4.3 METHODS

4.3.1 Population

This study focuses on participants in the Pittsburgh Lung Screening Study (PLuSS). PLuSS is a low-dose computer tomography (CT) screening program conducted as part of the University of

Pittsburgh Lung Cancer SPORE research program. PLuSS participants were recruited from January, 2002 until April, 2005. Current and former cigarette smokers between the ages of 50 and 79 years were eligible for the PLuSS. Participants must have smoked at least one-half pack per day for at least 25 years. Baseline data collection included a questionnaire, CT scan and pulmonary function test. A CT scan and brief telephone follow-up questionnaire were completed approximately one year after the baseline CT.

Between August, 2005 and January, 2006 we measured expired air carbon monoxide in a subset of 228 PLuSS participants during their follow-up CT scan appointment. Participants were asked their smoking status prior to taking the test. A total of 229 carbon monoxide readings were compared to self-reported smoking status. A reading of 8 parts per million or higher was considered a positive test for smoking.[10] Of the 108 participants who reported not smoking 13 (12%) tested positive, and of the 121 participants who reported that they were smoking 9 (7%) tested negative.

We selected a subset of PLuSS participants who were smoking at baseline and had provided information regarding their smoking status at follow-up for the genotyping analysis excluding those who had lung cancer diagnosed in the one-year follow-up interval. This subset was limited to Caucasians due to the high proportion of Caucasians in the PLuSS population (over 90%). Smoking status was assessed during the one-year telephone follow-up questionnaire. Participants were classified as Abstinent at one year (cases) if they reported that were not smoking and had remained abstinent for more than 30 days prior to the follow-up questionnaire. Participants were classified as Not Abstinent at one year (controls) if they reported that they were currently smoking. Subset selection occurred in October, 2004 and July, 2005. The subset included all in the Abstinent at one year group. Participants in the Not Abstinent at one year group were matched in a three to one ratio to the Abstinent at one year group in gender, decade of age, and time of enrollment (in three month intervals) strata. If a stratum did not contain enough participants who were not abstinent at one year, matching was conducted using an adjacent time of enrollment or decade of age stratum. Of the 900 specimens that were requested (225 Abstinent, 675 Not Abstinent), 881 were available and successfully genotyped (219 Abstinent, 662 Not Abstinent).

4.3.2 Genotyping Procedures

The *SLC6A3* 40 bp VNTR in the 3'-untranslated region was determined by a PCR-based assay adapted from Kang, et al with minor modification.[11] Briefly, each 25 μl reaction contained 1X PCR Optimized Buffer B (InVitrogen, Carlsbad, CA), 5% DMSO, 200 μM of dNTP, 900 μM of primers, 1.2 units of AmpliTaq Gold (Applied Biosystems, Foster City, CA) and 20ng of genomic DNA. Thermal cycling was initiated with 94° C activation for 10 minutes, followed by 40 cycles of 1 minute at 94° C and 3 minutes at 72° C ending with a 72° C extension for 10 min. The PCR products were electrophoresed on 8% polyacrylamide gels using the 50bp gel marker (Fisher Science) to determine PCR product length. Each gel also included two known heterozygote genotype samples as positive controls (8/9 repeats and 9/10 repeats) and one negative PCR control sample. An additional 10% of samples was repeated to verify the reproducibility of the assay. All results were interpreted independently by two laboratory personnel who were blinded to the case-control status of participants. In the event of a discrepancy, the genotyping assay was repeated until concordance was reached.

4.3.3 Data Analysis

Matching variables included Gender (male vs. female), Age (by decade of life at baseline), and Consent Date (by quarter). Potential risk factors for quitting included the following demographic variables: Education (high school or less vs. other), and Marital Status (married/living as married vs. divorced/separated/widowed vs. never married). Other potential risk factors included health-related variables such as Family History of Any Cancer (yes vs. no), Family History of Lung Cancer (yes vs. no), Personal History of Any Cancer Except Lung (yes vs. no), Number of Symptoms of lung disease (0 vs. 1 vs. 2 or more), Number of Health Conditions (0 vs. 1 vs. 2 or more), and Referral required due to abnormal CT finding (yes vs. no). Smoking history variables included Start Age (continuous), Baseline Cigarettes Per Day (less than 20 vs. 20 to 39 vs. 40 or more), Smoking Duration (continuous) and Smoking Duration (less than 46 years or 46 years or more). The *SLC6A3* allele was examined by genotype (10/10 vs. 10/9 vs. 9/9) and by presence or absence of 9- repeat allele. To examine possible interaction between *DRD2* and *SLC6A3, DRD2* genetic variables were examined by genotype (*TaqIA*: *A1A1* vs. *A1A2* vs. *A2A2*; *TaqIB*: *B1B1* vs.

B1B2 vs. *B2B2*; *C957T*: *CC* vs. *CT* vs. *TT*; *-141C ins/del*: *CC*, *C-*, --) and by presence or absence of the variant allele.

The relationships between all variables and one-year smoking status were initially assessed using univariate analysis techniques (chi-squared tests, t-tests, and Fisher's exact tests as appropriate). The focus of the analysis was to determine the impact of *SLC6A3* genotype on smoking cessation, so univariate tests also included an evaluation of relationships between the *SLC6A3* variables and other variables. Logistic regression was used to develop an overall risk model for smoking cessation. Matching variables were forced into the regression model. Potential risk factors that were significantly associated with smoking status in the univariate analysis were included in the model building analysis as were predictor variables that showed associations with smoking status in a previous evaluation of the PLuSS population. Once the best fitting model was established without the genetic variables, the genetic variables were added.

Building on a previous analysis that investigated the relationship of *DRD2* to smoking cessation, we added *SLC6A3* to a model that included only the *DRD2* variables to determine if *SLC6A3* had an impact on the relationship between these variables and smoking cessation.

4.4 RESULTS

Table 18 displays the allele frequencies which are consistent with frequencies reported in the literature for Caucasians. Aside from the most common 9- and 10-repeat alleles other alleles in our sample included 6-, 7-, 8-, 11-, and 12-repeat alleles. Table 19 shows the relationships of risk factor variables and genetic variables with smoking cessation. Number of symptoms at baseline (p=0.006) was the only predictor variable that was significantly associated with smoking behavior at one year. *SLC6A3* was not significantly associated with smoking cessation or with any of the matching or risk factor variables (See tables 19 and 20).

Odds ratios were calculated for *SLC6A3* genotypes and the presence of the 9-repeat allele (table 21). No statistically significant differences in abstinence at one year were found between *SLC6A3* genotypes, and no statistically significant association between the presence of the 9-repeat allele and abstinence at one year were observed. Compared to participants with the 10/10 genotype, the crude odds ratio for quitting in participants with the 10/9 genotype was 1.08 (95%

CI: 0.78-1.51) and with the 9/9 genotype was 0.89 (95% CI: 0.50-1.58). After adjusting for matching variables, compared to participants with the 10/10 genotype, the odds ratio for quitting in participants with the 10/9 genotype was 1.10 (95% CI: 0.79-1.54) and with the 9/9 genotype was 0.90 (95% CI: 0.50-1.62). Similarly both the adjusted and unadjusted odds ratios for smoking abstinence in the presence of a 9-repeat allele were close to 1.00. See table 21.

Based on a previous analysis of those data, the model building analysis began with a base model that included the matching variables (gender, decade of age, and quarter of consent) and number of symptoms. When we added *SLC6A3* to the base model, we found that it did not contribute to the model as either a three level variable (10/10 vs. 10/9 vs. 9/9) or as a two-level variable (presence of 9-repeat allele). *SLC6A3* was not significant in additional analyses comparing those who tried (regardless of success) to those who did not try to quit and comparing those who had made a quit attempt but were not abstinent at one year to those were abstinent at one year (data not shown).

The *DRD2* variables were added into the model that contained *SLC6A3*, the matching variables, and number of symptoms to see if the *DRD2* variables influenced the relationship between *SLC6A3* and smoking cessation. Consistent with a previous analysis of these data, *TaqIA* was significantly associated with smoking behavior at one year both by genotype and by presence or absence of the *A1* allele; however, the interaction term with *SLC6A3* was not significant. *TaqIB* (by genotype) showed a trend for an association with smoking behavior until *TaqIA* was added back into the model. *C957T* and *–141C Ins/Del* were not significantly associated with smoking cessation in a model that included *SLC6A3*, and no interaction with *SLC6A3* was evident. (Data not shown.)

Although interaction terms were not significant in the regression model, previous studies have reported interaction between *SLC6A3* and *DRD2*.[7, 8] To investigate this interaction, we evaluated the relationships between presence or absence of the 9-repeat allele and smoking behavior stratified by presence or absence of the *DRD2 TaqIA A1* allele (table 22). No statistically significant interaction between *DRD2 TaqIA* and *SLC6A3* was evident (p=0.381).

4.5 DISCUSSION

Our findings are consistent with those of Jorm et al. in that we found no direct association between smoking and variation in the *SLC6A3* gene. These findings are contrary to initial reports by Lerman et al. and Sabol et al.

Differences in study populations must be considered in comparing the current findings to previous findings. The initial Lerman study compared Caucasian and African-American non-smokers (smoked less than 100 cigarettes per day) to current smokers (at least 5 cigarettes per day for at least 1 year). Self-reported non-smokers with an exhaled air carbon monoxide level of 8 ppm or higher were excluded. The total study sample was smaller than the current study (521 total or 444 Caucasians in the Lerman study vs. 881 Caucasians in the current study).[7] The most significant difference between the Lerman study and the current study is that the Lerman study did not specifically address smoking cessation. If the genetic influences on smoking initiation, specifically initiation of smoking on a regular basis, and on smoking cessation differ, then comparisons cannot be made between the two studies.

The Sabol study looked at smoking cessation by comparing former smokers to current smokers. In contrast to the current study, the Sabol study was not restricted to Caucasians but was predominately Caucasian (83.9%). When comparisons in the Sabol study were limited to only current and former smokers, the sample size was 514 (283 current, 231 former). The unadjusted odds ratio for being a former smoker with a 9-repeat allele versus no 9-repeat allele was 1.49 (95% CI: 1.05-2.11).[6] When we used the same methods to calculate the unadjusted odds ratio our odds ratio (OR: 1.02, 95% CI: 0.75-1.39) comes close to the 95% CI reported by Sabol but does not reach statistical significance.

The Jorm study was restricted to Caucasians in Australia. As in the Sabol study, the Jorm study looked at smoking cessation by comparing current smokers to former smokers. For this comparison the sample size was 409 (198 current, 211 former). The unadjusted odds ratio for the Jorm study is consistent with our finding (OR: 1.16, 95% CI: 0.79-1.72).[9]

In both, a study of non-smokers versus smokers and a study of individuals who quit smoking after bupropion treatment versus those who did not quit after bupropion treatment, Lerman reported a *DRD2*(*TaqIA*) by *SLC6A3* gene-gene interaction. In both reports, the 9- repeat allele was only significant among individuals with the *TaqIA A2A2* genotype.[7, 8] We found no

significant effect of *SLC6A3* on smoking status even after stratifying by the presence of the *A1* allele (table 22). Therefore, larger single population studies or pooled analyses are needed to clarify the nature of the relationship between *TaqIA* and *SLC6A3* with respect to smoking behaviors.

The disparate findings across these four studies highlight the need for replication studies in gene-disease association studies. Small differences in sample selection with regard to the definition of cases and controls, ethnic composition and sample size could influence the study findings.

Table 18: SLC6A3 allele frequencies

Allele	Frequency	Percent
10	1281	72.7
9	463	26.3
Other	18	1.0

Table 19: Subjects (all subjects, Not Abstinent at one year, and Abstinent at one year) distributed according to selected baseline characteristics.

Characteristic	N	%	Smoking behavior on Follow-up (%) Not Abstinent (N=662)	Abstinent (N=219)	p-value
Overall	881	100.0	75.1	24.9	
Matching Variables					
Gender					0.858
men	423	48.0	48.2	47.5	
women	458	52.0	51.8	52.5	
Age (years)					0.981
50-59	530	60.2	60.3	59.8	
60-69	253	28.7	28.5	29.2	
70-79	98	11.1	11.2	11.0	
Consent Quarter					0.751
01/01/2002 - 03/31/2002	22	2.5	2.0	4.1	
04/01/2002 - 06/30/2002	108	12.3	12.7	11.0	
07/01/2002 - 09/30/2002	108	12.3	13.0	10.0	
10/01/2002 - 12/31/2002	82	9.3	8.8	11.0	
01/01/2003 - 03/31/2003	88	10.0	9.7	11.0	
04/01/2003 - 06/30/2003	128	14.5	14.8	13.7	
07/01/2003 - 09/30/2003	106	12.0	12.1	11.9	
10/01/2003 - 12/31/2003	116	13.2	13.1	13.2	
01/01/2004 - 03/31/2004	80	9.1	9.1	9.1	
04/01/2004 - 06/30/2004	43	4.9	4.8	5.0	
Baseline smoking behavior					
Intensity (cigarettes per day)					0.060
<20	285	32.3	30.2	38.8	
20-39	535	60.7	62.5	55.3	
40+	61	6.9	7.3	5.9	
Medical factors					
Number of symptoms					0.006
0	139	15.8	15.0	18.3	
1	193	21.9	19.8	28.3	
2+	549	62.3	65.3	53.4	
Number of conditions					0.177
0	605	68.7	67.2	73.1	
1	153	17.4	17.7	16.4	
2+	123	14.0	15.1	10.5	
CT screening referral					0.124
yes	427	48.5	47.0	53.0	
no	454	51.5	53.0	47.0	
Genetic Variables					
Genotype*					0.773
10/10	481	55.7	54.8	53.9	
10/9	307	35.5	34.3	36.5	
9/9	76	8.8	8.9	7.8	
Any SLC6A3-9					0.900
Yes	387	43.9	43.8	44.3	
No	494	56.1	56.2	55.7	

*Excludes 17 participants with alleles other than 10-repeat or 9-repeat

Table 20: Control subjects (Not Abstinent at one year by SLC6A3 genotype) distributed according to selected baseline characteristics

			Any SLC6A3 9-repeat allele (N=649)		
Characteristic	N	%	No (N=372)	Yes (N=290)	p-value
Matching Variables					
Gender					0.968
men	319	48.2	48.1	48.3	
women	343	51.8	51.9	51.7	
Age (years)					0.671
50-59	399	60.3	61.0	59.3	
60-69	189	28.5	28.8	28.3	
70-79	74	11.2	10.2	12.4	
Consent Time					0.823
01/01/2002 - 03/31/2002	13	2.0	2.4	1.4	
04/01/2002 - 06/30/2002	84	12.7	13.7	11.4	
07/01/2002 - 09/30/2002	86	13.0	13.2	12.8	
10/01/2002 - 12/31/2002	58	8.8	9.1	8.3	
01/01/2003 - 03/31/2003	64	9.7	8.9	10.7	
04/01/2003 - 06/30/2003	98	14.8	14.8	14.8	
07/01/2003 - 09/30/2003	80	12.1	11.3	13.1	
10/01/2003 - 12/31/2003	87	13.1	13.2	13.1	
01/01/2004 - 03/31/2004	60	9.1	9.7	8.3	
04/01/2004 - 06/30/2004	32	4.8	3.8	6.2	
Baseline smoking behavior					
Intensity (cigarettes per day)					0.917
<20	200	30.2	29.8	30.7	
20-39	414	62.5	63.2	61.7	
40+	48	7.3	7.0	7.6	
Medical factors					
Number of symptoms					0.426
0	99	15.0	16.1	13.4	
1	131	19.8	20.7	18.6	
2+	432	65.3	63.2	67.9	
Number of conditions					0.213
0	445	67.2	67.7	66.6	
1	117	17.7	19.1	15.9	
2+	100	15.1	13.2	17.6	
CT screening referral					0.555
yes	311	47.0	46.0	48.3	
no	351	53.0	54.0	51.7	

Table 21:Crude and adjusted odds ratios for the effect of SLC6A3 genotype on one-year quit status.

SLC6A3	Abstinent	Not Abstinent	Crude OR	p-value^	Adjusted OR+	p-value^
			OR 95% CI		OR 95% CI	
Genotype'				0.773		0.757
10/10	118	363	1.00 (reference)		1.00 (reference)	
9/10	80	227	1.08 (0.78-1.51)		1.10 (0.79-1.54)	
9/9	17	17	0.89 (0.50-1.58)		0.90 (0.50-1.62)	
Any 9-repeat allele				0.900		0.822
No	97	290	1.00 (reference)		1.00 (reference)	
Yes	122	372	1.02 (0.75-1.39)		1.04 (0.76-1.41)	

+Adjusted for factors (gender, decade of age, and quarter of enrollment) used to match the case and control groups.
^Wald Test
'Participants who had an SLC6A3 allele other than 9-repeat or 10-repeat were excluded. N=864

Table 22: An evaluation of the interaction between TaqIA and SLC6A3

TaqIA	Abstinent at one year	SLC6A3-9		p-value^
		No	Yes	
A2/A2				
	Yes	70 (26.8)	91 (27.2)	0.908
	No	191 (73.2)	243 (72.8)	
OR (95% CI)*		1.00	1.02 (0.71-1.48)	
A2/A1 or A1/A1				
	Yes	31 (19.4)	27 (21.4)	0.668
	No	129 (80.6)	99 (78.6)	
OR (95% CI)*		1.00	1.08 (0.60-1.97)	

^ p-value for chi-square test
*Adjusted for gender, decade of age, and time of enrollment (3 month intervals)
p=0.381 (Log liklihood ratio test) for interaction between TaqIA and SLC6A3

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5.0 OVERALL DISCUSSION

5.1 MAJOR FINDINGS

In an investigation of non-genetic factors associated with smoking cessation one year after entry into a lung screening study (PLuSS), we found that having a computed tomography (CT) scan of the lungs that resulted in a referral was significantly associated with abstinence at one year. The relative risk of being in the Successful Quitter group after receiving a CT referral was 1.39 (95% Confidence Interval (CI): 1.14-1.70). Even after controlling for marital status, gender, symptoms of lung disease, and pulmonary conditions, a CT referral was significantly related to quitting behavior ($p<0.001$). Relative to participants with CT results not resulting in a referral, participants who had CT results necessitating a referral were more often in the Unsuccessful Quitter group (OR: 1.43, 95% CI: 1.17-1.74, compared to Nonquitter) and to be in the Successful Quitter group (OR: 1.24, 95% CI: 0.96-1.61, compared to Unsuccessful Quitter). This association supports the notion that CT screening can influence smoking behaviors thereby creating a "teachable moment" for smoking interventions.

We also investigated the impact of *DRD2* polymorphisms (*TaqIA A1* vs. *A2*, *TaqIB B1* vs. *B2*, *C957T* C vs. T, *–141C Ins/Del C* vs. –) on smoking status one year after entry into PLuSS. After controlling for the matching variables and other genotypes, the *DRD2 TaqIA* polymorphism was significantly associated with being abstinent at one year ($p=0.01$). Compared to participants who had the homozygous wild type *TaqIA* genotype (*A2A2*), participants who carried at least one variant allele (*A1*) were less likely to quit (Odds Ratio: 0.47, 95% CI: 0.24-0.94). This association supports the hypothesis that genetic variation in the dopamine pathway influences smoking cessation.

We also looked at the contribution of the dopamine transporter *SLC6A3* VNTR polymorphism which occurs primarily as a 9- or 10- repeat allele. *SLC6A3* genotype was not

associated with smoking status at one-year (p=0.757). After adjusting for matching variables, compared to participants with the 10/10 genotype the odds ratio for being Abstinent at one year in participants with the 10/9 genotype was 1.10 (95% CI: 0.79-1.54) and with the 9/9 genotype was 0.90 (95% CI: 0.50-1.62). Contrary to previous reports no significant gene-gene interaction with the *DRD2 TaqIA* variant was observed.

5.2 STRENGTHS

The focus of this study is smoking cessation. Therefore, a strength of this study is that it isolates the period of time on the smoking continuum in which participants quit smoking. Participants in this study are fairly homogenous with respect to their smoking histories; thus, limiting the potential effect of genotype on smoking duration and intensity. Genetic association studies are frequently criticized for attempting to find an association between a single gene and disease because it is highly likely that groups of genes or variants contribute to disease. Although still limited in number, a strength of this study is that it investigates the contribution of several genetic variants on the dopamine pathway. This study, with a sample size of nearly 900 participants, is also among the largest of the studies that have looked at associations between the dopamine pathway and smoking behavior.

5.3 LIMITATIONS

Potential misclassification bias: I classified participants as smokers or quitters based on their answer to the telephone follow-up questionnaire question, "Are your currently smoking?" I estimated the overall accuracy of self-report in the PLuSS population by conducting exhaled air carbon monoxide tests in a subset of participants who returned for the one-year CT scan. Overall, I found approximately 90% agreement between self-reported smoking status and carbon monoxide levels using 8 parts per million (ppm) or higher to classify smokers with slightly lower agreement among those with a reading of 8 ppm or higher (88%) compared to those with a

reading below 8 ppm (93%). Because participants are unaware of their genotype, it is unlikely that misclassification will be differential with respect to genotype. Hence, misclassification with respect to smoking status would be expected to bias the observed association between genes and smoking outcome toward the null (no association).

Limited number of genes: The inability to quit smoking is most likely influenced by many factors and multiple genes. No single gene or set of genes has been identified as the most important determinant of smoking cessation. *DRD2* was initially selected because a strong biological rationale exists for the involvement of the dopamine pathway in smoking cessation, smoking behaviors in general, and because assays for most of the variants had already been developed. *SLC6A3*, also part of the dopamine pathway, was selected because of the potential for interaction with *DRD2*.

Limited generalizability: Frequencies of the genetic variants of interest are known to differ among ethnic groups. The sample for the genetic studies was limited to Caucasian subjects because the small number of non-Caucasians in the parent populations made subgroup analyses impossible. However, because of this limitation the results cannot be applied to the general population.

This study also included only individuals who self-selected into a lung cancer screening study. These individuals may differ from individuals in the general population with regard to their motivations for quitting and their experiences with smoking cessation attempts in the past.

Potential selection bias: Because my primary endpoint was smoking status at one year, participants who did not complete the one year follow-up questionnaire were excluded from the study. Without data from the one-year follow-up it is impossible to determine if smoking status at one year is associated with completing the follow-up questionnaire. However, the completion rates for the one-year follow-up were quite high particularly when only those participants who were eligible for follow-up were considered (97%). Of the baseline smokers who lacked follow-up data, many were not yet due for follow-up.

Lack of environmental data: The parent study, PLuSS, was designed as a lung cancer screening study, not a smoking cessation study. Therefore, limited data were collected regarding smoking behaviors, exposure to second hand smoke, social pressure and motivations related to smoking behavior. These environmental data could modify the effects of genotype on smoking cessation.

Possible confounding by cessation strategy: The ultimate goal of genotyping analyses related to smoking cessation is to develop tailored cessation strategies by genotype. Although we did not find significant results for some variants when we assessed quit status, if data were available regarding quit strategy we may have found associations between genotype and quit strategy.

5.4 FUTURE RESEARCH

This study provides a basis on which I can expand my investigation of smoking cessation in several ways. As I described previously, there are a number of candidate genes that could influence smoking cessation. A study of this population is currently underway investigating the effect of CYP2A6 polymorphisms on smoking cessation. Additional studies could expand the gene panel further.

For my dissertation work I chose to limit the genotyping analysis to PLuSS participants who were smoking at baseline. However, with additional funding I could expand my sample to include individuals who had quit smoking before the baseline assessment.

Future studies could be designed to obtain additional information regarding cessation attempts, environment, and health provider messaging for tobacco abstinence during the period of interest. These studies could also include additional evaluations of smoking status, such as a cotinine test, among all participants at the time of self-report.

5.5 APPLICATION TO PUBLIC HEALTH

Cigarette smoking is the leading preventable cause of death in the United States.[1] Although strategies are in place to prevent children and young adults from initiating smoking, half of all adults have smoked in their lifetime and over half continue to smoke. A vast majority of these smokers want to quit, but have been unable to do so.[2]

Although a variety of behavioral and pharmacological smoking cessation aides are available, most individuals who quit successfully do so without any assistance.[1] The limited effectiveness of cessation aides in the population may be due to interindividual differences in various aspects of smoking behavior including both the strength of addiction and the burden of withdrawal symptoms. These interindividual differences are partially dictated by genetics. Therefore, identification of genetic influences on smoking behavior and consideration of those influences in the selection of quitting regimens may improve success rates thereby reducing the morbidity and mortality due to continued cigarette smoking.

5.6 REFERENCES

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