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**Abstract**

Smoking of tobacco products continues to be a major cause of worldwide health problems. Epidemiological studies have shown that tobacco smoking is the greatest risk factor for the development of pancreatic cancer. Smokers who are able to quit smoking can reduce their risk of pancreatic cancer by nearly 50% within two years, however, their risk of developing pancreatic cancer remains higher than that of non-smokers for 10 years. Nicotine is the major psychoactive substance in tobacco, and is responsible for tobacco dependence and addiction. Recent evidence suggests that individuals have genetically based differences in their ability to metabolize nicotine, as well as genetic differences in the psychological reward pathways that may influence individual response to smoking initiation, dependence, addiction and cessation. Numerous associations have been reported between smoking behavior and genetic polymorphisms in genes that are responsible for nicotine metabolism. In addition, polymorphisms in genes that encode neurotransmitters and transporters that function in psychological reward pathways have been implicated in differences in smoking behavior. However, there is a large degree of between-study variability that demonstrates the need for larger, well-controlled case-control studies to identify target genes and deduce mechanisms that account for the genetic basis of individual differences in smoking behavior. Understanding the genetic factors that increase susceptibility to tobacco addiction may result in more effective tobacco cessation programs which will, in turn, reduce the incidence of tobacco related disease, including pancreatic cancer.

**INTRODUCTION**

The use of tobacco products constitutes the most preventable cause of premature death worldwide. It is estimated that half of all Americans who continue to smoke will die of smoking-related diseases\[^1\], including cancer, emphysema and heart disease. The U.S. Surgeon General reported in 2004 that cigarette smoking has caused more than 12 million premature deaths in the United States since the publication of the Surgeon General’s report on smoking and health in 1964\[^2\]. Although the dangers inherent in smoking are well known, the use of tobacco continues because these products are delivery systems for nicotine, an extremely addictive drug. Long term smoking is the most established risk factor for lung cancer, however smoking also increases the risk of developing cancers of the esophagus, uterine cervix, kidney, bladder, stomach and pancreas\[^3\]. This review will briefly discuss the effects of cigarette smoke components on the pancreas, and then focus on the genetic basis for nicotine addiction that is responsible for tobacco product use.

Pancreatic cancer is a relatively rare tumor; the lifetime risk of dying from pancreatic cancer is between 1% to 2% of the general population. However, pancreatic tumors are extremely aggressive, with a 5-year survival rate of less than 5% and a mortality rate of nearly 100%\[^4\]. Since there are presently no tests for early screening, and once detected, therapeutic choices are limited, the best hope for reducing mortality from pancreatic cancer is prevention. Smoking of tobacco products is the most important risk factor for developing pancreatic cancer\[^5,6\], with risk increasing with higher levels of tobacco use and more years of exposure. A 40 year epidemiological study of British physicians
determined that the pancreatic cancer rate for smokers was 35 per 100,000 person years, while the risk for ex-smokers and non-smokers was reduced to 23 and 16 per 100,000 person years respectively[7]. A large prospective study by Fuchs et al[9] determined that current smokers had a 2.5 times greater relative risk of developing pancreatic cancer compared to study subjects who had never smoked. They also found that although smoking cessation reduces the risk of pancreatic cancer by 48% within 2 years, smokers who quit have an increased risk for approximately 10 years compared to non-smokers. Smoking, as a single risk factor or in combination with alcohol, increases the risk of idiopathic pancreatitis, a precancerous condition[13,15,16]. Clearly, one way to reduce the risk of pancreatic cancer is to develop more effective smoking cessation strategies; however, smoking is a complex behavior, which is likely influenced by both environmental and genetic factors. There is wide inter-individual variation in the risk of becoming dependent on nicotine. Genetic factors that influence nicotine metabolism and the psychological reward system of nicotine use are both thought to be important in the development of nicotine dependence.

MECHANISMS OF TOBACCO INDUCED PANCREATIC CANCER

Cigarette smoke is a complex mixture of carcinogenic compounds and nicotine, many of which have deleterious effects on the exocrine pancreas. Nicotine forms carcinogenic N-nitroso compounds during processing[17,18], which induce pancreatic cancer in the Syrian golden hamster model of pancreatic carcinogenesis[19,20]. A particular component of tobacco smoke, 4-(methylnitrosamino)-1-butanone (NNK), is implicated in pancreatic carcinogenesis by its ability to form DNA adducts[21] which have been associated with activating RAS mutations that are found in most human pancreatic adenocarcinomas[18]. NNK also induces proliferation in pancreatic ductal epithelial cells by stimulating EGF mediated signal transduction pathways through binding to β-adrenergic receptors[19,20].

Nicotine, although not carcinogenic, exerts toxic effects on the pancreas. Exposure of rat pancreatic acini to nicotine results in increased protein secretion[21]. We have demonstrated that nicotine induces cytoplasmic vacuolization, cellular edema and increases the cellular amylase content in the exocrine pancreas[22]. We also demonstrated that the increase in pancreatic enzymes in nicotine treated rats was accompanied by reduced CCK mediated enzyme secretion, which may be the causative factor in nicotine induced pancreatic cell pathology. Exposure of rats to tobacco smoke produced fibrosis and scarring of pancreatic acinar structures, characteristic of chronic pancreatitis[19]. In humans, nicotine inhibits the secretion of bicarbonate and affects the composition of pancreatic secretions[23,24], and in patients with pancreatitis, nicotine exposure resulted in increased pancreatic enzyme secretion, including amylase[25] and lipase[26].

THE GENETICS OF NICOTINE DEPENDENCE

Nicotine is the major psychoactive component of tobacco that is responsible for dependence through a nicotine stimulated reward system that is thought to be mediated by the dopaminergic system of the brain. Recent research has identified behavioral, environmental and genetic factors that influence the various stages of smoking behavior, including smoking initiation, development of addiction and smoking cessation. Early evidence for the influence of heredity on tobacco dependence came from studies of tobacco use among families[26], adopted siblings and among mono and dizygotic twins[27,28]. In a study of mono and dizygotic twins, True et al[31] found that 50% of the risk of smoking initiation and 70% of the risk for continuing to smoke were due to genetic factors.

POLYMORPHIC GENES INVOLVED IN NICOTINE METABOLISM

Individuals with increased tolerance to nicotine because of a greater capacity to metabolize the drug may experience fewer adverse reactions to their first encounter with nicotine, and therefore may have a greater propensity to continue using tobacco products. Conversely, slow metabolizers of nicotine would be expected to smoke less and would be less likely to become nicotine dependent. Smokers tend to adjust their smoking behavior in order to maintain a certain level of nicotine in the brain[32], so that an individual's capacity to metabolize nicotine will influence their intake and exposure. Polymorphic expression of genes that are responsible for nicotine metabolism may be responsible for the wide variability in nicotine tolerance between individuals.

The hepatic enzymes cytochrome P450 2A6 (CYP2A6) and cytochrome P450 2D6 are the major isoforms responsible for the metabolism of nicotine to cotinine, however hepatic CYP2A6 is responsible for 90% of the first pass metabolism of nicotine[33,34]. Both of these enzymes are polymorphic in the human population, with genetic differences that are responsible for high and low activity alleles.

CYTOCHROME P450 2A6 (CYP2A6)

The CYP2A6 gene is polymorphic in the human population, with large inter-individual differences in the levels of hepatic CYP2A6 protein and enzyme activity[34,35]. A deletional allele was found to be responsible for low or non-existent CYP2A6 activity[36]. A number of studies have found that individuals with genetically determined slow or absent CYP 2A6 activity have a reduced risk of becoming smokers, and those who do smoke tend to smoke fewer cigarettes per day and have a higher smoking cessation success rate[37-40]. Piancza et al[41] reported that a tobacco dependent population had an under representation of low activity CYP2A6 alleles, and that those smokers who had low activity alleles tended to smoke fewer cigarettes per week, suggesting a role for CYP2A6 in nicotine tolerance and dependence. However, other studies have failed to detect an association between genetically low CYP2A6 activity and nicotine use or dependence[42,43]. One reason for this discrepancy was the use in early studies of a
genotyping protocol that overestimated the number of low activity alleles in the study population\[44,45\]. A meta-analysis by Carter et al\[46\] also failed to find a significant association between CYP2A6 genotype and smoking behavior. In another meta-analysis, Munafò et al\[47\] concluded that the influence of individual genes on smoking behavior may be subtle, and that larger studies that have the power to detect the effects of multiple genes on smoking behavior will be necessary. However, in a number of studies, they found strong evidence that the reduced activity allele of CYP2A6 was associated with smokers who were able to quit smoking.

Recently, the use of CYP2A6 inhibitors has been explored as a chemoprevention strategy for smoking cessation. A study by von Weymarn et al\[48\] reported that the benzyl and phenylethyl isothiocyanates that are found in cruciferous vegetables such as broccoli and cabbage, were effective competitive inhibitors of both CYP2A6 and CYP 2A13. CYP2A13 is the enzyme responsible for the activation of the tobacco procarcinogen NNK to its ultimate carcinogenic form. This report suggests that inhibition of CYP2A6 may convert the phenotype of smokers to one which confers less metabolic tolerance to nicotine, leading to fewer cigarettes smoked per day. The resultant reduction in nicotine metabolism may possibly increase success with smoking cessation. At the same time, inhibition of CYP2A13, which is found primarily in the lung, may result in less activation of NNK, potentially protecting individuals who continue to smoke from developing lung cancer. Sellers et al\[49\] reported that another CYP2A6 inhibitor, methoxsalen, was effective in increasing the bioavailability of nicotine in smokers, resulting in a decrease in the number of cigarettes they smoked per day. These studies demonstrate that the use of CYP2A6 inhibitors may be a useful strategy to reduce tobacco exposure and may have the potential to increase the success rate of smoking cessation programs.

**CYTOCHROME P450 2D6 (CYP2D6)**

Caporaso et al\[50\] determined inter-individual differences in CYP2D6 phenotype by measuring the metabolism of dextromethorphan, a CYP2D6 substrate. They concluded that polymorphisms in CYP2D6 were not major determinants of nicotine metabolism in smokers except in ultranetabolizers. These are individuals who have a duplication of the CYP2D6 gene that is present in 3%-8% of Caucasians and up to 30% of other ethnic groups\[51\]. This duplication results in the production of high levels of functional CYP2D6 protein and results in the increased metabolism of CYP2D6 substrates, including nicotine. In a case-control study of lung and larynx cancer, the CYP2D6 gene duplication was found in 13% of cancer patients compared to 6% of healthy control subjects. The frequency of a genetic polymorphism that codes for a high activity CYP2D6 allele called CYP2D6*49 was also higher in cases compared to controls\[52\]. Approximately 3%-10% of Caucasians are CYP2D6 poor metabolizers, due to inheritance of two defective alleles. Saarikowski et al\[53\] found the same proportion of poor metabolizers in groups of smokers and never-smokers, however among men, a trend toward more poor metabolizers in the non-smoking group was observed. They also found twofold more ultranetabolizers among heavy smokers compared to non-smokers. Overall, the results of these studies suggest that CYP2D6 affects nicotine metabolism among individuals who have high activity due to gene duplication, however the influence of the low activity allele remains controversial.

**GENES INVOLVED IN NICOTINE DEPENDENCE**

Nicotine is thought to induce a euphoric state in users and by that is thought to be the result of activation of the mesolimbic dopaminergic reward system in the nucleus accumbens of the brain\[54-56\]. Nicotine binds to nicotinic receptors that, when activated, enhance dopamine release in areas of the brain that are thought to be involved in reward\[55,57\]. The involvement of the dopaminergic system in the reinforcement activity of nicotine may be related to the highly addictive properties of the drug\[58\]. Genetic polymorphisms in genes that affect this reward system, including dopamine receptors and transporters, nicotinic receptors and serotonin receptors may modulate an individual’s risk of becoming nicotine dependent.

**DOPAMINE RECEPTOR GENE POLYMORPHISMS**

The human dopamine D2 receptor (DRD2) has a TaqI polymorphism with two minor alleles termed the TaqIA allele (A1) and the TaqIB allele (B1). The TaqIA*A1 allele has been shown to be associated with reduced expression of dopamine D2 receptor in the striatum\[58-61\]. It has been hypothesized that subjects with reduced numbers of dopamine receptors may compensate for this deficiency by using nicotine to increase brain dopamine levels. The presence of the DRD2 TaqI allele has been associated with an earlier age of smoking initiation\[62\], increased risk of being a current smoker, and reduced duration of smoking abstinence\[63\]. Spitz et al\[64\] conducted a case control study of lung cancer patients and found that a greater percentage of chronic smokers had the B1:B1 genotype compared with non-smokers, whether they were cancer cases or controls. The least common A1 or B1 alleles were associated with individuals who were younger when they started smoking and had attempted to quit smoking fewer times compared with smokers with the more common DRD2 alleles. Other studies have failed to find an association between the DRD2 TaqI allele and smoking behavior. Bierut et al\[65\] analyzed a family study by the transmission disequilibrium test and found no difference in the frequency of DRD2 alleles transmitted to habitual smokers. In a small British study, Singleton et al\[66\] found no increase in the DRD2 TaqI allele in smokers compared to non-smokers, and Johnstone et al duplicated these findings in a larger study\[67\]. Munafò et al\[68\] conducted a meta-analysis of the genetic basis for smoking behavior and concluded that there is some evidence for an association between the DRD2 TaqI*A1 allele and smoking behavior, but larger, better designed
studies in a variety of populations are needed to confirm this relationship.

Other dopamine receptors are genetically polymorphic, but have received less attention than the DRD2 gene with respect to smoking behavior. A 5’ polymorphism of no known function, located in the dopamine D1 receptor gene (DRD1), has been associated with smoking[69]. The DRD3 receptor is genetically polymorphic and is highly expressed in the nucleus accumbens, however, no association with smoking behavior has been reported[69].

A polymorphism characterized as a variable number of tandem repeats has been reported in the dopamine D4 receptor (DRD4)[70,71]. The receptor containing 7 repeats has been characterized as having a reduced response to dopamine binding[72]. Shields et al[73] compared DRD4 genotype for a population of smokers compared to non-smokers and found that African American smokers had a higher incidence of the DRD4 allele containing 7 repeats than African American non-smokers. In addition, African American smokers with this allele had an earlier age of smoking initiation and less success at smoking cessation than African Americans with shorter repeat sequences. The same analysis of a Caucasian population showed no association with smoking status. This data suggests that individual genotypes may be a factor in the success of smoking cessation strategies, and more effective strategies may need to be tailored to an individual’s genetic background. The human DRD5 gene has at least 4 missense or nonsense polymorphisms, however, no association with smoking behavior has been reported[74].

DOPAMINE TRANSPORTER POLYMORPHISMS

The dopamine transporter gene (SLC6A3) has a variable number of tandem repeats polymorphism in the 3’ noncoding region of the gene[75] that is associated with reduced transporter levels in the brain[76,77]. The role of this polymorphism in determining smoking behavior is not clear, however, in some studies, the polymorphism containing 9 repeats was found to be associated with greater levels of cigarette craving among African American smokers[77]. Other studies found that individuals with the SLC6A3 9 repeat allele were less likely to be smokers, especially if they also had the DRD2 Taq1 A1 allele[78]. These individuals were less likely to start smoking at an early age, and those who did smoke had greater periods of smoking cessation. Sabol et al[79] confirmed these findings in a diverse population of smokers, non-smokers, and ex-smokers, however Vandenbergh et al[80] failed to replicate these results in spite of another report on the function of the 9 repeat sequence as a transcriptional enhancer[81,82].

CATABOLISM OF DOPAMINE

The action of dopamine is terminated by the action of catabolic enzymes, primarily catecholamine-O-methyltransferase (COMT) with lesser roles for monoamine oxidase A and B (MAO) and dopamine β hydroxylase (DBH). Genetic polymorphisms have been discovered in these enzymes, and the effects on smoking behavior have been tested in a number of studies. In a study of smokers, McKinney et al[83] reported that polymorphisms in DBH and MAO were related to maintenance of nicotine levels and predicted the quantity of cigarettes smoked. No association between the amount of tobacco consumed and the functional COMT A1947G single nucleotide polymorphism (SNP), which results in the substitution of a methionine for valine at codon 108 (Met108Val) in the COMT protein, was detected in this study. These results were unexpected in light of a report that the G allele at this locus results in a three to fourfold increase in COMT activity[84], and a reported association of this allele with addiction to other drugs[85]. The lack of association between this SNP and smoking initiation, persistence and cessation was replicated in a larger study of current smokers, ex-smokers and lifetime non-smokers[86]. More recent studies include the finding of a positive association between the high activity COMT allele and nicotine dependence in a Caucasian population, however, these results were not replicated in a second independent study by the same researchers[87]. In a case-control study of women, those who were homozygous for the lower activity Met allele were more likely to be ex-smokers rather than current smokers, and in a nicotine replacement clinical trial reported by the same group, Met homozygotes at the COMT locus had more success at smoking cessation[88]. Bueten et al[89] analyzed five allelic variants in the COMT gene, including the Met108Val SNP, and found a significant association with nicotine dependence. The lack of reproducibility of this data, even when the same researchers analyze independent study populations, may be due to the lack of power to detect relatively small effects of COMT on smoking behavior. These results demonstrate the need for large, adequately powered replication studies to determine the genetic basis of smoking behavior and nicotine addiction.

SEROTONIN TRANSPORTER PROMOTER POLYMORPHISMS

Nicotine increases the secretion of serotonin in the brain[90] therefore, the serotonergic system may have a function in determining smoking behavior. Lower serotonin reuptake has been associated with an increased risk of depression as well as increased impulsive or aggressive behavior[91]. This combination of behavioral traits is termed neuroticism, and has been associated with increased incidence of smoking, nicotine dependence, and difficulty in quitting smoking[92]. Evidence for a genetic link to neuroticism and smoking behavior centers around a 44-bp deletion/insertion polymorphism that corresponds to short (S) and long (L) versions of the serotonin transporter gene (5-hydroxytryptamine transporter or 5-HTT) promoter[93,94]. Functional characterization of this polymorphism has demonstrated that the short promoter variant reduces the transcriptional activity of the gene and results in decreased 5-HTT expression and decreased serotonin uptake[94]. Hu et al[95] found a relationship between the genotype for the 5-HTT promoter polymorphism and degree of...
neuroticism and smoking behavior. This finding was confirmed by another study which reported that smokers who were heterozygous or homozygous for the 5-HTT S-allele were more likely to be dependent on nicotine than subjects who were homozygous for the L allele. However, in a Japanese population, the presence of the S allele was associated with non-smokers or ex-smokers, indicating that individuals who were homozygous for the S allele were less likely to begin smoking, or were more successful at smoking cessation. Other studies were unable to detect any association between the 5-HTT promoter polymorphism and smoking behavior in Caucasian or African American populations, in spite of significant differences in the distribution of 5-HTT promoter alleles between racial groups.

CONCLUSIONS
The variability of results reported for most candidate genes that are hypothesized to affect smoking behavior demonstrates the need for larger, well controlled studies designed to define the genetic basis for inter-individual differences in nicotine and dopamine metabolism as they relate to smoking behavior and nicotine dependence. Results of these future studies will be useful in identifying individuals who are at increased risk of becoming dependent on nicotine and will also facilitate the development of smoking cessation strategies that are targeted to individual differences in nicotine and neurotransmitter action and metabolism. Considering that smoking is the greatest risk factor for pancreatic cancer, reduction of tobacco use through both abstinence programs and successful smoking cessation is the best hope for reducing the risk of developing this devastating disease.

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