Dependence on tobacco, like many other drug dependencies, is a complex behavior with both genetic and environmental factors contributing to the variance. The heritability estimates for smoking in twin studies have ranged from 46 to 84%, indicating a substantial genetic component to smoking. Candidate gene studies have detected functional polymorphisms in genes coding for the cytochrome P450 enzymes, and variations in these genes that lead to more rapid nicotine metabolism have been implicated in smoking. Similarly, smoking has been associated with polymorphisms in dopaminergic genes that may influence the dopamine receptor number and/or function. Animal experiments have localized specific subunits of the nicotinic receptors that may mediate the reinforcing properties of nicotine and have investigated their role in nicotine dependence. However, environmental factors have also been found to contribute to the risk of initiation and persistence of smoking. We review the scientific evidence that supports a role for genetic influences on smoking, discuss the specific genetic and neurobiological mechanisms that may mediate susceptibility to nicotine dependence, identify possible gene/environmental interactions that may be important in understanding smoking behavior, and suggest directions for future research. Insights into the genetic contributions to smoking can potentially lead to more effective strategies to reduce smoking.

Key words: addiction; gene; nicotine; smoking; tobacco

Abbreviations: COMT = catechol-O-methyl transferase; CYP = cytochrome P-450; DRD2 = dopamine D2 receptor; 5-HTT = serotonin transporter; MAO = monoamine oxidase; OCR = overall concordance ratio; TH = tyrosine hydroxylase

Annually, tobacco smoking is responsible for approximately 3 million deaths worldwide, with > 430,000 occurring in the United States alone. Because smoking is a modifiable risk factor, treatment and prevention represent an enormous opportunity for public health promotion. Despite public awareness of the health risks of smoking, approximately 25% of adult Americans continue to smoke, making an accurate understanding of various factors that influence smoking behavior critical.

Although environmental factors such as peer influences and advertising may contribute to smoking, a significant determinant of continued tobacco use is dependence on nicotine. Clinical research has highlighted interesting individual differences in the ability to become dependent on nicotine and on the ability to quit. While hereditary causes of such clinical variations were suggested > 40 years ago, advances in behavioral genetics and molecular biology have renewed interest in the genetic basis for nicotine dependence.

GENETIC MODELS

Tobacco smoking is believed to be a complex, multifactorial behavior with both genetic and envi-
environmental determinants. The approaches to understand the genetic contributions to smoking include the following: (1) study of individuals who share genes; this includes twin, family, and adoption studies; association studies, case-control studies based on a comparison of unrelated affected and unaffected individuals from a population; an allele A at a gene of interest is said to be associated with the trait if it occurs at a significantly higher frequency among affected individuals compared to control subjects; (3) animal studies, analysis of inbred, transgenic and gene knock-out animals; animal studies offer advantage of studying large number of genetically identical animals under controlled conditions; (4) linkage analysis, study of the inheritance pattern of phenotypes and genotypes in pedigrees. While family, adoption, and twin studies each have contributed evidence to evaluate the involvement of genetic factors, association and linkage studies have improved our understanding of these complex disorders. Development of sophisticated mathematical models to analyze genetic data has permitted the evaluation of the relative importance of both genetic and environmental contributions.

TWIN STUDIES

The twin pair study has long been a popular research design to investigate genetics in the cause of diseases. Twin studies usually examine concordance rates for traits of interest. If the proportion of monozygotic twins concordant for a given trait is greater than the proportion of dizygotic twins, it is likely that genes influence the trait. If there is no significant difference in concordance rates between monozygotic and dizygotic twins, then the trait is likely to be influenced by environmental factors. Fisher (1958) first reported that concordance for smoking was significantly higher in monozygotic than dizygotic male twin pairs among the German population. These findings were subsequently confirmed among female twins from Germany and replicated in studies from the United States, Scandinavian countries, Australia, Britain, and Japan in populations of adults, elderly, and adolescent smokers.

While initial reports suggested that the influence of heredity on smoking was modest, more recent twin studies have included larger sample sizes, better characterization of the phenotype, and more sophisticated models of data analysis. These studies have found significant genetic influences on several aspects of smoking behavior, such as the initiation and persistence of smoking and number of cigarettes smoked in both men and women. The heritability estimates for smoking (i.e., the proportion of the variance in smoking that is attributed to genetic factors) in these studies have ranged from 46 to 84%, comparable to the heritability estimates for asthma, hypertension, or alcoholism.

For example, in a large study of 4,960 male twin pairs of World War II veterans followed up over 16 years, Carmelli et al. reported that the concordance rate was significantly higher among monozygotic than dizygotic twin pairs for never-smoking, current smoking, and quitting. In a recent review of >17,500 reared-together monozygotic and dizygotic twins from 14 different studies, it was estimated that genetic, familial-environmental, and individual-specific environmental risk factors accounted for 56%, 24%, and 20% of the variance in smoking. Studies of twins reared apart that control for familial influences have also found that genetic factors contribute to about 60% of the variance in smoking.

To examine the effect of genetic influences, twin studies have employed an overall concordance ratio (OCR) that tests the difference between monozygotic and dizygotic concordance rates. If the OCR is significantly > 1, potential genetic influences are considered to be present. Most studies have reported OCR values of 1.3 to 1.6 for smoking, indicating a moderate effect of genetic factors on different aspects of smoking behaviors.

It must be noted that social influences such as peer and family smoking, lower educational levels, lack of parental concerns about smoking, and perceptions about smoking that are promoted by tobacco companies also contribute to initiation of smoking. Once regular smoking is established, the two important predictors of persistent smoking are dependence on nicotine and lower educational levels. Researchers have examined different statistical models to study the interaction of genetic and environmental factors on smoking. It seems that there may be some genetic factors that influence both initiation and smoking and some that are unique to one or the other aspect of smoking behavior. Also, the risk for initiation of smoking appears to be influenced by both environmental and genetic factors, but the latter may be more important in contributing to the risk of persistence of smoking. Though the twin studies clearly indicate that genetic influences contribute to smoking, the findings are limited by the assumption that monozygotic and dizygotic twins are equally exposed to similar environmental influences. Studies have found that monozygotic twins may share a more similar environment than the dizygotic pair, which may inflate the heritability rate.
ANIMAL STUDIES

Since human studies do not permit the manipulation of individual genes and gene products, animal experiments offer the opportunity to systematically examine the biological influence of specific genes on nicotine addiction. Several approaches to the study of animal genetics have been useful in the understanding of nicotine dependence. The most useful include those done with inbred strains, transgenic mice, and knock-out mice.

Inbred Strains

Inbred strains are produced by within-family mating so that the animals are homozygous at all genetic loci. Thus, all members of an inbred strain are genetically identical. In a series of experiments, Morrison and Lee,24 Hatchell and Collins,25 and Robinson et al26 showed that different strains of inbred animals differ in their sensitivity to behavioral and physiologic effects of nicotine, including the development of tolerance and sensitivity to aversive responses such as nicotine-induced seizures.24–26 For example, using $^{[125I]}$-bungarotoxin as a ligand for nicotinic receptors,27,28 Miner et al29 (1984) showed that variance in nicotine-induced seizure sensitivity correlated very highly ($r^2 = 0.64, p < 0.05$) with the number of bungarotoxin binding sites in the hippocampus of 19 different inbred strains. Furthermore, genetic influences seem to determine the number of brain nicotinic receptors that mediate the effects of nicotine.27 These observed differences between inbred strains support the hypothesis that genetic factors may contribute to the differences in susceptibility to nicotine dependence among humans.

Transgenic and Knock-out Animals

The objective of knock-out experiments is to replace the specific gene of interest with one that is inactive or altered; the biochemical deficits observed in these animals can reveal the function of the protein expressed by the gene. For example, using knockout mice, Picciotto et al30 demonstrated that the $\beta$-2 subunit neuronal nicotinic acetylcholine receptor may mediate the reinforcing properties of nicotine while the $\alpha$-7 subunit was found to be important in mediating nicotinic actions in the hippocampus.31 Other models of genetically engineering where a segment of DNA from a different organism has been introduced into the germline of the animals have permitted researchers to examine specific receptors that may mediate the addictive properties of nicotine. For example, transgenic mice that over express tyrosine hydroxylase, a rate-limiting, enzyme-controlling dopamine synthesis, appear to be less sensitive to physiologic effects of nicotine.32 Nicotine has also been shown to increase the expression of tyrosine hydroxylase (TH) in cultured cell models,33 indicating that dopaminergic mechanisms may be important mediators of the central effects of nicotine. However it is worth noting that experimental manipulation of a gene may produce multiple unsuspected phenotypic changes that limit the validity of these models and its applicability to humans.

CANDIDATE GENES FOR SMOKING

Genes Influencing Metabolism of Nicotine

There is increasing evidence that tobacco consumption may be influenced by genetically determined variations in the cytochrome P-450 (CYP) group of enzymes, in particular the CYP2A6 enzyme that metabolize nicotine to cotinine34 (Fig 1). Along with the normal functional allele CYP2A6*1, two
variants of the CYP2A6 gene have been identified: CYP2A6*2 and *3; both are associated with reduced activity of the enzyme. Pianezza et al\textsuperscript{35} reported that the frequency of individuals with impaired nicotine metabolism (ie, carriers of CYP2A6*2 or *3 alleles) was significantly lower among tobacco-dependent individuals than control subjects. This possible protective effect of the CYP2A6*2 and *3 alleles was also demonstrated in studies that reported that individuals with these alleles smoked significantly fewer cigarettes per day and had increased likelihood of quitting smoking.\textsuperscript{36}

Another P450 enzyme, CYP2D6, is also involved in oxidation of nicotine to cotinine. Individuals who are homozygous for the recessive defective alleles (CYP2D6*3, *4 and *5) are termed poor metabolizers.\textsuperscript{37,38} Individuals carrying one or two copies of the functional CYP2D6*1 or *2 genes are termed extensive metabolizers, and those with more than two copies are termed ultrarapid metabolizers.\textsuperscript{39} Several studies have investigated this polymorphism in tobacco dependence with conflicting results. Turgeon et al\textsuperscript{40} (1995) reported that poor metabolizers were underrepresented among smokers compared to non-smokers, supporting the hypothesis that individuals who metabolize nicotine slowly are less likely to become addicted. However, Cholerton et al\textsuperscript{41} (1996) failed to find a difference in the CYP2D6 genotype among smokers and nonsmokers. More recently, however, the same group concluded that although CYP2D6 status does not influence whether or not a person becomes a smoker, once an individual starts to smoke, subsequent smoking behavior may be influenced by their CYP2D6 genotype.\textsuperscript{42} Further support for the involvement of this genotype in smoking comes from reports that the prevalence of ultrarapid metabolizers among heavy smokers was fourfold compared to nonsmokers and twofold compared to smokers with variable smoking habits.\textsuperscript{43} It seems that individuals may regulate their rates of smoking depending on genetically mediated differences in blood levels of nicotine. Whether the CYP2D6 genotype may be related to risk of lung cancer has been investigated with contradictory results and the associated risk for developing tobacco-related diseases remains to be determined.\textsuperscript{44–47}

**Dopamine Genes**

The mesolimbic dopaminergic system is critical to the reinforcing effects of several addictive drugs including nicotine.\textsuperscript{48} As shown in Figure 2, nicotine has been shown to stimulate dopamine release in the nucleus accumbens,\textsuperscript{49} possibly through activation of

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**Figure 2.** Effects of nicotine on dopaminergic neurotransmission. Nicotine increases the release of dopamine (DA). TH mediates the conversion of tyrosine to L-dopa, the precursor of dopamine; this is the rate-limiting step in dopamine synthesis. Dopamine transporter (DAT) is the site for reuptake of dopamine and regulates the amount of synaptic dopamine. COMT and MAO are involved in the metabolism of dopamine. Dopamine binds to postsynaptic receptors that mediate neurotransmission. The genes coding for the enzymes and receptors have been studied in smoking. DR = dopamine receptor.
acetylcholine receptors located in mesolimbic dopaminergic pathways. Not surprisingly, investigators have examined the association of variations in several genes controlling the dopamine metabolisms with nicotine dependence.

Dopamine Receptor Genes: Polymorphisms in several dopamine receptor genes have been detected and studied in addictive diseases. For example, Blum et al.51 described a restriction fragment length polymorphism (TaqI) in the 3′ untranslated region of the dopamine D2 receptor (DRD2) gene, with two alleles DRD2*A1 and *A2; the former has been associated with reduced DRD2 density.52 Noble et al.53 first reported a significantly higher prevalence of DRD2*A1 allele among current smokers and ex-smokers compared to nonsmokers. These findings were replicated in a study of non-Hispanic whites, which found that 49% of smokers carried the A1 allele compared to 26% of control subjects.54 The study also found a significant, inverse relationship between the prevalence of the DRD2*A1 allele and the age of onset of smoking, and the maximum duration of abstinence from smoking. These relationships have also been also observed among Mexican-American smokers.55 It has been suggested that individuals with the DRD2*A1 allele may have less number of dopamine receptors and receptor binding,52 and may need to use larger amounts of nicotine to increase synaptic dopamine and thus become rapidly tolerant. However, the validity of early population-based association studies has been questioned because of the potentially confounding effects of “population admixture.” This term refers to the unequal distribution of genes within a population due to differences in ethnicity or racial background of the individuals that make up the population. Since persons affected and unaffected by a disease may come from different ethnic backgrounds and there seem to be ethnic differences in allele frequencies, the genetic heterogeneity in a population can lead to spurious findings. Studies using family-based approaches to avoid population stratification, have not observed an association of the DRD2 polymorphism with susceptibility to nicotine dependence.50,51 It appears that the association between nicotine addiction and DRD2 genotype may not be as strong as suggested in earlier studies.

Investigations of other dopamine receptor genes among smokers have been relatively limited. Shields et al.58 evaluated the association between smoking and the long (L) and short (S) variable tandem repeat variants of the dopamine D4 receptor gene. The study found that African-American individuals, who had at least one L allele, had a higher risk of smoking and earlier age of smoking initiation compared to individuals homozygous for the S allele. Interestingly, this association was not observed among whites. Since the L allele has been associated with personality trait of “novelty seeking,” the authors suggested that the ethnic differences in their study might have been related to genetically mediated differences in the novelty-seeking trait between the two ethnic groups. The role of dopamine D1 receptor gene in nicotine dependence was investigated by Comings et al.60 as a part of a study that examined dopamine receptor genes among smokers, Tourette syndrome probands, and pathologic gamblers. In all three groups, there was a significant increase in the frequency of individuals with the 1/1 or 2/2 genotype compared to control subjects, suggesting that there may be an overlap in genetic susceptibility to addictive behaviors, possibly mediated by allelic variants in the D1 gene.

The Dopamine Transporter Gene: The dopamine transporter protein is expressed by the SLC6A3 gene, and variations in the dopamine transporter gene have been reported to mediate concentrations of and responses to synaptic dopamine. A variable number tandem repeat polymorphism in this gene has been described61; the 9-repeat allele (SLC6A3–9) has been associated with dopamine excess disorders,62 and the 10-repeat allele (SLC6A3–10) has been linked to conditions with insufficient dopamine.63,64 Lerman et al.65 found that smokers were significantly less likely to have SLC6A3–9 genotype than nonsmokers. Also smokers with the SLC6A3–9 genotype were more likely to have started smoking after 16 years of age and had quit smoking in the past for significantly longer periods than those with other genotypes. Notably, the association of SLC6A3 gene with smoking was pronounced in persons with DRD2*A2 genotypes. The investigators suggested that the increased synaptic dopamine linked to the SLC6A3–9 genotype may be protective against smoking, and this may be more likely to occur among individuals with normal DRD2 densities. These findings were partly confirmed by Sabol et al.66 in a population of current, former, and nonsmokers. Their data showed that there was no association between the SLC6A3 gene and smoking initiation, but a significant association was observed for smoking cessation. In both these studies, the effect size was small and mixed samples of volunteers were recruited, raising the possibility of volunteer bias. A more recent study on a community sample67 failed to replicate these findings, indicating that more consistent evidence is necessary to understand the association between the dopamine transporter gene and smoking.

Genes Influencing Metabolism of Dopamine: Different enzymes such as TH, dopamine β-hydroxy-
lase, catechol-O-methyl transferase (COMT), and monoamine oxidase (MAO)-A and MAO-B are involved in the synthesis and metabolism of dopamine. Although these genes have been investigated in several disorders such as alcoholism, depression, and schizophrenia, limited data are available among smokers. Exposure to tobacco smoke has been reported to reduce levels of MAO-A and MAO-B in the brain. Consistent with these findings, Costa-Mallen et al reported a modest association between a polymorphism in the gene for MAO-B and smoking. Similarly, an association between heavy smoking and variations in genes for MAO-A and dopamine β-hydroxylase, but not COMT, was found in a British study, with a stronger relationship among whites and women. The only study that investigated polymorphisms in the TH gene among smokers reported negative findings.

Serotonergic and Nicotinic Genes

Increasing evidence indicates that nicotine increases serotonin release in the brain and that symptoms of nicotine withdrawal may be modulated by diminished serotonergic neurotransmission. The serotonin transporter (5-HTT) has attracted attention of researcher because it regulates the magnitude and duration of serotonin neurotransmission. A polymorphism in the 5’ promotor region of the 5-HTT yielding a short (S) and a long (L) variant of the allele has been reported. The S variant has been associated with reduced serotonin expression and uptake. The few studies that examined the role of the 5-HTT gene in smoking have yielded contradictory results. While Lerman et al found no significant difference in the distribution of 5-HTT genotypes among smokers and nonsmokers, an association between the L allele and smoking was reported among Japanese population. This conflicting data may be due to different study populations and differences in genotype grouping. More recent studies have reported an interaction between 5-HTT gene polymorphism and neuroticism in nicotine addiction, suggesting that nicotine addiction may be influenced by the combination of 5-HTT gene and anxiety-related personality traits than by either factor alone. Variations in the 5-HTT gene may also affect platelet aggregation a risk factor for heart disease; since a single gene is responsible for the expression of 5-HTT on platelets and brain, it may be interesting to explore whether risk for heart disease and nicotine addiction may share genetic susceptibility mediated through the 5-HTT gene.

Silverman et al detected five novel single nucleotide polymorphisms in the gene for the α2 subunit of the nicotinic acetylcholine receptor, reported to mediate the reinforcing properties of nicotine in knock-out experiments. However in a large well-designed study, the investigators did not find an association between four of these polymorphisms and smoking; more data in this area are required to draw any meaningful conclusions about the role of acetylcholine receptor genes in smoking. Table 1 summarizes the major findings of candidate gene studies.

Linkage Analysis

Developments in cloning, hybridization, and sequencing techniques have permitted chromosomal localization of several genes influencing neurotransmission. Two studies have suggested evidence for linkage of nicotine addiction to chromosomal regions. Using the Collaborative Study on the Genetics of Alcoholism data, Bergen et al performed a sibling-pair linkage analysis of two smoking-related traits, ever/never smokers and pack-year. There was some evidence of linkage of the ever/never smoking trait to regions on chromosome 6, 9, and 14. Similarly, in a family based pedigree study, Duggirala et al found strong evidence for linkage of smoking behavior to a genetic location on chromosome 5q and weaker evidence for linkage of smoking behavior to locations on chromosome 4, 15, and 17. However, an extensive linkage study conducted among 130 families from New Zealand employing a genome scan failed to find a strong evidence of linkage of smoking to selected individual regions on chromosome 2, 4, 10, 16, 17, and 18; the discrepant findings may be related to differences in clinical characteristics of phenotype, the presence of comorbidity such as alcohol abuse, or differing contributions of environmental influences. The data from linkage studies have been inconclusive so far, but has the potential to provide more definitive evidence of the involvement of specific genes in mediating nicotine addiction.

Conclusion

Twin and animal studies have consistently found a substantial genetic influence on the development of nicotine dependence. Although definitive evidence is not yet available, variations in several candidate genes may contribute to smoking. Perhaps the most consistent evidence exists for the genes coding for the CYP group of enzymes that lead to increased metabolism of nicotine and the DRD2 genes that regulate dopamine function. However, it is worth noting that environmental factors are also important mediators of smoking. A better understanding of the genetic and environmental influences and their in-
teractions should reinforce the concept of tobacco smoking as a chronic addictive disease that needs to be addressed. Furthermore, insights into the genetic contributions to smoking can potentially lead to more effective strategies to reduce smoking.

**Future Directions**

Most of the genetic data have been obtained from research designs that have limitations in the study of complex behaviors. Also, the inconsistencies in the current body of evidence have limited the clinical utility of the findings. It is increasingly recognized that smokers are not a homogeneous group; moreover, genetic influences on different stages of smoking such as initiation, maintenance, and cessation may not be identical.\(^\text{19}\) Researchers may need to examine the behavioral differences leading to tobacco dependence by studying well-defined subgroups of smokers with clearly characterized phenotypes and incorporating methodologies designed to avoid problems of population stratification.

There are suggestions that possible candidate genes may also include genes encoding the opioid, cannabinoid, and glutamate receptors.\(^\text{89}\) Since several genes may mediate nicotine dependence, utilizing newer techniques such as arrays, which permit rapid screening and detection of multiple gene variations, may be more efficient and cost-effective. Also, the extent to which genetic risk factors are common to all drugs of abuse or specifically determine the variance of individual drugs, including nicotine, requires further clarification.\(^\text{90–92}\)

Considering the evidence for ethnic differences in metabolism of nicotine, it seems timely that genetic investigations are expanded to include different ethnic groups. More importantly, along with determining the interactions of genes with each other, genetic research will have to examine the relationships of genes with other biological and environmental factors.\(^\text{85}\) Such an integrated approach may help to develop better strategies to reduce smoking. Finally, investigators have to be sensitive to the possibility that genetic markers for smoking have the potential for misuse and issues of confidentiality and access to genetic information are likely to become increasingly prominent in the future.

**References**


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<th>Table 1—Candidate Gene Association Studies in Smoking*</th>
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*CI = confidence interval; US = United States; AA = African American; HA = Hispanic American; UK = United Kingdom; NA = not available; NS = not significant.

\(\text{†}\)Data from patients enrolled in a case-control study for lung cancer.

\(\text{‡}\)Control population from a lung cancer case-control study.

\(\text{§}\)Family-based association approach.

\(\text{¶}\)Nonvounteer sample.

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